Cardiovascular disease, inflammation, and periodontal infection

David W. Paquette, Nadine Brodala & Timothy C. Nichols

Cardiovascular and periodontal diseases are common inflammatory conditions in the human population. In atherogenesis, inflammation plays a continuous role from endothelial cell expression of adhesion molecules to the development of the fatty streak, established plaque, and finally plaque rupture. Exposures to infections like periodontal disease have been postulated to perpetuate inflammatory events in atherogenesis. Recent observational studies and meta-analyses continue to demonstrate a modest but statistically significant increased risk for cardiovascular disease among persons exposed to periodontal disease or infection. Experiments with animal models further indicate that periodontal infection can increase atherosclerosis in the presence or absence of hypercholesterolemia. While the available pilot data in patients suggest that periodontal interventions can improve surrogate serum biomarkers and vascular responses associated with cardiovascular disease, the effect of these interventions on true outcomes of cardiovascular diseases like myocardial infarction and stroke is presently unknown. Nevertheless, clinicians and patients should be aware of the consistent association between cardiovascular and periodontal diseases along with the potential preventive benefits of periodontal interventions.

Cardiovascular disease accounts for 29% of deaths worldwide and ranks as the second leading cause of death after infectious and parasitic diseases (100). Atherosclerosis, which is a major component of cardiovascular disease, affects one in four persons and contributes to 39% of deaths annually in the United States (4). In atherosclerosis, large to medium-sized muscular and large elastic arteries become occluded with fibro-lipid lesions called atheromas. End-stage complications or events associated with atherosclerosis include coronary thrombosis, acute myocardial infarction, and stroke. Traditional risk factors related to behaviors, diet, lifestyle, and family history do not appear to fully account for the development of atherosclerosis. Furthermore, despite continued preventive efforts addressing modifiable risk factors, mortality rates from cardiovascular disease have remained virtually unchanged over the past decade in developed countries. Clinicians and investigators currently appreciate that inflammation appears to play a pivotal role in the development of atherosclerosis. This appreciation has intensified the search for chronic exposures or infections that potentially cause inflammation in vessels. Putative infections that may at least exacerbate atherosclerosis include cytomegalovirus, herpes simplex virus, Chlamydia pneumoniae, Helicobacter pylori, and periodontal disease (71). The major objective of this review is to provide the reader with a strong fundamental understanding of the pathogenesis, risk factors, and current interventions for cardiovascular disease. This review will also present the latest relevant research data implicating a relationship between cardiovascular and periodontal diseases.

Inflammation and the pathogenesis of cardiovascular disease

Inflammation plays a central and continuous role in the pathogenesis of atherosclerosis from its initiation to the development of clinical complications (Table 1) (58, 60). Normally, endothelial cells, which form the innermost surface of the artery wall, resist adhesion by circulating leukocytes. Several exposures or risk factors for atherosclerosis upset this homeostasis. Factors, such as a smoking, hypertension,
high-saturated-fat diet, obesity hyperglycemia and insulin resistance, promote endothelial cell expression of adhesion molecules that allow attachment of leukocytes to the arterial wall, a seminal event in inflammation. One such adhesion molecule is vascular cell adhesion molecule-1, which binds monocytes and T lymphocytes, the types of leukocytes found in early atherosclerotic plaques. Cybulsky and Gimbrone demonstrated that endothelial cells expressed vascular cell adhesion molecule-1 in certain vascular areas prone to lesion formation in rabbits fed an atherogenic diet (18). In the same rabbit model, vascular cell adhesion molecule-1 expression clearly precedes the appearance of macrophages in the artery intima (the layer underneath the endothelium) (56). Similarly, mice prone to atherosclerosis (i.e. because they cannot produce low-density lipoprotein receptor or apolipoprotein E) but engineered to express a poorly functioning form of vascular cell adhesion molecule-1 show a significant reduction in atherosclerosis as compared to normal vascular cell adhesion molecule-1-producing controls (19). This difference in lesion formation occurs despite similar cholesterol concentrations, lipoprotein profiles and circulating leukocyte counts in the mice. One recognized molecular initiator of vascular cell adhesion molecule-1 expression for animals placed on athero-

ogenetic diets is the accumulation of modified lipoprotein particles in the arterial intima. Other initiators include oxidized lipids (via nuclear factor-κB-mediated pathways) and pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α (85).

Atherosclerotic lesions generally develop in specific areas in animals and humans secondary to the blood flow characteristics. Laminar blood flow produces shear stress coinciding with several protective mechanisms in vessels such as nitric oxide synthase expression (33). Enzymatic production of the vasodilator, nitric oxide, can down-regulate vascular cell adhesion molecule-1 gene expression by inhibiting nuclear factor-κB activation and platelet aggregation (22). In contrast, areas of the vasculature prone to atherogenesis experience disturbed blood flow and a reduction in these protective mechanisms. For example, cultured endothelial cells subjected to disturbed flow exhibit increased expression of nuclear factor-κB compared with cells exposed to laminar flow (30).

### Development of the fatty streak

The accumulation of monocytes in the vessel intima is a hallmark event in the development of the early
atherosclerotic lesion called the ‘fatty streak.’ Following adherence to arterial endothelium, monocytes penetrate the vessel lining via diapedesis or migration between endothelial cells (Table 1). This cellular event requires a chemoattractant gradient largely because of monocyte chemoattractant protein-1. Mice with inactive low-density lipoprotein receptors and also lacking the ability to express monocyte chemoattractant protein-1 have approximately 80% less lipid deposition and fewer macrophages in the walls of their aortas despite consuming the same high-fat diet as compared to monocyte chemoattractant protein-1-producing mice (13). In contrast, homozygous apolipoprotein-E-deficient mice also lacking the ability to express the receptor for monocyte chemoattractant protein-1 (CCR2), exhibit significantly less atherogenesis than do mice with a normal CCR2 gene (11). Within the intima, monocytes mature into macrophages, express scavenger receptors, and engulf modified lipoproteins. Cholesterol esters accumulate in the cytoplasm of these macrophages, which transform into ‘foam cells’ (i.e. lipid-laden macrophages in the vessel intima). At the same time, the macrophages multiply and release several growth factors and cytokines, which amplify and sustain pro-inflammatory signals. One growth factor, macrophage colony-stimulating factor appears to be an important mediator of these transformation and proliferation steps. It is also over-expressed in animal models and human atherosclerotic plaques (17, 79). Mice prone to atherosclerosis as a result of reduced expression of the low-density lipoprotein receptor or the apolipoprotein E gene and also lacking the ability to express macrophage colony-stimulating factor show slower atherogenesis and reduced macrophage accumulation as compared to mice with normal macrophage colony-stimulating factor expression (75, 90).

T lymphocytes also participate in the pathogenesis and inflammatory events of atherosclerosis. These immune cells enter the inflamed artery wall and join macrophages via a number of interferon-\(\gamma\)-inducible chemokines (e.g., \(\gamma\)-IP-10, MIG, and I-TAC) that interact with the CXCR3 receptor on T lymphocytes (64). Several other adhesion molecules, chemokines, cytokines, and growth factors participate in this process. For example, the interaction between interleukin-8 and its receptor, CXCR2, can also contribute to lesion formation in mice (10). Nevertheless, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1, and macrophage colony-stimulating factor appear to be the key inflammatory signals in the initiation and development of the fatty streak (58).

Progression to complex plaque

While accumulation of foam cells is the hallmark of the fatty streak, the accumulation of fibrous tissue in vessels typifies the advanced atherosclerotic lesion called the ‘complex plaque.’ Smooth muscle cells synthesize the bulk of the extracellular matrix of complex plaques; hence, their arrival and elaboration of extracellular matrix provides the transition to a fibrolipid lesion. Growth factors and cytokines (e.g. platelet-derived growth factor) liberated from endothelial or infiltrating monocytes stimulate the migration of smooth muscle cell from the vessel tunica media into the intima. Mediators including platelet-derived growth factor, transforming growth factor-\(\beta\) and interleukin-1 stimulate the smooth muscle cells to produce interstitial collagen. The formation of complex plaques can occur at an early age as demonstrated by classic autopsy studies (99). Indeed, one of six teenagers in the United States already exhibits pathological intimal thickening in their coronary arteries (94).

Endothelial cells do not appear to be passive responders to immunological stimuli from leukocytes in the formation of complex plaques. For instance, human endothelial cells exposed to bacterial endotoxin express interleukin-1\(\beta\) and interleukin-1\(\alpha\) messenger RNA (59). Other cytokines expressed by vascular wall cells have been identified, including tumor necrosis factor-\(\alpha\), tumor necrosis factor-\(\beta\), interleukin-6 along with macrophage colony-stimulating factor and monocyte chemoattractant protein-1 (60). Another pro-inflammatory cytokine, CD40 ligand (CD154), can also contribute to this phase of atherosclerosis because interruption of CD40/CD154 signaling slows the initiation and progression of atherosclerosis (63). Accordingly, low-density lipoprotein-receptor-deficient mice fed a high-cholesterol diet and treated with antibody to CD154 show significantly smaller atherosclerotic lesions as compared to control groups (treated with either rat immunoglobulin or saline only) (82). This provocative finding reported by Schonbeck et al. illustrates that inflammation influences the progression of atherosclerosis and that inhibiting inflammatory events not only prevents the formation of new lesions but also slows the progression of existing atherosclerosis.

Plaque rupture

While atheromatous plaques narrow the lumina of affected vessels and compromise blood flow, the
major clinical sequelae of atherosclerosis (coronary thrombosis, myocardial infarction, and stroke) develop following plaque rupture and thrombosis (Table 1). In coronary arterial thromboses, the underlying atherosclerotic lesion often does not produce critical arterial narrowing (36). Coronary arteries for the most part can enlarge and compensate for developing plaques (i.e. up to 40% stenosis) thus preserving blood flow to the myocardium (34).

Physical disruption of the atherosclerotic plaque causes most acute coronary syndromes via thrombus formation and sudden expansion of the lesion (58). In the non-ruptured plaque, the ‘fibrous cap’ protects the blood from the lipid core of the plaque. An intact fibrous cap owes its biomechanical strength and stability to interstitial collagen. When the fibrous cap fractures, blood comes into contact with the lipid core, and a thrombus forms. Plaques that have ruptured and caused fatal thromboses in general have thin fibrous caps (61).

Inflammation interferes with the integrity of the fibrous cap in two ways: first, by blocking the creation of new collagen fibers, and second by stimulating the destruction of existing collagen. For example, interferon-γ produced by T lymphocytes in the plaque inhibits both basal collagen production and the stimulatory effects transforming growth factor-β, platelet-derived growth factor and interleukin-1 (3). T lymphocytes also promote the destruction of existing collagen in vulnerable plaques via the production of interleukin-1 and CD40 ligand. These mediators in atherosclerotic plaques stimulate macrophages to produce collagen-degrading enzymes or matrix metalloproteinases 1, 8, and 13 (42, 92). In addition, mast cells in plaques may release the matrix metalloproteinase-inducer, tumor necrosis factor-α, as well as serine proteinases, tryptase and chymase that can activate matrix metalloproteinase pro-enzymes (53, 80). CD40 ligand from T lymphocytes can also promote thrombogenicity of the lipid core via stimulation of macrophage tissue factor expression. This potent procoagulant when exposed to factor VII in the blood, initiates the coagulation cascade and thrombus formation (62). In summary, inflammation influences all of the events in atherogenesis including the final one, plaque rupture.

Risk factors and interventions for cardiovascular disease

Traditional major risk factors for cardiovascular disease include cigarette smoking, hypertension (> 140/90 mmHg), high low-density lipoprotein cholesterol (> 100 mg/dl), low high-density lipoprotein cholesterol (<40 mg/dl), diabetes mellitus, family history of premature coronary heart disease, age (men > 45 years, women > 55 years), obesity (body mass index > 30 kg/m²), physical inactivity and an atherogenic diet. It is also recognized that these factors can interact with each other to increase the risk of cardiovascular disease in patients (89). For example, the Framingham Heart Study (n = 32,995) showed that for various cholesterol levels between 185 and 335 mg/dl, cardiovascular risk was elevated with the addition of each of the following risk factors: glucose intolerance, elevated systolic blood pressure, cigarette smoking, and left ventricular hypertrophy on electrocardiography (50). Data from the Framingham Heart Study and two other large prospective cohort studies, the Chicago Heart Association Detection Project in Industry (n = 35,642) and the Multiple Risk Factor Intervention Trial (n = 347,978) indicate that the majority of patients with fatal coronary artery disease or non-fatal myocardial infarction present with at least one of four risk factors including cigarette smoking, diabetes mellitus, hyperlipidemia, and hypertension (35). With fatal coronary artery disease, exposure to at least one risk factor ranged from 87% to 100% for all three cohorts. For non-fatal myocardial infarction in the Framingham Heart Study cohort, previous exposure to at least one risk factor was found in 92% of men and 87% of women aged 40–59 years at baseline. Furthermore, another recent analysis involving 14 international randomized clinical trials (n = 122,458) showed that one of these four conventional risk factors was present in 84.6% of men and 80.6% of women with coronary artery disease (52).

Recent attention has focused on elevated serum C-reactive protein as a strong and independent risk factor or predictor of cardiovascular disease events (68). C-reactive protein is an acute-phase reactant primarily produced by the liver in response to infection or trauma. Other tissues may be involved in its synthesis including smooth muscle cells from normal coronary arteries and diseased coronary artery bypass grafts (14, 47). C-reactive protein appears to be directly involved in augmenting the innate inflammatory response via induction of prothrombotic factors (e.g. plasminogen activator inhibitor-1, pro-inflammatory adhesion molecules, and monocyte chemoattractant protein-1) and interference with endothelial nitric oxide synthase (26, 72, 97, 102). In the Physicians’ Health Study, an epidemiological study of over 22,000 healthy middle-aged men
with no clinical evidence of disease, increasing levels of serum high-sensitivity C-reactive protein at study entry were associated with up to a threefold increase in the risk of incident myocardial infarction and a twofold increase in risk of ischemic stroke (76). When compared with other potential serum biomarkers [e.g. homocysteine, lipoprotein(a), interleukin-6, intracellular adhesion molecule-1, and serum amyloid A] and standard lipid measures, C-reactive protein proved to be the single strongest predictor of cardiovascular risk in apparently healthy participants in the Women’s Health Study (n = 28,263) (77, 78). Accordingly, the relative risk ratio for the highest versus lowest quartile of serum C-reactive protein concentrations was 4.4 (95% CI 1.7–11.3). Moreover, the addition of serum C-reactive protein to traditional cholesterol screening enhanced cardiovascular risk prediction and proved to be independent of low-density lipoprotein cholesterol. Indeed, the poorest event-free survival in women was among those with high low-density lipoprotein cholesterol and high C-reactive protein levels, and the best event-free survival was among those with low low-density lipoprotein cholesterol and low C-reactive protein levels. Individuals with low low-density lipoprotein cholesterol levels but high C-reactive protein levels were at higher risk than those with low high-density lipoprotein cholesterol levels but low C-reactive protein levels. These data suggest that elevated C-reactive protein levels may be particularly useful for identifying asymptomatic individuals who may be at high risk for future cardiovascular events but who have average cholesterol levels.

Preventive interventions (primary or secondary) for cardiovascular disease focus on recognition and reduction of modifiable risk factors in patients. These approaches include blood pressure screening, weight reduction, exercise, smoking cessation, diet modification, and patient counseling and education. Initiating and maintaining these lifestyle changes are not easy tasks for patients. Pharmacological intervention with the statin class of drugs is used to further reduce serum lipids and the likelihood of cardiovascular events, even in those with average low-density lipoprotein concentrations. Numerous clinical trials have consistently demonstrated that statins reduce cardiovascular events by at least 25% (55, 69, 85). In contrast, the effect of statins and other lipid-lowering therapies on the extent of vessel stenosis caused by a plaque is much smaller (13); hence, statins may have secondary anti-inflammatory effects. Indeed, C-reactive protein concentrations decrease 15–50% with statin therapy (2, 70, 93, 96). For patients with end-stage cardiovascular disease, tertiary preventive interventions involve physically expanding stenotic vessels via angioplasty (with or without stenting) versus revascularization via coronary bypass surgery.

**Association between periodontal and cardiovascular diseases**

Patients with periodontal disease share many of the same risk factors as patients with cardiovascular disease including age, gender (predominantly male), lower socioeconomic status, stress, and smoking (7). Additionally, a large proportion of patients with periodontal disease also exhibit cardiovascular disease (95). These observations suggest that periodontal disease and atherosclerosis share similar or common etiological pathways. In 2003, Scannapieco et al. conducted a systematic review of the evidence supporting or refuting any relationship (81). In response to the focused question, ‘Does periodontal disease influence the initiation/progression of atherosclerosis and therefore cardiovascular disease, stroke, and peripheral vascular disease?’ the investigators identified 31 human studies. Table 2 lists select influential studies identified in the review plus additional recent observational studies discussed below. Although the authors did not perform any meta-analysis because of the differences in reported outcomes, the authors noted relative (not absolute) consistency and concluded, ‘Periodontal disease may be modestly associated with atherosclerosis, myocardial infarction, and cardiovascular events.’ An accompanying consensus report approved by the American Academy of Periodontology recommends, ‘Patients and health care providers should be informed that periodontal intervention may prevent the onset or progression of atherosclerosis-induced diseases.’

Since this review and consensus report, at least three meta-analyses on the cardiovascular–periodontal disease association have been conducted and published. Meurman et al. reported a 20% increase in cardiovascular disease risk among patients with periodontal disease (95% CI 1.08–1.32), and an even higher risk ratio for stroke varying from 2.85 (95% CI 1.78–4.56) to 1.74 (95% CI 1.08–2.81) (67). Similarly, Vetter and Khader et al. reported relative risk estimates of 1.19 (95% CI 1.08–1.32) and 1.15 (95% CI 1.06–1.25), respectively (51, 98). These meta-analyses of the available observational human data suggest a modest but statistically significant increase in the risk for cardiovascular disease with periodontal disease.
Table 2. Summary of observational studies (case–control and cohort) investigating an association between periodontal and cardiovascular diseases in human populations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Population</th>
<th>Periodontal outcome or exposure</th>
<th>Cardiovascular outcome</th>
<th>Findings and conclusions</th>
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<tbody>
<tr>
<td>Matilla et al. (65)</td>
<td>Case–control</td>
<td>100 cases and 102 controls</td>
<td>Dental Severity Index (sum of scores for caries, periodontal disease, periapical pathosis, and pericoronitis)</td>
<td>Evidence of myocardial infarction from ECG and elevated enzyme levels (creatine phosphokinase isoenzyme MB)</td>
<td>Dental health significantly worse in patients with myocardial infarction versus controls after adjusting for smoking, social class, smoking, serum lipids, and diabetes</td>
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<tr>
<td>Matilla et al. (66)</td>
<td>Case–control</td>
<td>100 cases</td>
<td>Dental Severity Index</td>
<td>Clinical diagnosis or radiographically confirmed myocardial infarction</td>
<td>Significant association between dental infections and severe coronary atheromatosis in men (but not women)</td>
</tr>
<tr>
<td>Arbes et al. (5)</td>
<td>Case–control</td>
<td>5,564 subjects (NHANES III)</td>
<td>Per cent attachment loss of all teeth (&gt;3 mm) and categorized according to four levels</td>
<td>Self-reported myocardial infarction</td>
<td>Positive association between periodontal disease and coronary heart disease (OR 3.8 for severe attachment loss) after adjusting for age, gender, race, etc.</td>
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<tr>
<td>DeStefano et al. (24)</td>
<td>Cohort</td>
<td>9,760 subjects (NHANES I)</td>
<td>Subjects classified with no periodontal disease, with gingivitis, periodontitis ≥4 mm probing depth or edentulous</td>
<td>Hospital admission or death due to coronary heart disease</td>
<td>Periodontitis is associated with small increased risk for coronary heart disease (RR 1.7) among men</td>
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<tr>
<td>Beck et al. (6)</td>
<td>Cohort</td>
<td>1,147 men (Normative Aging Study)</td>
<td>Percent radiographic alveolar bone loss</td>
<td>Incidence of total and fatal coronary heart disease and stroke</td>
<td>Periodontal disease associated with moderate risk for coronary heart disease (OR 1.5–1.9) and stroke after adjusting for age and cardiovascular disease risk factors (OR 2.9)</td>
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<tr>
<td>Joshipura et al. (48)</td>
<td>Cohort</td>
<td>44,119 subjects (Health Professionals Follow-up Study)</td>
<td>Self-reported number of teeth and history of periodontal disease</td>
<td>Fatal and non-fatal myocardial infarction or sudden death (revascularization cases excluded)</td>
<td>A small association between tooth loss and coronary heart disease risk for men (RR 1.7)</td>
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<td>Wu et al. (101)</td>
<td>Cohort</td>
<td>9,962 subjects (NHANES I and follow-up)</td>
<td>Subjects classified with no periodontal disease, with gingivitis, periodontitis (&gt;4 teeth with overt pocketing) or edentulous</td>
<td>Incident cases of stroke</td>
<td>Compared to periodontal health, relative risk for stroke with periodontitis was 2.1 and significant</td>
</tr>
<tr>
<td>Beck et al. (8)</td>
<td>Cohort</td>
<td>6,017 subjects (ARIC Study)</td>
<td>Severe periodontitis defined as clinical attachment loss ≥3 mm at ≥30% of sites</td>
<td>Carotid artery intima-media wall thickness ≥1 mm</td>
<td>Periodontitis may influence atheroma formation (OR 1.3)</td>
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<td>Hujoel et al. (44)</td>
<td>Cohort</td>
<td>8,032 dentate adults (NHANES I)</td>
<td>Periodontal pocketing and attachment loss</td>
<td>Death or hospitalization due to coronary heart disease or revascularization obtained from medical records</td>
<td>Periodontitis was not associated with a significant increased risk for coronary heart disease</td>
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<td>Howell et al. (43)</td>
<td>Cohort</td>
<td>22,037 male subjects (Physician’s Health Study I)</td>
<td>Self-reported presence or absence of periodontal disease at baseline</td>
<td>Incident fatal and non-fatal myocardial infarction or stroke</td>
<td>No significant association between self-reported periodontal disease and cardiovascular disease events</td>
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<tr>
<td>Hung et al. (45)</td>
<td>Cohort</td>
<td>41,407 men from the HPFS and 58,974 women from the NHS</td>
<td>Self-reported tooth loss at baseline</td>
<td>Incident fatal and non-fatal myocardial infarction or stroke</td>
<td>For men with tooth loss, the relative risk for coronary heart disease was 1.36. For women with tooth loss, the relative risk was 1.64</td>
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<tr>
<td>Pussinen et al. (73)</td>
<td>Cohort</td>
<td>6,950 Finnish subjects in the Mobile Clinic Health Survey</td>
<td>Serum antibodies to <em>P. gingivalis</em> or <em>A. actinomycetemcomitans</em></td>
<td>Incident fatal or non-fatal stroke</td>
<td>Seropositive subjects had an OR of 2.6 for stroke</td>
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<td>Beck et al. (9)</td>
<td>Cohort</td>
<td>15,792 subjects (ARIC Study)</td>
<td>Serum antibodies to periodontal pathogens</td>
<td>Carotid artery intima-media wall thickness ≥1 mm</td>
<td>Presence of antibody to <em>C. rectus</em> was associated with carotid atherosclerosis (OR 2.3)</td>
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<tr>
<td>Engebretson et al. (29)</td>
<td>Cohort</td>
<td>203 subjects from INVEST</td>
<td>Radiographic alveolar bone loss</td>
<td>Carotid plaque thickness via ultrasonography</td>
<td>Severe periodontal bone loss was independently associated with carotid atherosclerosis (OR 3.64)</td>
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Recent findings from several worldwide population studies warrant detailed consideration. These studies include the Atherosclerosis Risk in Communities Study, the Health Professional Follow-up Study, the Nurses Health Study, and the Oral Infections and Vascular Disease Epidemiology Study conducted in the United States. Other studies have involved populations from Sweden, Finland, and China.

Beck et al. have collected periodontal probing data on 6,017 persons, 52–75 years of age, participating in the Atherosclerosis Risk in Communities study (8, 9, 27). These investigators assessed both the presence of clinical coronary heart disease (myocardial infarction or revascularization procedure) and subclinical atherosclerosis (carotid artery intima-media wall thickness using B-mode ultrasound) as dependent variables in the population. Individuals with both high attachment loss (≥10% of sites with attachment loss >3 mm) and high tooth loss exhibited elevated odds of prevalent coronary heart disease as compared to individuals with low attachment loss and low tooth loss (odds ratio 1.5, 95% CI 1.1–2.0 and odds ratio 1.8, CI 1.4–2.4, respectively) (27). A second logistic regression analysis indicated a significant association between severe periodontitis and thickened carotid arteries after adjusting for covariates like age, gender, diabetes, lipids, hypertension, and smoking (8). Accordingly, the odds ratio for severe periodontitis (i.e. 30% or more of sites with ≥3 mm clinical attachment loss) and subclinical carotid atherosclerosis was 1.31 (95% CI 1.03–1.66). In a third report, these investigators quantified serum immunoglobulin G antibody levels specific for 17 periodontal organisms using a whole bacterial checkerboard immunoblotting technique (9). Analyzing the mean carotid intima-media wall thickness (≥1 mm) as the outcome and serum antibody levels as exposures for this same population, the investigators noted that the presence of antibody to *Campylobacter rectus* increased the risk for subclinical atherosclerosis twofold (odds ratio 2.3, 95% CI 1.83–2.84). In particular, individuals with both high C. rectus and *Peptostreptococcus micros* antibody titers had almost twice the prevalence of carotid atherosclerosis as compared to those with only a high C. rectus antibody (8.3% versus 16.3%). Stratification by smoking indicated that all microbial models significant for smokers were also significant for never smokers except for *Porphyromonas gingivalis*. Thus, clinical signs of periodontitis are associated with coronary heart disease and subclinical atherosclerosis in the Atherosclerosis Risk in Communities population, and exposures to specific periodontal

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pathogens significantly increase the risk for atherosclerosis in smoking and non-smoking subjects.

Hung et al. assessed self-reported periodontal disease outcomes and incident cardiovascular disease in two extant databases, the Health Professional Follow-up Study (n = 41,407 men followed for 12 years) and the Nurses Health Study (n = 58,974 women followed for 6 years) (45). After controlling for important cardiovascular risk factors, men with a low number of reported teeth (≤10 at baseline) had a significantly higher risk of coronary heart disease (relative risk 1.36 95% CI 1.11–1.67) as compared to men with a high number of teeth (25 or more). For women with the same reported extent of tooth loss, the relative risk for coronary heart disease was 1.64 (95% CI 1.31–2.05) as compared to women with at least 25 teeth. The relative risks for fatal coronary heart disease events increased to 1.79 (95% CI 1.34–2.40) for men and 1.65 (95% CI 1.11–2.46) for women with tooth loss respectively. In a second report, the investigators evaluated the association between self-reported periodontal disease and serum elevations in cardiovascular disease biomarkers cross-sectionally in a subset of Health Professional Follow-up Study participants (n = 468 men) (49). Serum biomarkers included C-reactive protein, fibrinogen, factor VII, tissue plasminogen activator, low-density lipoprotein cholesterol, von Willebrand factor, and soluble tumor necrosis factor receptors 1 and 2. In multivariate regression models controlling for age, cigarette smoking, alcohol intake, physical activity, and aspirin intake, self-reported periodontal disease was associated with significantly higher levels of C-reactive protein (30% higher among periodontal cases compared with non-cases), tissue plasminogen activator (11% higher), and low-density lipoprotein cholesterol (11% higher). These analyses reveal significant associations between self-reported number of teeth at baseline and risk of coronary heart disease and between self-reported periodontal disease and serum biomarkers of endothelial dysfunction and dyslipidemia.

One population study, the Oral Infections and Vascular Disease Epidemiology Study (or INVEST), has been planned a priori and conducted exclusively to evaluate the association between cardiovascular disease and periodontal outcomes in a cohort population. Engebretson et al. reported that for a group of 203 stroke-free subjects (ages 54–94 years) at baseline, mean carotid plaque thickness (measured with B-mode ultrasound) was significantly greater among dentate subjects with severe periodontal bone loss (≥50% measured radiographically) as compared to those with less bone loss (<50%) (29). Indeed, the group noted a clear dose–response relationship when they plotted subject tertiles of periodontal bone loss against carotid plaque thickness graphically. The investigators next collected subgingival plaque from 1,056 subjects and tested for the presence of 11 known periodontal bacteria using DNA techniques (25). The investigators found that cumulative periodontal bacterial burden was significantly related to carotid intima-media wall thickness after adjusting for cardiovascular disease risk factors. Whereas mean intima-media wall thickness values were similar across burden tertiles for putative (orange complex) and health-associated bacteria, values rose with each tertile of etiological bacterial burden (Actinobacillus actinomycetemcomitans, P. gingivalis, Treponema denticola and Tannerella forsythia). Similarly, white blood cell values (but not serum C-reactive protein) increased across these burden tertiles. These data from INVEST provide evidence of a direct relationship between periodontal microbiology and subclinical atherosclerosis independent of C-reactive protein.

Consistent associations between periodontal outcomes and atherosclerosis have been recently demonstrated among populations in Europe and Asia. For 131 adult Swedes, mean carotid intima-media wall thickness values were significantly higher in subjects with clinical and/or radiographic evidence of periodontal disease as compared to periodontally healthy controls (91). Multiple logistic regression analysis identified periodontal disease as a principal independent predictor of carotid atherosclerosis with an odds ratio of 4.64 (95% CI 1.64–13.10). Pussinen et al. monitored antibody responses for A. actinomycetemcomitans and P. gingivalis among 6,950 Finnish subjects for whom cardiovascular disease outcomes over 13 years were available (Mobile Clinic Health Survey) (73). Compared with the subjects who were seronegative for these pathogens, seropositive subjects had an odds ratio of 2.6 (95% CI 1.0–7.0) for a secondary stroke. In a second report on 1,023 men (Kuopio Ischemic Heart Disease Study), Pussinen et al. observed that cases with myocardial infarction or coronary heart disease death were more often seropositive for A. actinomycetemcomitans than those controls who remained healthy (15.5% versus 10.2%) (74). In the highest tertile of A. actinomycetemcomitans antibodies, the relative risk for myocardial infarction or coronary heart disease death was 2.0 (95% CI 1.2–3.3) compared with the lowest tertile. For P. gingivalis antibody responses, the relative risk was 2.1 (95% CI 1.3–3.4). Abnet et al. recently
published findings from a cohort study of 29,584 healthy, rural Chinese adults monitored for up to 15 years (1). Tooth loss was evaluated as an exposure outcome for periodontal disease, and mortality from heart disease or stroke were modeled as dependent variables. Individuals with greater than the age-specific median number of teeth lost exhibited a significantly increased risk of death from myocardial infarction (relative risk 1.28, 95% CI 1.17–1.40) and stroke (relative risk 1.12, 95% CI 1.02–1.23). These elevated risks were present in both men and women irrespective of smoking status. Collectively, these findings indicate consistent associations for periodontal disease and pathogenic exposures with cardiovascular disease for European and Asian populations.

**Biological plausibility and experimental evidence**

Since periodontal infections result in low-grade bacteremias and endotoxemias in affected patients (83, 86), systemic effects on vascular physiology via these exposures appear biologically plausible. Four specific pathways have been proposed to explain the plausibility of a link between cardiovascular disease and periodontal infection. These pathways (acting independently or collectively) include:

- direct bacterial effects on platelets,
- autoimmune responses,
- invasion and/or uptake of bacteria in endothelial cells and macrophages,
- endocrine-like effects of pro-inflammatory mediators.

In support of the first pathway, two oral bacteria, *P. gingivalis* and *Streptococcus sanguis*, express virulence factors, the collagen-like platelet aggregation associated proteins, that induce platelet aggregation *in vitro* and *in vivo* (39, 40). Second, autoimmune mechanisms may play a role because antibodies that cross-react with periodontal bacteria and human heat-shock proteins have been identified (41, 87). Deshpande et al. have thirdly demonstrated that the *P. gingivalis* serum can invade aortic and heart endothelial cells via fimbriae (23). Several investigative groups have independently identified specific oral pathogens in atheromatous tissues (16, 37). In addition, macrophages incubated *in vitro* with *P. gingivalis* and low-density lipoprotein take up the bacteria intracellularly and transform into foam cells (31). In the last potential pathway, systemic pro-inflammatory mediators are upregulated for endocrine-like effects in vascular tissues, and studies consistently demonstrate elevations in C-reactive protein and fibrinogen among periodontally diseased subjects (88, 101).

Experiments with animal models demonstrate that specific infections with periodontal pathogens accelerate atherogenesis. For example, inbred heterozygous and homozygous apolipoprotein-E-deficient mice exhibit increased aortic atherosclerosis when challenged orally or intravenously with invasive strains of *P. gingivalis* (15, 32, 54, 57). While *P. gingivalis* challenges increased aortic atherosclerosis in apolipoprotein-E-deficient mice in a hypercholesterolemic background only, normocholesterolemic pigs were recently shown to develop both coronary and aortic lesions with *P. gingivalis* challenges (12). This finding suggests that *P. gingivalis* bacteremias may exert an atherogenic stimulus independent of the high cholesterol levels in pigs. It is worth noting that a wide range of *P. gingivalis* doses was used in these animal studies. While the clinically relevant dose for human subjects is unknown at present, it probably varies greatly (21, 38, 46). Importantly, *P. gingivalis* challenge enhances atherosclerosis, despite different routes of administration and dosing regimens, in both species. The *P. gingivalis* 16 ribosomal DNA was detected by polymerase chain reaction in atheromas from some but not all of these mutant mice and pigs. These experiments suggest that both the host response and the virulence of the specific *P. gingivalis* strains appear to be important variables in these infection–atherogenesis models.

Evidence in humans demonstrating the beneficial effects of periodontal therapy on cardiovascular disease outcomes is limited and indirect at present. D’Auto et al. recently demonstrated that periodontitis patients treated with scaling and root planing exhibited significant serum reductions in the cardiovascular disease biomarkers, C-reactive protein and interleukin-6 (20). In particular, patients who clinically responded to periodontal therapy in terms of pocket depth reductions were four times more likely to exhibit serum decreases in C-reactive protein relative to patients with a poor clinical periodontal response. Elter et al. also report decreases in these serum biomarkers plus improved endothelial function (i.e. flow-mediated dilation of the brachial artery) for 22 periodontitis patients treated with ‘complete mouth disinfection’ (i.e. scaling and root planing, periodontal flap surgery and extraction of hopeless teeth within a 2-week period) (28). Similarly, Seinost et al. tested endothelial function in 30 patients with severe periodontitis and compared this with 31 periodontally healthy control subjects (84). Before the interventions, flow-mediated dilation was
significantly lower in patients with periodontitis than in control subjects. Periodontitis patients with favorable clinical responses to non-surgical periodontal therapy (i.e. scaling and root planing, topical and peroral antimicrobials plus mechanical retreatment) exhibited concomitant improvements in flow-mediated dilatation of the brachial artery and serum C-reactive protein concentrations. While the effects of periodontal therapy on cardiovascular disease events in patients have yet to be determined, the available pilot data suggest that periodontal therapies can improve surrogate cardiovascular disease outcomes like serum biomarkers and endothelial dysfunction.

Conclusions

Inflammation plays a central role in atherogenesis from endothelial cell expression of adhesion molecules to the development of the fatty streak, established plaque, and finally plaque rupture. Human observational studies and experimental animal models continue to implicate periodontal infection as a systemic exposure that may perpetuate these inflammatory events in vessels. Although treatments aimed at decreasing periodontal infection and inflammation can reduce serum inflammatory biomarkers predictive of cardiovascular disease and improve vascular responses, the clinical relevance of these surrogate changes to reduced risks for myocardial infarction or stroke are not known at this time. Nevertheless, clinicians and patients should be knowledgeable about this consistent association and the potential preventive benefits of periodontal interventions.

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