



Inflammation in Chronic Periodontitis and Significant Systemic Diseases

MICHAEL P. RETHMAN, DDS, MS

ABSTRACT Endogenous chemical mediators play seminal roles in the initiation, persistence, and resolution of inflammation. Recent studies have revealed parallels between inflammatory mediators and mechanisms common to oral and systemic diseases. These relationships imply that novel therapeutics that profoundly modulate inflammatory mediators may improve clinical outcomes. Key source for this article is a 2008 conference reported in a *Journal of Periodontology* supplement titled *Proceedings of the 2008 Workshop on Inflammation; Inflammation and Periodontal Diseases: A Reappraisal*.

AUTHOR

Michael P. Rethman, DDS, MS, is a periodontist in Kaneohe, Hawaii; a diplomate, American Board of Periodontology; and an adjunct assistant professor at both the College of Dentistry, The Ohio State University, and at Baltimore College of Dental Surgery, University of Maryland.

The Immune System¹⁻³

Inflammation consists of biochemical and cellular processes initiated by tissue irritation, injury, or infection. Nearby capillaries, soluble proteins and inflammatory cells respond to chemical signaling and edema results. Inflammatory cells are attracted from nearby tissues and blood. Complex cascades begin that are aimed at facilitating the destruction and removal of foreign organisms, removal and replacement of necrotic cells and damaged structural components, all aimed at the eventual restoration of tissue homeostasis and health.

Inflammation also activates other components of the immune system and may provide functional capabilities for these systems. Indeed, many of the more primitive mechanisms of the inflammatory response, such as lytic proteins collectively named complement, are tools that are used

by more targeted immune mechanisms that developed later in evolution. Unfortunately, some of these tools, although usually adequate to the task at hand, are often not ideal. Many aspects of inflammation are nonspecific in their actions and can damage or destroy important host tissues while attempting to restore homeostasis. Examples include the periodontal ligament in periodontitis, and joint components in rheumatic arthritis. Furthermore, inflammation may never fully succeed at restoring tissue health/homeostasis and chronic inflammation may result.

To better understand the context for inflammation as part of the immune system, it's important to recall a basic understanding of entire immune system itself. Therefore, two general divisions of the immune system will be described, namely the innate, or nonspecific, and adaptive, or specific, components.

The innate immune system consists of evolutionary older mechanisms that respond locally and immediately to infection or trauma. A key feature of the innate immune system feature is complement. The soluble protein components of complement circulate in the serum and may be activated by numerous pathways. Bacteria themselves can directly activate the complement. When activated, complement proteins self-assemble into pore-like tubular structures that can penetrate bacterial membranes causing them to perish. Although bacteria themselves can activate the complement, the complement is also an important example of an innate capability that can be activated or amplified by other immune system components.

Using chemical signals called cytokines, the innate system recruits immune cells, activates complement, facilitates the removal of foreign substances, and activates the adaptive immune system. Phagocytic immune cells such as neutrophils, monocytes, and macrophages release cytokines termed interleukins that in turn play other roles including the clearing of pathogens or marking them for destruction by other cells.

The adaptive immune system amplifies the capabilities of the innate immune system because it is able to distinguish between host and foreign substances. This system is highly adaptable because of an exquisitely refined genetic mechanism that permits a small number of genes to generate a vast number of different antigen receptors, each of which is uniquely expressed on individual lymphocytes. When challenged by a specific antigen, such lymphocytes are activated. However, there are functionally and anatomically distinct T-lymphocyte (T-cell) and B-lymphocyte (B-cell) systems. B-cells originate in bone marrow, inhabit the spleen, and circulate in the blood. T-cells originate in the thymus and reside in the lymph nodes.

B-cells produce antibodies and remain quiescent until becoming fully activated by a specific antigen whose molecular structure docks with a unique antigen receptor complex on the B-cell surface.

Once a B-cell encounters its matching antigen and receives an additional signal from a T-helper cell, it can further differentiate into a plasma B-cell or a memory B-cell. The former produces prodigious amounts of antibodies that quickly bind to invading cells that display a matching antigen and thereby facilitate

**WHEN ACTIVATED,
complement proteins
self-assemble into
pore-like tubular structures
that can penetrate bacterial
membranes causing
them to perish.**

their elimination via a number of mechanisms, including facilitated phagocytosis and complement-mediated lysis. Memory B-cells are long-lived and function as prompt-responders to assure a quick and overwhelming antibody response should the same antigen be detected again. Most vaccines take advantage of this aspect of B-lymphocytes.

Unlike B-cells, T-cells fail to recognize antigen in the absence of a formalized antigen presentation, with the important exception of superantigens that can trigger a T-cell response much more directly. (Many bacteria produce superantigens, including the normally nonoral *Staphylococcus aureus* and *Streptococcus pyogenes*. Superantigens may cause serious acute and chronic diseases including toxic shock syndrome, rheumatoid arthritis, diabe-

tes and several types of skin disorders.) T-cells are more typically activated by the presentation of a processed antigen.

Although many cell types can present antigens, dermal dendritic cells, certain B-cells, and macrophages play key roles. Dendritic cells are commonly found in the epithelium, including the oral mucosa. Presentation cells process antigenic proteins and present peptides to T-cells residing in nearby lymph nodes. When a proper match is made, T-cells proliferate and attack invaders that display the specific antigenic peptides on their cell membranes. Unfortunately, this exquisite system is not always perfect. This is because processed antigens similar to proteins displayed by a host's cells are thought responsible for many autoimmune diseases (e.g., rheumatic heart disease).

Genes, Epigenetics and Gene-Environment Interactions in Inflammation and Disease

Genes, also known as alleles, function as the primary blueprints for proteins responsible for the anatomy and functionality of each organism. The 30,000 human genes consist of deoxyribonucleic acid (DNA) polymers. DNA molecules contain three-unit nucleotide sequences that code for the 20 amino acids used to produce all human proteins. Only a small fraction of each DNA molecule is known to contain genes, the remainder appears to have other physiological functions that are as yet not well understood.

Human DNA's iconic double helix is usually coiled around protein complexes called histones. DNA-histone complexes form chromatin packaged into 23 pairs of chromosomes. The genes themselves, epigenetic factors, and gene-environment interactions all have roles in inflammation and disease.

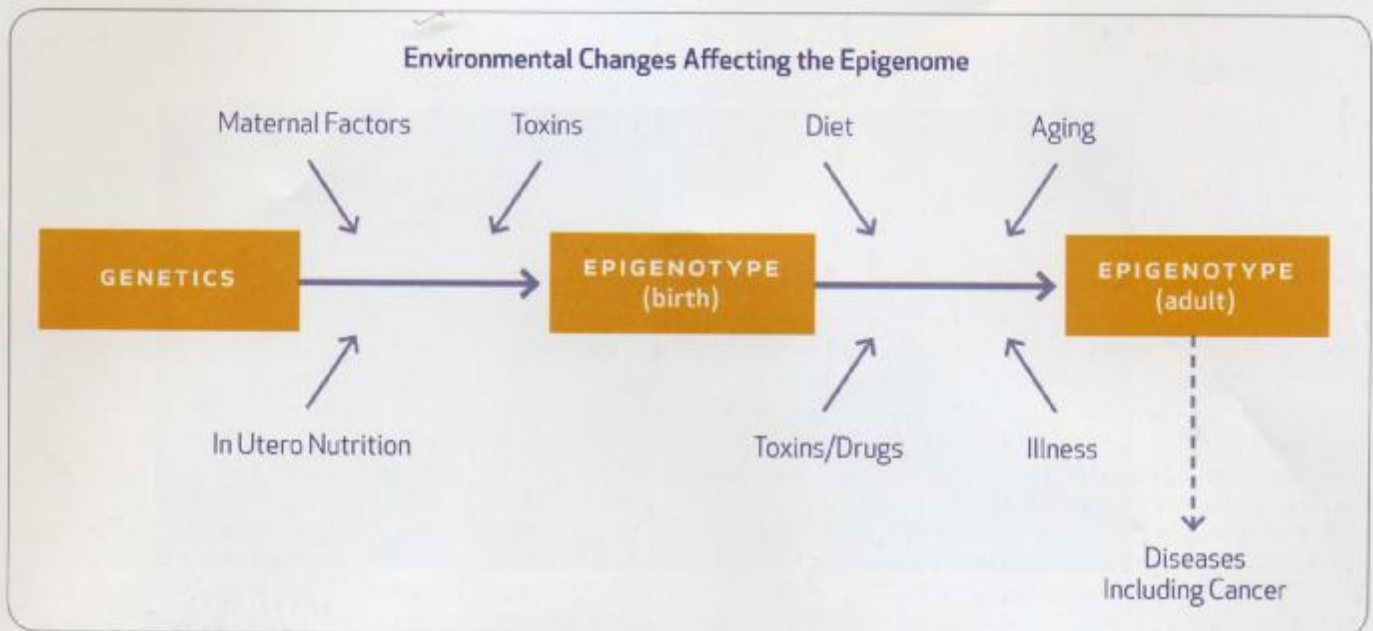


FIGURE 1. The epigenome changes in response to various environmental stimuli. Smoking, illnesses, drugs, diet, age, and in utero nutrition may affect the epigenetic signature to varying degrees at different points in development. Induced epigenetic modifications may be passed on to subsequent cell generations with potentially detrimental effects. Reprinted with permission from the American Academy of Periodontology 79:1517, 2008.

Gene-Caused Diseases

There are dozens of gene-caused disorders such as hemophilia A and sickle cell anemia. The latter is a gene-caused disease that results from a single nucleotide variation that appears in certain individuals in whom the amino acid valine replaces glutamate in a component of hemoglobin. Individuals who inherit this genetic variation from both parents lead shorter lives and are prone to serious vascular problems when their red blood cells assume sickle shapes. However, consistent with evolutionary pressures that affect human genes, children who inherit the variant allele from only one parent are substantially less likely to incur life-threatening malarial infections.⁴

Epigenetics and Disease⁵

Every cell with a nucleus contains all of an organism's genes. However, for cells to specialize as nerve cells, epithelial cells, muscle cells, etc., gene activity must be regulated. Epigenetics considered certain types of chemical modifications relevant

to how genes are activated.⁶ What this means is that every human cell has the same instruction manual, but different cell types are using different "chapters." For example, the secretory cells in the parotid gland contain the DNA instructions necessary to make bone, but for these cells and most others, the "bone genes" are turned off. Epigenetic changes are preserved when cells divide but most epigenetic changes only occur within the course of an individual organism's lifetime.⁷

New evidence suggests key roles for epigenetics in human pathologies, including inflammatory and neoplastic disorders. The epigenome is influenced by environmental factors throughout life. Nutritional factors can have profound epigenetic effects on the expression of specific genes and these traits can be passed on to subsequent generations of cells. Some cancers are associated with altered epigenetic profiles that lead to altered expression of genes involved in cell growth or differentiation. Epigenetic changes are necessary for the inactivation of one of

the two X chromosomes in females and the monoallelic expression of certain regulatory genes (e.g., insulin growth factor-2 expressed from the paternal gene only).

Epigenetic changes are likely causes of the increased frequency of autoimmune and neoplastic with increasing age. Indeed, studies in aging monozygotic twins reveal increasing epigenetic differences apparently resulting from environmental influences (FIGURE 1).

Acetylation of histone proteins and methylation of DNA are two central epigenetic mechanisms. The former relaxes histone structures thereby encouraging gene expression by making the DNA more accessible for gene transcription. On the other hand, DNA methylation inhibits transcription.

Although epigenetics is an emerging field of study in inflammation research, some activities have been identified. Experiments examined the gene-specific control of lipopolysaccharide (LPS)-induced tolerance by chromatin.⁸ (Many bacterial species associated with

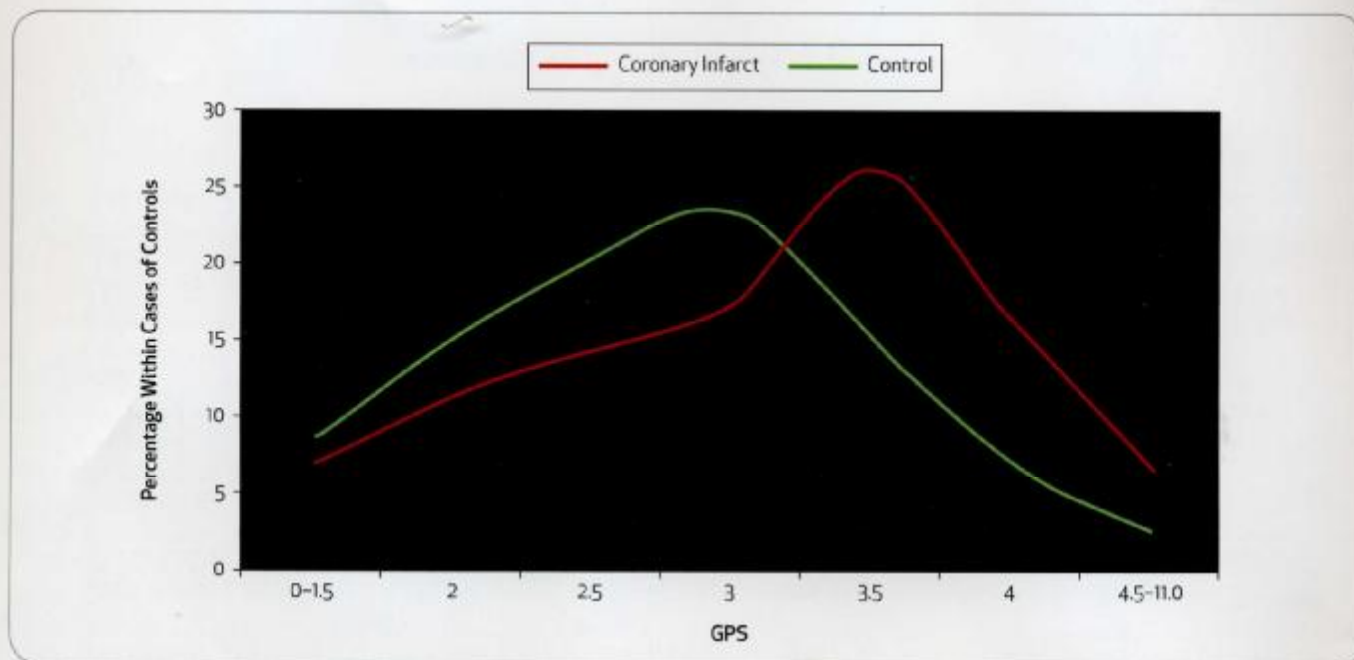


FIGURE 2. Distribution of cases with coronary infarct and control subjects by GPS. The subjects with coronary infarct (red line) had a GPS distribution that skewed toward the higher values, whereas the distribution of the control subjects (green line) was skewed toward lower values. Data from Trichopoulos et al.¹⁰ Reprinted with permission from the American Academy of Periodontology 79:151Q, 2008.

periodontitis produce LPS.) Although macrophages responded to LPS stimulation, they become hyporesponsive upon repeat LPS stimulation. Two distinct patterns of chromosomal modifications occurred during this hyporesponsive state. A group of genes responsible for inflammatory molecule production (e.g., TNF α and IL-6) was transiently silenced (i.e., tolerized). A second group of genes that includes various anti-microbial capabilities remained nontolerized. Of note is that tolerization seems to limit additional pathology associated with excessive inflammation, whereas nontolerized genes continue to produce anti-microbial enzymes that can do more harm than good.

Gene-Environmental Interactions⁹

This section will discuss the effects of the environment on genetic expression relevant to maladies linked to oral inflammation, namely type 2 diabetes, cardiovascular diseases, and metabolic syndrome, MetS.

Numerous studies have shown that

individuals respond differently to drugs and diet. Despite tightly controlled conditions, dietary interventions to reduce serum cholesterol demonstrate wide-ranging yet modest average effects. The wide range of effects is encouraging, especially for those with a genotype that is highly responsive. However, the middling average response suggests significant genetic complexity underlying common clinical phenotypes such as "those with high cholesterol." In recent years, the complexity of genetic bases for type 2 diabetes has suggested that many genes play roles making it nearly impossible to study the effects of any single gene because of each gene's relatively small effect.

A recent study scored and ranked individuals by genetic predisposition scores (GPS) based on certain candidate genes, some of which produce cytokines involved in inflammation (e.g., IL-1 β , IL-6 and TNF).¹⁰ The incidence of myocardial infarction was assessed. These results suggest a "genetic threshold" for predisposition to myocardial infarction. This study was also important because it

revealed that a diet high in plant foods, olive oil, and moderate wine intake (but low in meat and dairy products) was cardioprotective even in individuals with high GPS scores (FIGURE 2).

MetS is a constellation of abnormalities, generally considered to include abdominal obesity, high blood glucose/impaired glucose tolerance, dyslipidemia, and high blood pressure. Together these increase the risk for type 2 diabetes and cardiovascular disease. A growing body of evidence from experimental and epidemiologic studies suggests that a nexus of all these abnormalities is a proinflammatory state. The hypothesis that chronic low-level inflammation underlies the pathophysiology of MetS is supported by the finding that as the characteristics of MetS rise in a population, plasma concentrations of proinflammatory markers, high sensitivity C-reactive protein (CRP) and IL-6, also increase, as the concentration of adiponectin (an adipocyte-derived protein important in glucose regulation and fatty acid (FA) catabolism) decreases.

A number of gene-environmental interactions have been surmised from an ongoing study that examines how gene-environmental interactions influence susceptibility to MetS. Known as the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, it aims to characterize the genetic bases for the variable response of triglycerides (TG) levels following two dietary challenges, one that acutely raises TG via a fat-laden diet versus lowered TG resulting from fenofibrate administration.¹² Twelve hundred genetically homogeneous subjects with and without MetS were compared:

- Waist circumference, saturated fatty acid levels in erythrocyte cell membranes, levels of CRP, IL-6 and TNF α were all higher in subjects with MetS. Levels of polyunsaturated fatty acids (PUFA) were lower.

- Not only was MetS associated with higher levels of IL-1 β , but the risk for MetS was also associated with several genetic variants of the genes that encode IL-1 β .

- In light of the above, investigators wondered if diet could counter increased risks among those with differing alleles. Data indicated that diets high in certain forms of PUFA could do just that.¹²

Other reports derived from the GOLDN data investigated the effects of TG-lowering fenofibrate treatment on risk factors for cardiovascular diseases. Fenofibrate (eg, brand-name pharmaceuticals Antara, Fenoglide, Lipofen, Lofibra, TriCor, Triglide) lowers serum lipid levels and targets the atherogenic "lipid triad" (high serum TGs, low high-density lipoprotein levels with small and dense low-density lipoprotein particles) and inflammation. Because both phenotypes are important components of diabetes and MetS that potentially link these

metabolic disorders to cardiovascular disease, fibrates were hypothesized to be therapies that might reduce cardiovascular disease risk in these patients.

Unfortunately, the study results were mixed. Some individuals with certain CRP alleles responded well. (CRP's role in atherogenesis, independent of lipid-based risk factors has been associated with multiple risk factors for cardiovascular disease including obesity, insulin resistance, and high blood pressure, and is a predictor of MetS.¹³) These data suggest that resistance

**ALTHOUGH INCOMPLETE,
research encouragingly
indicates that genetic
predisposition to MetS
and other disorders can be
substantially decreased
via dietary changes.**

to the anti-inflammatory drug fenofibrate depends on variable CRP genetic expression among MetS subjects. Similar to CRP expression, differences in alleles associated with proinflammatory IL-6 gene appear responsible for modulating serum levels of IL-6 and also modulate various serum lipid levels associated with MetS.

Another aspect of lipid metabolism is that the perilipin proteins coating intracellular lipid droplets in fat cells have numerous allelic variants that appear to play roles in lipid metabolism. These have been linked to postprandial TG levels, body weight, obesity, risk for MetS, and serum inflammatory levels.

Although incomplete, research encouragingly indicates that genetic predisposition to MetS and other disorders can be substantially decreased via dietary changes.¹⁴

Cytokines in Periodontal Tissue Destruction^{15,16}

Between the initial infection and the tissue destruction characterizing periodontitis is the production of numerous cytokines that mediate inflammatory mechanisms. Cytokines are functionally subdivided into chemokines, innate immune cytokines, and acquired immune cytokines. Animal experiments have suggested roles for all in periodontitis.

Chemokines are chemotactic cytokines, such as interleukin-8, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1. Chemokines are produced by cells normally present noninflamed tissue and recruit leukocytes and modulate osteoclast formation. Numerous cell types in the periodontium produce chemokines, including fibroblasts, endothelial cells, macrophages, osteoclasts, epithelial cells, neutrophils, monocytes, lymphocytes, and mast cells. Some stimulate osteoclast formation and survival.

Neutrophils, monocytes, and other cells produce innate immune cytokines such as IL-1, IL6, IL-11 and TNF α after being summoned to the site of injury or infection by the chemokines.

Experimental suppression of IL-1 appears to slow periodontal destruction; IL-6 appears pro-destructive; IL-11 appears protective. TNF α spurs osteoclast formation and accelerates periodontal breakdown as experiments in a murine model infected with periodontal pathogen *Aggregatibacter actinomycetemcomitans* (Aa) have shown. Indeed, greater numbers of Aa were observed in test mice genetically modified to decrease TNF α reactivity. However, despite higher bacterial levels, lower levels of bone-resorption-inducing cytokines were detected compared with control mice.

Acquired immune cytokines are produced by antigen-activated T- and B-cells as described above. They include IL-1,

IL-6 and TNF α in addition to IL-17 and nuclear factor-kappa B ligand (RANKL). IL-1 and IL-6 play roles in bone resorption via stimulation of RANKL, although lymphocytes also secrete numerous osteoclast-formation inhibitors such as osteoprotegerin (OPG), IL-4, IL-10, IL-13 and interferon. RANKL, which binds to RANK, is one of the most potent inducers of osteoclast formation and activity. OPG binds to RANKL and inhibits osteoclast activities. It seems clear that various immune cytokines can inhibit or enhance periodontal destruction.

Cytokines, such as IL-1, are also involved in a phenomenon termed bone decoupling. Bone decoupling is the unbalancing of osteoblastic bone formation with osteoclastic bone destruction as is seen in the bone loss that characterizes periodontitis. Experiments in diabetic mice have suggested that TNF α plays a role in inducing an increased morbidity among osteoblasts that may lead to decoupling.¹⁷ Similar evidence in primates has been reported.¹⁸

Multiple lines of evidence clearly indicate that increases in RANKL production raise the RANKL/OPG ratio and stimulate the differentiation maturation and longevity of osteoclasts leading to net bone loss. On the other hand, lowering of the ratio by either reducing RANKL or increasing OPG results in osteoclast apoptosis and is thereby osteoprotective.

Historically, periodontal practitioners have focused almost entirely on mitigating the bacterial etiologies of periodontitis. Although such tactics remain reasonable, it seems that reduction of inflammation and attenuation of the host's immune reaction to the microbial plaque, leading to a decrease in the ratio of RANKL to OPG resulting in a decrease in bone loss would be clinically useful as well. Future periodontal therapeutic tactics may directly target the RANK/RANKL/OPG axis.

The Relationship of Inflammation to Important Systemic Diseases That may be Associated With Chronic Periodontitis

Diabetes¹⁹

Diabetes is a serious health care concern. Its worldwide incidence is predicted to increase in concert with increased prevalence of obesity. Diabetes is a major individual and public health burden because of its serious microvascular sequelae. These include nephropathy, retinopathy, neuropathy, cardiovascular disease, and

THESE FINDINGS suggest that inflammatory processes may play a greater role in the long-term progression of type 1 diabetes than in its onset.

periodontitis. Total annual costs exceed \$132 billion in the United States alone.

Many factors, such as genetics, diet, sedentary lifestyle, the perinatal environment, age, and obesity are associated with diabetes. Nevertheless, an inflammatory basis for diabetes and its complications is gaining traction. Inflammation is associated with both type 1 and type 2 diabetes.

Type 1 diabetes is typically found in adolescents and young adults and arises from the autoimmune destruction of pancreatic islet cells that produce insulin. The increasingly common type 2 diabetes occurs mainly in adults, although its prevalence among young people is increasing in concert with childhood obesity rates. Type 2 diabetes is characterized by increased cellular nonresponsiveness

to insulin (known as insulin resistance) that overwhelms the ability of pancreatic beta cells to secrete sufficient insulin.

Although there is controversy surrounding the precise role of inflammatory processes in type 1 diabetes, intriguing findings have emerged from studies of the inflammatory biomarker, CRP. Although CRP concentrations in individuals with the new onset (within days of diagnosis) of type 1 diabetes were similar to those observed in healthy controls, levels in individuals with long-term type 1 diabetes were significantly higher ($P=0.04$).

These findings suggest that inflammatory processes may play a greater role in the long-term progression of type 1 diabetes than in its onset. To wit, increases in inflammatory markers are observed in conjunction with the complications of type 1 diabetes. For example, increases in circulating levels of CRP, soluble vascular cell adhesion molecule-1, and nitrotyrosine were seen in patients with microvascular disease compared to those without diabetes.²⁰ Increases in monocyte release of interleukin (IL)-1 β and superoxide anions were also reported in patients with type 1 diabetes.

Type 2 Diabetes

Increases in inflammatory markers have appeared in apparently healthy individuals who later developed type 2 diabetes.^{21,22} This suggests that inflammation ramps up early in the disease process. For example, in adult Pima Indians (epigenetically prone to type 2 diabetes), individuals with higher white blood cell (WBC) counts (an indicator of greater inflammation), were more likely to develop type 2 diabetes over a 20-year period compared with those who had lower WBC counts. Similarly, in a prospective study of apparently healthy, middle-aged women, inflammatory markers IL-6 and CRP were associated with an increased risk for developing type 2 diabetes over a

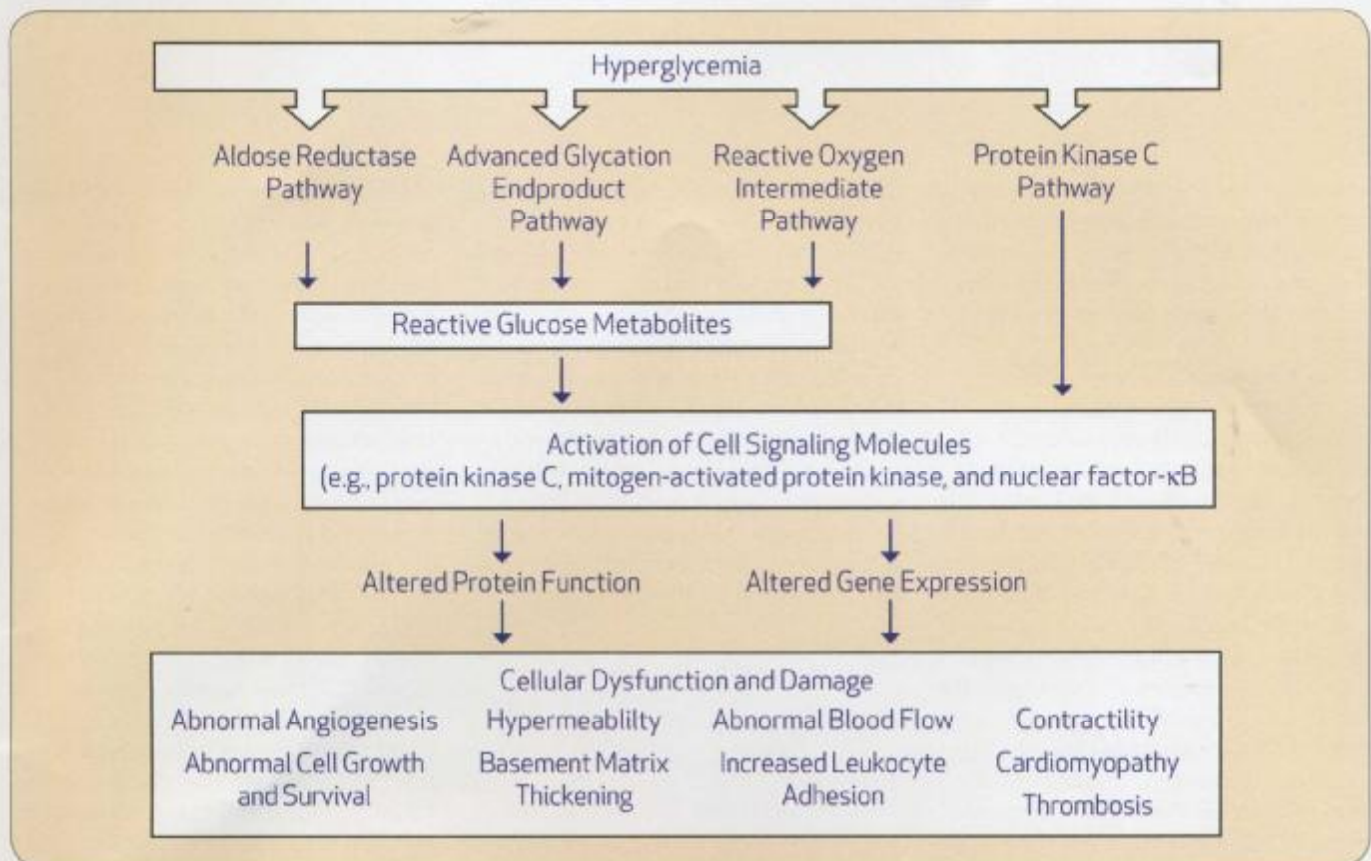


FIGURE 3. Major pathways initiated by hyperglycemia that contribute to complications of diabetes. Reprinted with permission from Blackwell Publishing.

four-year period. These findings are similar those seen in healthy, middle-aged men.

Type 2 diabetes is linked to obesity. Obesity and MetS have been linked to higher levels of inflammatory markers as discussed earlier. However, other data indicate that type 2 diabetes may develop in some independent of such associations. Nevertheless, evidence from both animal and human studies suggest possible roles for $\text{TNF}\alpha$, other inflammatory mediators, circulating markers of obesity (free fatty acids), bacterial lipopolysaccharides, protein kinases and/or oxidants in the development of insulin resistance in obesity and type 2 diabetes.

In this model, nuclear factor-kappa B is activated by these mediators and results in the transcription of genes that promote insulin resistance and the production of even more inflammatory markers. Fur-

thermore, both animal and human trials have shown that pharmacological disruption of this pathway improves insulin sensitivity and lowers inflammatory load.²⁴

The hyperglycemia that characterizes poorly controlled diabetes is considered a major risk factor for the development of diabetic complications including cardiovascular disease. **FIGURE 1** schematically represents pathways and mechanisms.

The actions of inflammatory pathways at the local tissue level are key to understanding their contribution to the pathogenesis of diabetic complications. Evidence suggests that increases in systemic markers of inflammation, such as CRP and IL-6, are associated with complications such as diabetic nephropathy. However, systemic inflammatory factors are only weakly associated with the development of diabetic retinopathy, and the relation-

ship remains unclear for periodontitis.

As noted in **FIGURE 3**, altered gene expression and altered protein function are thought to play roles at local levels where diabetic complications are manifested. Among numerous other cytokines, kinase beta ($\text{PKC}\beta$) is thought to play a key role in microvascular complications. The promising drug ruboxistaurin may inhibit this pathway and is in human clinical trials.

Although the most widely studied diabetic complications share a microvascular component adversely affected by hyperglycemia, periodontitis may be different. Indirect evidence linking periodontitis to obesity among individuals who are nondiabetic supports this distinction.

Nevertheless, it's not unlikely that obesity and insulin resistance enhance periodontitis risk and that hyperglycemia of diabetes worsens periodontitis. Additional

studies that tease out the relative contributions of the proinflammatory effects of obesity alone versus the effects of insulin resistance and hyperglycemia would be helpful to better understand these relationships.

*Inflammation and Alzheimer's Disease*²⁵

Alzheimer's disease is the most common cause of progressive intellectual failure and a major cause of dementia. As demographics in the developed world shift toward more aged populations, Alzheimer's may become even more prevalent. The classic pathologic hallmarks of Alzheimer's are two: β -amyloid plaques and the neurofibrillary tangles. In an Alzheimer's patient, these are profusely distributed in the frontal neocortex and limbic system. These brain regions are associated with the higher mental functions that Alzheimer's impairs. Furthermore, a recently recognized aspect of Alzheimer's pathology is inflammation, specifically, an innate inflammatory response that may reflect attempts to remove amyloid deposits from the brain.

In recent years, numerous innate inflammatory mediators have been reported to be upregulated in pathologically vulnerable regions of the brain in Alzheimer's disease. These data led to a re-examination of the dogma of brain immunologic privilege and new studies that examined the roles of the innate inflammatory response in a number of other neurologic disorders, particularly Parkinson's disease and human immunodeficiency virus dementia.

Discoveries about neuroinflammation are beginning to move to the clinic. More than 20 epidemiologic surveys have demonstrated that common nonsteroidal anti-inflammatory drugs (NSAIDs) may protect against the development of Alzheimer's. By contrast, anti-inflammatory treatment trials for existing Alzheimer's have typically shown little to no effect on halting or reversing

the disorder, although the drugs tested have often not been those suggested by epidemiological or other scientific results.

The extensive literature on innate inflammation and neurologic disease aside, key questions remain. First, are innate inflammatory responses a cause of neurologic disease or merely an effect? Second, can anti-inflammatory agents effectively treat existing neurologic disease, or is a protective strategy in high-risk patients the only reasonable option? Third, whether for protection or treatment, what is the best choice of anti-inflammatory agent?

ALZHEIMER'S DISEASE has not been associated with serum levels of proinflammatory mediators or with chronic periodontitis.

Of interest to dental practitioners, Alzheimer's disease has not been associated with serum levels of proinflammatory mediators or with chronic periodontitis.

*Inflammation, C-reactive Protein, and Atherosclerosis*²⁶

Cardiovascular events, such as myocardial infarction and stroke, remain leading causes of morbidity and death in the United States. Evidence suggesting etiologic links between chronic periodontitis and cardiovascular disease exists. Data derived from a meta-analysis of five prospective cohort studies, five case-control studies, and five cross-sectional studies suggested a positive correlation between periodontitis and coronary heart disease.²⁷ After adjusting for risk factors, such as smoking, dia-

betes, alcohol intake, obesity, and blood pressure, subjects with periodontitis had a 1.14- to 1.59-fold greater risk for developing coronary heart disease compared to those without periodontitis.

Although the mechanisms underlying this association are not clearly understood, it was reported that certain colonizers of periodontal pockets (*Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) have been detected in atherosclerotic plaques.^{28,29} These pathogens produce lipopolysaccharides, that, in turn, induce macrophages to secrete cytokines (interleukin [IL]-1 α and -1 β and tumor necrosis factor [TNF]) that can play important roles in atherothrombogenesis.

Elevated cell- and cytokine-mediated markers of inflammation, including CRP, fibrinogen, and various cytokines, are associated with periodontitis.³⁰ The same elevated proinflammatory factors in periodontitis have also been linked with atherothrombogenesis. The connection between vascular events and periodontitis is also supported by evidence that oral bacteria enhance the expression of platelet aggregation-associated protein.

Atherosclerosis appears to be a chronic inflammatory disorder, suggesting that plasma markers of inflammation would be useful for vascular disease risk assessment. For example, in a large prospective study involving healthy men, IL-6 levels were elevated among men who subsequently experienced a myocardial infarction compared with age-matched controls.³¹ In another large prospective study, healthy middle-aged women who subsequently developed cardiovascular events exhibited increased levels of soluble P-selectin, soluble CD40L, or macrophage-inhibitory cytokine compared with matched controls.³²⁻³⁴

TNF- α is another factor associated with cardiovascular disorders. Plasma concentrations of TNF- α were measured from 272 patients who developed recurrent nonfatal myocardial infarction or another cardiovascular event.³⁵ TNF- α levels were persistently elevated among postmyocardial infarction patients at increased risk for recurrent coronary events. These data indicate that changes in baseline levels of the inflammatory biomarkers discussed above may be potential biomarkers indicative of future risk for cardiovascular events, and may even be therapeutic targets aimed at cardiovascular disease prevention.

The high-sensitivity CRP (hsCRP) assay more accurately measures CRP than older assessment techniques. Increased hsCRP appears to be independent predictor for cardiovascular events. The relative risk for a first myocardial infarction and ischemic stroke increases as baseline concentrations of hsCRP rise (suggesting strongly that atherothrombosis — a typical precursor to myocardial infarction and stroke — is, at least in part, an inflammatory disorder).³⁶

Elevations of other biomarkers significantly associated with vascular events include Lp(a) lipoprotein, homocysteine, IL-6, total cholesterol, serum amyloid A, apolipoprotein B-100, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and the ratio of total cholesterol/HDL cholesterol.³⁶ In 2002, data derived from a longitudinal study of nearly 30,000 healthy women also supports CRP as a cardiovascular risk indicator.³⁷ CRP is also a stronger predictor for cardiovascular events and death than are measures of LDL. Indeed, women in the high CRP/low LDL subgroup were at higher absolute risk than those in the low CRP/high LDL subgroup.

After adjusting for components of the

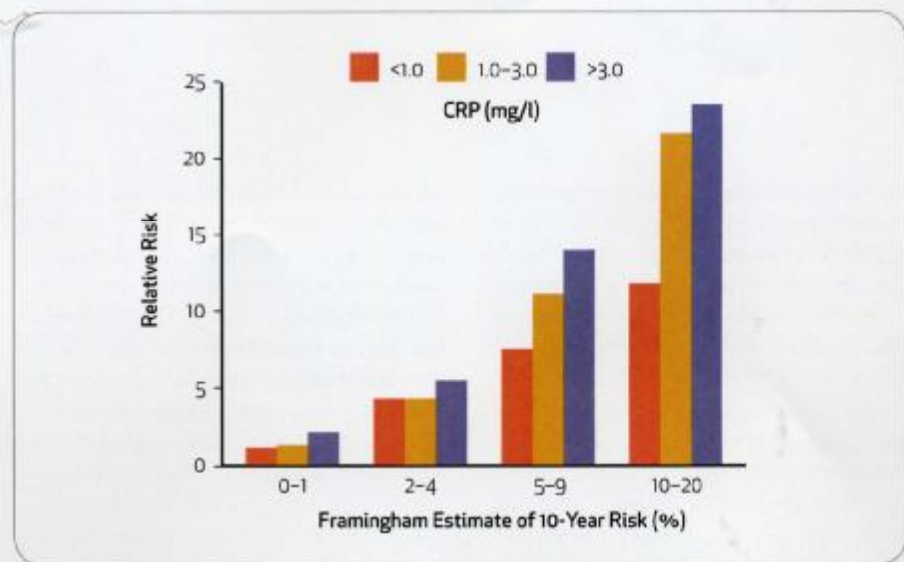


FIGURE 4. CRP levels are a stronger predictor of cardiovascular events than are LDL levels and add to prognostic information supported by the Framingham risk score. A) Event-free survival among women (N=27,939) with CRP and LDL levels above or below the median for the study population.¹⁴ B) Multivariable-adjusted relative risks for cardiovascular disease according to CRP levels and the estimated 10-year risk based on the Framingham risk score, currently defined by the National Cholesterol Education Program and according to CRP levels and categories of LDL.³⁹ Copyright 2002 Massachusetts Medical Society. All rights reserved. Ridker PM, Rifai N, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 347(20):1557-65, Nov. 14, 2002.

Framingham risk score, including age, smoking status, blood pressure, presence or absence of diabetes mellitus, and HDL and LDL levels, quintiles of CRP remained an independent prognostic factor for risk. Moreover, increasing levels of CRP were associated with an increased risk for cardiovascular events at all levels of estimated 10-year risks (FIGURE 4).

Since these reports, studies based on at least a dozen more population cohorts around the world have corroborated the usefulness of hsCRP as a predictor of myocardial infarction, ischemic stroke, and cardiovascular death. A recent, large, prospective, randomized, double-blind, placebo-controlled multicenter trial (the JUPITER trial) compared the effects of an oral statin drug versus a placebo in apparently healthy patients with elevated hsCRP levels and non-elevated levels of LDL cholesterol. This study was terminated early because of the beneficial effects of the statin drug rosuvastatin in reducing in reducing the rate of serious cardiovascular events.³⁸

Stroke and Ischemic Events

Inflammatory processes also appear to heighten the risk for stroke and cerebral small-vessel disease. In a sample of elderly people, ages 60 to 90, hsCRP levels were associated with the presence and progression of white matter lesions in the brain presumed to be the result of ischemia.³⁹ In another study, CRP levels were compared to the likelihood of ischemic strokes or transient ischemic attacks.^{40,41} After adjusting for other risk factors, those in the highest quartiles of CRP levels had a two- to threefold greater risk for stroke. Overall, these data support the hypothesis that CRP, as a marker of low-level inflammation, predicts an increased risk for cardiovascular events in apparently healthy individuals.

Inflammation Appears to be a Risk Factor for Diabetes

As noted earlier, evidence supports roles for inflammation in the pathogenesis of diabetes. Similar to cardiovascular disease, increased hsCRP is a predictor of risk

for type 2 diabetes. In a large prospective cohort study in women initially free of diagnosed diabetes, baseline levels of hsCRP and IL-6 were significantly higher among cases than controls.⁴² Later studies investigating a direct association between hsCRP levels and diabetes used exogenous injections of recombinant human CRP (rhCRP). Following injections of rhCRP, numerous cytokine markers of inflammation became significantly elevated compared with controls.⁴³ In a follow-up study by the same group, rhCRP injections administered to healthy males resulted in increased plasma glucose levels and decreased insulin production. Additional evidence implicated CRP as a prognostic marker for MetS. Collectively, this evidence suggested the need to develop strategies aimed at decreasing vascular risk among individuals with elevated levels of CRP.

Reynolds Risk Score Improves on Framingham Risk Score

Whether to add hsCRP assessment to traditional risk-prediction models, such as the Framingham risk score, remains a topic of current research. To address this issue, a series of 35 risk factors were evaluated at baseline among 25,558 initially healthy women over age 45 who were followed for future cardiovascular events over a 10-year period. Using these data, a new risk-prediction algorithm, the Reynolds risk score, was developed and validated. In brief, of the new biomarkers of risk, the most important additions were hsCRP and parental history of myocardial infarction before age 60. When these two factors were added to the usual risk markers, the Reynolds risk score proved to be more accurate than the Framingham risk score, particularly for those at "intermediate risk" where 70 percent of all events occur.

Furthermore, among those at intermediate risk, almost half of all participants

were predicted to be at higher or lower risk than anticipated when the Reynolds risk score was used, and in almost all cases, this reclassification was correct.⁴⁴ Since the January 2008 AAP workshop (that is the seminal basis for this article), additional evidence has been published validating the Reynolds risk score as an improved risk-prediction system in men.⁴⁵ Treatment guidelines recommend statins for patients at higher risk. The Reynolds risk score should facilitate more effective and efficient use of these drugs.

**FOLLOWING
injections of rhCRP,
numerous cytokine markers
of inflammation became
significantly elevated
compared with controls.**

Statins

Statins possess potent lipid lowering and anti-inflammatory properties. When 3,745 patients with acute coronary syndrome (an umbrella term used to cover clinical symptoms compatible with acute myocardial ischemia) were treated with statins, the levels of LDL cholesterol and hsCRP were decreased.⁴⁶ Treated subjects who achieved a target level of hsCRP ≤ 2 mg/l had a significant improvement in event-free survival, independent of levels of LDL cholesterol. Subjects who achieved LDL cholesterol levels ≤ 70 mg/dl and hsCRP levels ≤ 2 mg/l did even better. These findings were corroborated in a second multinational trial that reinforced the significance of hsCRP as an indicator of risk and inflammation.⁴⁷

Genetics and CRP

A number of studies have linked variances in hsCRP to genetic differences. Moreover, a recent genome-wide assessment of >6,400 women, data suggested close genetic links among CRP, diabetes, and early atherothrombosis.⁴⁸ However, analysis of these studies has suggested that only between 20 percent and 40 percent of the population variance in CRP has a genetic basis.

Therapeutic Implications

Thus far, it is unproven that inhibiting inflammation in general or CRP in particular will decrease the rate of vascular events. However, early research is promising. For example, a CRP inhibitor resulted in smaller infarcts and less cardiac damage (in rats dosed with rhCRP). Other approaches include the use of novel IL-6 or TNF inhibitors. Alternately, low-dose methotrexate, often used to treat rheumatic arthritis, is known to decrease parameters linked with systemic inflammation in humans, including erythrocyte sedimentation rate, CRP concentrations, and signs of clinical inflammation. An early study was promising.⁴⁹ In light of the similarities between rheumatic arthritis and atherosclerosis (such as the involvement of cytokines and elevated levels of CRP), conducting a trial comparing low-dose methotrexate to placebo in the secondary prevention of cardiovascular disease would contribute significant understanding in this arena.

Conclusion

The human immune system, the epigenome, the environment and cytokines play complex and interwoven roles in the myriad processes of inflammation. Inflammation is now known to be a common feature of many diseases associated with aging such as chronic

periodontitis, type 2 diabetes, cardiovascular disease, and Alzheimer's disease. Furthermore, there is increasing evidence that chronic oral inflammation and the resulting systemic increases in inflammatory mediators may enhance the morbidity of certain systemic diseases commonly associated with advancing age. Therefore, it will behoove dental practitioners to remain alert in coming years for the advent of improved predictive, preventive, and mitigative tactics that will emerge from this exciting and dynamic field of study. As of now, there remains much to be learned, but the benefits will likely be profound. ■■■■

REFERENCES

1. Van Dyke TE, Inflammation and periodontal diseases: a reappraisal. *J Periodontol* 79:1501-2, 2008.
2. Van Dyke TE, Kornman KS, Inflammation and factors that may regulate inflammatory response. *J Periodontol* 79:1503-8, 2008.
3. Goldsby R, Kindt TJ, et al, Immunology, sixth ed, New York: WH Freeman pages 1-574, 2006.
4. Pennisi E, DNA study forces rethink of what it means to be a gene. *Science* 316:1556-7, 2007.
5. Wilson AG, Epigenetic regulation of gene expression in the inflammatory response and relevance to common diseases. *J Periodontol* 79:1511-9, 2008.
6. Mutsaers V, Raaijmakers BM, et al, The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. *Stem Cells* 25:3223-33, 2007.
7. Graves D, Cytokines that promote periodontal tissue destruction. *J Periodontol* 79:1585-91, 2008.
8. Foster SL, Hargreaves DC, Medzhitov R, Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 447:972-8, 2007.
9. Ordoñez JM, Shen J, Gene-environment interactions and susceptibility to metabolic syndrome and other chronic diseases. *J Periodontol* 79:1508-10, 2008.
10. Trichopoulos A, Yiannakouris N, et al, Genetic predisposition, nongenetic risk factors and coronary infarct. *Arch Intern Med* 168:891-6, 2008.
11. GOLDN, Genetics of lipid lowering drugs and diet network. <http://www.biostat.wustl.edu/goldn/>. Accessed Feb. 1, 2010.
12. Shen J, Arnett DK, et al, Interleukin beta genetic polymorphisms interact with polyunsaturated fatty acids to modulate risk of the metabolic syndrome. *J Nutr* 137:1846-51, 2007.
13. Verma S, Szmitko PE, Ridker PM, C-reactive protein comes of age. *Nat Clin Pract Cardiovasc Med* 2:29-36, 2005.
14. DeBusk RM, Fogarty CP, et al, Nutritional genomics in practice: where do we begin? *J Am Diet Assoc* 105:589-98, 2005.
15. Graves D, Cytokines that promote periodontal tissue destruction. *J Periodontol* 79:1585-91, 2008.
16. Cochran DL, Inflammation and bone loss in periodontal disease. *J Periodontol* 79:1569-76, 2008.
17. Liu R, Bal HS, et al, Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *J Dent Res* 85:510-4, 2006.
18. Assuma R, Oates T, et al, IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *J Immunol* 160:403-9, 1998.
19. King GL, The role of inflammatory cytokines in diabetes and its complications. *J Periodontol* 79:1527-34, 2008.
20. Devaraj S, Cheung AT, et al, Evidence of increased inflammation and microcirculatory abnormalities in patients with type 1 diabetes and their role in microvascular complications. *Diabetes* 56:2790-6, 2007.
21. Pradhan AD, Manson JE, et al, C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327-34, 2001.
22. Vojarova B, Weyer C, et al, High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:455-61, 2002.
23. Thorand B, Löwel H, et al, C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: Results from the MONICA Augsburg cohort study, 1984-1998. *Arch Intern Med* 163:93-9, 2003.
24. Fleischman A, Shoelson SE, et al, Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care* 31:289-94, 2008.
25. Rogers J, The inflammatory response in Alzheimer's disease. *J Periodontol* 79:1535-43, 2008.
26. Ridker PM, Silverman JD, Inflammation, C-reactive protein and atherothrombosis. *J Periodontol* 79:1544-51, 2008.
27. Bahekar AA, Singh S, et al, The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am Heart J* 154:830-7, 2007.
28. Schenkein HA, Barbour SE, et al, Invasion of human vascular endothelial cells by actinobacillus actinomycetemcomitans via the receptor for platelet-activating factor. *Infect Immun* 68:5416-9, 2000.
29. Spahr A, Klein E, et al, Periodontal infections and coronary heart disease: role of periodontal bacteria and importance of total pathogen burden in the coronary event and periodontal disease (CORODONT) study. *Arch Intern Med* 166:554-9, 2006.
30. Graves DT, Cochran D, The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol* 74:391-401, 2003.
31. Ridker PM, Rifai N, et al, Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation* 101:2149-53, 2000.
32. Ridker PM, Buring JE, Rifai N, Soluble P-selectin and the risk of future cardiovascular events. *Circulation* 103:491-5, 2001.
33. Schonbeck U, Varo N, et al, Soluble CD40L and cardiovascular risk in women. *Circulation* 104:2266-8, 2001.
34. Brown DA, Breit SN, et al, Concentration in plasma of macrophage inhibitory cytokine-1 and risk of cardiovascular events in women: A nested case-control study. *Lancet* 359:2159-63, 2002.
35. Ridker PM, Hennekens CH, et al, C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342:836-43, 2000.
36. Ridker PM, Cushman M, et al, Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336:973-9, 1997.
37. Ridker PM, Rifai N, et al, Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 347:1557-65, 2002.
38. Ridker PM, Danielson E, et al, Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 359:2195-207, 2008.
39. van Dijk EJ, Prins ND, et al, C-reactive protein and cerebral small-vessel disease: the Rotterdam scan study. *Circulation* 112:900-5, 2005.
40. Rost NS, Wolf PA, et al, Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke* 32:2575-9, 2001.
41. Pradhan AD, Manson JE, et al, C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327-34, 2001.
42. Biscendial RJ, Kastelein JJ, et al, Activation of inflammation and coagulation after infusion of C-reactive protein in humans. *Circ Res* 96:714-6, 2005.
43. Ridker PM, Buring JE, et al, Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds risk score. *JAMA* 297:611-9, 2007.
44. Ridker PM, Paynter NP, et al, C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds risk score for men. *Circulation* 118:2243-51, 2008.
45. Ridker PM, Cannon CP, et al, C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 352:20-8, 2005.
46. Morrow DA, de Lemos JA, et al, Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor trial. *Circulation* 114:281-8, 2006.
47. Ridker PM, Pare G, et al, Loci related to metabolic syndrome pathways including LEPR, HNF1A, IL6R, and GSKR associate with plasma C-reactive protein: the women's genome health study. *Am J Hum Genet* 82:1185-92, 2008.
48. Choi HK, Herman MA, et al, Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet* 359:1173-7, 2002.
49. Pradhan AD, Manson JE, et al, C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327-34, 2001.

TO REQUEST A PRINTED COPY OF THIS ARTICLE, PLEASE

CONTACT Michael P. Rethman, DDS, MS, 47-140 Hono Place, Kaneohe, Hawaii, 96744-5608.