Oral health and cardiovascular disease

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Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Kardiologhuset ingång 50, Akademiska sjukhuset, Uppsala, Friday, May 23, 2008 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

In the past two decades studies have indicated that oral health might be associated with the prevalence for cardiovascular disease (CVD), although the biological link still remains unknown. Bacteria and inflammatory mediators causing periodontal disease have also been suggested to influence the progression of atherosclerosis.

The aims of this thesis were to study oral inflammation and associations between different oral health parameters and CVD.

Inflammatory mediators such as interleukin-1 (IL-1) as well as bone resorption activity (BRA) were significantly higher in gingival crevicular fluid (GCF) from sites with periodontal disease compared to healthy sites. Treatment resulted in a reduction of BRA as well as the levels of IL-1 for most of the diseased pockets. The levels of IL-1 were not correlated to the amount of BRA.

Number of teeth (NT) was consistently associated to CVD and was the only oral health parameter that related to all-cause mortality and mortality in CVD in a dose-dependent manner. Subjects with <10 teeth had a 7-fold increase risk for mortality in coronary heart disease compared to those with >25 teeth. Furthermore, NT was also significantly associated to the levels of leukocytes and to the metabolic syndrome, which consists of a combination of cardiovascular risk factors.

Other investigated oral health parameters, such as severity of periodontal disease, number of deepened pockets, and bleeding on probing, were not related to CVD in a consistent way.

Oral health parameters as well as myocardial infarction (MI) were related to serum antibody levels against Porphyromonas gingivalis (Pg), indicating that Pg might be a link between oral health and MI.

In conclusion, treatment reduced the increased levels of IL-1 and BRA in GCF from sites with periodontal disease. Oral health was associated to CVD with number of teeth being the only oral health parameter that consistently was associated to CVD. Serum antibody levels against P. gingivalis were related to myocardial infarction (MI) as well as to oral health parameters, suggesting that this bacteria could be a link between oral health and CVD.

Keywords: Oral health, periodontal disease, number of teeth, cardiovascular disease, mortality

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ISSN 1651-6206
ISBN 978-91-554-7188-0
urn:nbn:se:uu:diva-8708 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8708)
To my beloved family
PAPERS


IV. Holmlund A, Holm G, Lind L. Impaired oral health as a predictor for cardiovascular mortality in a cohort of 7,674 subjects followed for 12 years. *Manuscript*

V. Holmlund A, Hedin M, Pussinen P, Lerner UH, Lind L. *Porphyromonas gingivalis (Pg)* a possible link between impaired oral health and acute myocardial infarction. *Manuscript*

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Abbreviations

*Aa, Aggregatibacter actinomycetemcomitan*
ACS, Acute coronary syndrome
AGE, Advanced Glycation End product
AL, Attachment Loss
AMI, Acute Myocardial Infarction
APO-E, Apolipoprotein E
ARIC, Atherosclerosis Risk in Community
B-cells, B-Lymphocytes
BOP, Bleeding On Probing
BMI, Body Mass Index
BRA, Bone Resorbing Activity
CAC, Coronary Artery Calcification
CAD, Coronary Artery Disease
CAL, Clinical Attachment Loss
CCA, Common Carotid Arteries, CHD, Coronary Heart Disease
CEJ, Cemento-Enamel Junction
CEVD, Cerebrovascular Disease
cIMA, Cross sectional intima
CRP, C - reactive protein
CP, Chronic Periodontitis

*CPI, Community Periodontal Index*
*CPTIN, Caries and Periodontal Index Treatment of Needs*
CVA, Cerebral Vascular Attack
*CVD, Cardiovascular Disease*
CT, Computer Tomography
DM, Diabetes Mellitus
DMF, Decayed, Missing and Filled teeth
DNA, Deoxyribonucleic acid
ECA, External Carotid Artery
ECG, Electrocardiogram
FGF, Fibroblast Growth Factor
FMLP, N-Formyl-Methionyl-Leucyl-Phenylalanine
GCF, Gingival Crevicular Fluid
G-CSF, Granulocyte Colony-Stimulating Factor
GI, Gingivitis Index
HDL, High Density Lipoprotein
HT, Hypertension
*hsCRP, High sensitive C-reactive Protein*
ICA, Internal Carotid Artery
ICAM, Intercellular Adhesion Molecule
ICARAS, Inflammation and Carotid Artery Risk for Atherosclerosis Study
ICAM, Intercellular Adhesion Molecule
ICARAS, Inflammation and Carotid Artery Risk for Atherosclerosis Study
IgG, Immunoglobulin G
IL-1, Interleukin-1
IL-ra, Interleukin Receptor Antagonist
IMT, Cartoid artery Intima-Media Thickness
INF-γ, Interferon –γ
INVEST, Oral Infections and Vascular Disease Epidemiology Study
LDL, Low Density Lipoprotein
MBL, Marginal Bone Loss
MCP, Monocyte Chemotactic Protein
M-CSF, Macrophage Colony-Stimulating Factor
MI, Myocardial Infarction
mRNA, Messenger Ribonucleic Acid
NADH, Reduced form of Nicotinamide Adenine Dinucleotid
NCEP/ATPIII, National Cholesterol and Education Program/Annual Treatment Planning III
NHANES, National Health and Nutrition Examination Survey
RA, Rheumatoid Arthritis
RANK, Receptor Activator of Nuclear factor κB
RANKL, Receptor Activator of Nuclear factor κB ligand
RR, Relative Risk
OA, Osteoarthritis
OR, Odds Ratio
PAI, Plasminogen Activator Inhibitor
PHS, Periodontal Health Status
PD, Pocket Depth
Pg, Porphyromonas gingivalis
PDGF, Platelet Derived Growth Factor
PLI, Dental Plaque Index
PMN, Polymorph nuclear leucocytes
PTI, Panoramic Tomography Index
PVD, Periphery Vascular Disease
SBP, Systolic Blood Pressure
SLI, Silness-Löe index
TGF-β, Transforming Growth Factor-β
Th, T-lymphocyte helpercell
TIA, Transient Ischemic Attack
TNF, Tumor Necrosis Factor
VCAM, Vascular Cellular Adhesion Molecule
WBC, White Blood Cell
WHO, World Health Organization
VSMC, Vascular smooth muscle cell
Introduction

“It is what we think we know that prevents us from learning more”.
Albert Einstein

Periodontal disease

Periodontal diseases are very frequent and can affect up to 80% of the worldwide population. Gingivitis is the mildest form of periodontal disease and it is caused by a bacterial biofilm that accumulates on the teeth adjacent to the gingiva if not proper hygiene is performed. If the biofilm is left alone it will take between 10-20 days to develop gingivitis with clinical symptoms such as bleeding, swelling and redness of the gingiva. However, this condition is reversible and after removal of the biofilm the gingiva will be normalized in about a week.

In 10-15% of the population the gingival inflammation can progress to a more severe, and nonreversible condition called periodontitis. This is a chronic inflammatory disease where the inflammation that is triggered by the bacterial biofilm extends into the deeper parts of the supporting tissues of the tooth, leading to degradation of connective tissue and alveolar bone. If left untreated it could finally cause tooth loss. The disease often progresses without clear symptoms for the diseased person, which makes it difficult for them to know when to seek help. The classification of periodontal disease has changed overtime but according to the most recent classification, periodontitis could either be in a localized (i.e affects only a part of the dentition) or in a more generalized form (i.e affects a major part of the dentition). Both these forms can appear in an aggressive or a chronic type depending on the progression rate of the disease. Gingivitis can affect people at all ages. Periodontitis on the other hand is most common in middle aged and older people, but it can also appear in young children, although this is unusual.

Riskfactors for periodontitis

Periodontitis has a multifactor etiology where bacteria play an important role. The bacterial biofilm changes its composition over time from being colonized mainly by a gram-positive flora in the newly formed biofilm towards a dominant gram-negative anaerobic flora in the older biofilm. Bacte-
ria like *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythensis* (*Tf*) and *Aggregatibacter actinomycetemcomitans* (*Aa*) (earlier called *Actinobacillus actinomycetemcomitans*) have been acknowledged as causative factors for periodontitis. 6 However, there are a number of other micro-organisms that have been associated with periodontitis and most recently were also viruses suggested as possible etiology factors. 7, 8 As the pocket can harbor around 500 different species 9 and only a fraction of these micro-organisms has been thoroughly studied, it is still a matter of discussion if above suggested micro-organisms are the ones that actually cause the disease or merely are present because the environment in the diseased pockets favors these micro-organisms.

How serum antibody levels against periodontal pathogens relate to disease progression and development still remains unclear, although associations have been reported. 10 Bacteria are essential, but not sufficient to cause periodontitis. What finally decides if disease develops or not is how the host response handles the challenge from the micro-organisms in the biofilm. 11, 12 An important factor for the host response is the genetic heritage and it is estimated that 50% of the individuals with periodontitis have genetic factors that predispose for disease development. 13

Behavioral patterns like smoking seriously affect the risk for development and progression of periodontal disease. 14, 15 Stress has also been suggested as a risk factor for periodontitis. 16, 17 Systemic diseases can be a predisposing factor for periodontitis and it is well known that diabetes increases the risk for progression and development of periodontitis, especially if the diabetes is uncontrolled. 18, 19 Rheumatoid arthritis and osteoporosis are other systemic conditions that recently have been related to periodontal disease. 20, 21

**Cardiovascular disease**

Cardiovascular disease (CVD), here used as heart and vascular derangements related to atherosclerosis, is responsible for over 50% of the mortality in the industrialized part of the world. As living standard in the non-industrialized parts is increasing, CVD will also become a major health problem in these regions. Although the main symptom related to the heart disease is angina pectoris, it is the occlusion of arterial vessels leading to myocardial infarction that are the major cause for mortality in CVD.

CVD develops through changes in the large and medium sized artery walls by atherosclerosis, which today is considered to be an inflammatory process starting rather early in life. This leads to plaque formation in the artery wall and narrowing of the lumen. There has to be a considerable narrowing of the artery lumen before the blood flow is seriously affected and symptoms occur. In the legs, atherosclerosis with narrowing of the major
arteries could lead to a syndrome called *Claudicatio intermittens* with symptoms such as pain and reduced ability to walk.

**Risk factors for cardiovascular disease**

CVD has a multifactor etiology where major risk factors are smoking, obesity, diabetes, high blood pressure, high cholesterol levels, lifestyle, heritage and gender. Heritage and gender are factors we cannot influence, but obesity and smoking are probably the two most important life style factors with an extensive impact on the development of CVD that we can do something about. Obesity is closely link to the development of diabetes type II and to the metabolic syndrome. The metabolic syndrome (MetS) is a syndrome that consists of different combinations of cardiovascular risk factors such as high blood pressure, dyslipidemia, abnormal glucose control, and abdominal obesity with insulin resistance suggested as a common denominator of the syndrome. The MetS is present if an individual has three of the above motioned risk factors. In the last decade this syndrome has attracted more attention as it is closely related to an almost epidemic increase in prevalence of diabetes type II, which in turn is associated with an elevated risk for cardiovascular disease.

Although there are other definitions for the MetS, the two most commonly used definitions come from the World Health Organization (WHO) and The National Cholesterol and Education Program/Annual Treatment Planning III (NCEP/ATP III). The WHO definition states that the metabolic syndrome is present if an individual has a fasting blood glucose level >5.6 mmol/l in combination with two of the following conditions, abdominal obesity, hypertension, dyslipidemia, and micro albuminuria. The NCEP/ATP III criteria are almost the same except for the micro albuminuria, which is not included in the NCEP criteria, and that fasting blood glucose level >5.6 mmol/l is not compulsory for the diagnose.

Despite that these risk factors can explain a major part of the CVD, there is still a portion of CVD incidence that cannot be explained by above mentioned risk factors. Recently, chronic inflammation has been suggested as a missing piece in the puzzle.

**Inflammatory response**

**In periodontitis**

The tooth and its surrounding tissue is the only place in the body where hard tissue (i.e cementum and enamel) perforate epithelium and communicates with an external environment. A seal is therefore needed and is pro-
vided by the junction epithelium, which is the barrier between underlying connective tissue and the oral cavity. A small fluid transudate will flow from this seal even in gingival health, minimizing bacterial accumulation mainly by a mechanical factor. This gingival crevicular fluid (GCF) also contains leukocytes and macromolecular components derived from serum and underlying connective tissue and this is sufficient to maintain homeostasis in healthy conditions. However, if the bacterial challenge from the biofilm exceeds a threshold level, the immunological defense will react by initiating a series of events in the underlying connective tissue, including angiogenesis, increased vessel wall permeability and accumulation of inflammatory cells, in attempt to preserve the homeostasis. The release of endotoxin, proteaser and N-formyl-methioyl-leucyl-phenylalanine (FMLP) from the biofilm will trigger the defending leukocytes in the area to react with phagocytes and the production of small very active proteins called cytokines. The amount of GCF will increase as well as the levels of pro-and anti-inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alfa (TNF-α) and interleukin-6 (IL-6). GCF from sites with periodontitis contains higher levels of these and other cytokines than healthy sites. 27, 28 Similarly, these cytokines are also highly expressed in inflamed synovium and synovial fluid from patients with rheumatoid arthritis. 29, 30
Figure 1. Products from the biofilm triggers the recruitment of inflammatory cells into the connective tissue adjacent to the junction epithelium and stimulate the release of cytokines from stationary cells as well as inflammatory cells. These cytokines stimulate the osteoclastogenesis, bone resorption, degradation of the extracellular matrix and decrease bone formation, events leading to loss of tooth supporting tissues in sites with periodontitis.

When the inflammatory process has started, more leucocytes are recruited from the blood stream of the adjacent connective tissue due to released cytokines and endotoxin that will enhance the expression of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin on the endothelium surface causing more leucocytes to adhere and penetrate the vessel wall. The recruitment and movement of leucocytes towards the inflamed part of the tissue is guided by a chemotactic gradient constituted by products from the innate defense system called “the complement system” and by released chemokines like interleukin-8 (IL-8).  The cell content in the GCF will mainly be comprised of polymorphonuclear leucocytes (PMN) belonging to the innate defense. The PMN cells are very important players in the defense and attack opposing bacteria by phagocytosis and releasing enzymes such as elastase, lysozyme, lactoferrin and bacterial collagenas. The protective importance of these cells regarding periodontitis can be exemplified by the fact that individuals that lack PMN-cells (Kostmans syndrome) will develop a very aggressive form of periodontal disease early in life. Furthermore, individuals suffering from different impairment of PMN-cell function such as leukocyte adhesion deficiency, Chediak Higashi disease, chronic neutropenia, cyclic neutropenia and Papillon-Lefèvre syndrome are more prone to develop periodontal disease.  

Monocytes and lymphocytes are other leucocytes present in the GCF. The lymphocytes belong to the adaptive immunity and the antibodies present in the GCF, mainly immunoglobulin G (IgG) are produced by B-lymphocytes (B-cells). How the different types and levels of antibodies in GCF relate to disease development and progression of periodontitis is not fully understood today. In advanced stages of periodontitis, the predominant leucocyte in the periodontal tissue is the plasma cells (i.e antibody producing B-cells). The production of antibodies from B-cells is dependent on signals from a T-lymphocyte called T-helper cell (Th). By characterizing the cytokine profile, three types of Th-cells has been identified. Th1 secretes IL-2, IL-12, Tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ), the Th2 cells mainly produce IL-4, IL-5, IL-6, IL-10, IL-13; and the Th3 cells secrete transforming growth factor (TGF-β). Although, the Th2 subset is more abundant in periodontitis lesions, the relative importance of the Th1 and Th2 subsets in periodontal disease is poorly understood.
Inflammatory bone resorption

Within all sites of the skeleton, bone resorption and bone formation are ongoing processes throughout life in a process called bone remodeling. Normally, these processes are in balance and bone formation equals the bone resorption. However, processes like inflammation, malignances and hormonal imbalances can alter this balance, causing either of these events to be more pronounced. If bone formation carried out by cells called osteoblasts will exceed bone resorption, exerted by activated osteoclasts, the result will be increased bone mass. On the other hand, when bone resorption is the most pronounced process the result will be loss of bone mass. A lot of the earlier mentioned cytokines can influence the balance between bone formation and resorption. Cytokines such as IL-1, IL-6, IL-11, IL-17, TNF-α, leukemia inhibitory factor (LIF), and oncostatin M (OSM) are all stimulators of bone resorption. Furthermore, also kinins and thrombin from the kallekrein-kinin and coagulation cascades can stimulate bone resorption and synergistically interact with IL-1 and TNF-α. On the contrary, cytokines such as IL-4, IL-10, IL-12, IL-13, IL-18, INF-β and INF-γ inhibit osteoclastogenesis and thereby reduces bone resorption. The amount and combination of these stimulatory and inhibitory cytokines will determine the extent of bone loss in the inflammatory process.

The production of estrogen is decreased in women after menopause. As this hormone suppresses several of the cytokines that stimulate bone resorption, menopause might, apart from increased risk for osteoporosis, also be associated with an increased risk for marginal periodontitis, although final proof is still lacking.

Generally increased bone resorption is followed by an increased bone formation. Despite that inflammation induced sclerotic bone formation (increased bone mass as is sometimes the case in the vicinity of other inflammatory conditions), is never seen in radiographs from marginal periodontitis, increased bone formation can be observed in such sites by using an isotope that is incorporated during osteoblastic bone formation in combination with a sensitive bone scan. Similarly, increased bone formation can be seen in the peri-articular bone at affected joints in patients with rheumatoid arthritis, osteoarthritis and apical periodontitis. (figure 2)
Osteoclasts originate from myeloid hematopoetic stem cells. The differentiation of osteoclasts is very closely related to the differentiation of macrophages and the dendritic cells in the immune system. The mononucleated progenitor cells, differentiating along the osteoclastic lineage finally results in fusion of the cells into multinucleated osteoclasts at the periosteal and endosteal surfaces. No osteoclasts can be formed unless osteoclast progenitor cells are activated by the binding of receptor activating of nuclear factor κB (RANK) by RANK-ligand (RANKL), which is expressed by stromal cells/osteoblast and some other types of cells including T-lymphocytes. This stimulation can be reduced by osteoprotegerin (OPG), a protein acting as a decoy receptor for RANKL, thereby preventing RANK on the osteoclasts progenitor cells to bind to RANKL. (figure 3)
Increased expression of RANKL has been found in inflamed gingiva from individuals with periodontal disease,\textsuperscript{37} in GCF from patients with periodontitis\textsuperscript{38} as well as in synovium from patients with rheumatoid arthritis.\textsuperscript{39} Furthermore, in accordance with these observations, expression of OPG was reported to be reduced in gingiva from patients with periodontitis and the levels of OPG decreased in GCFs from sites with periodontal disease compared to healthy sites.\textsuperscript{37, 38}

As earlier mentioned, the inflammatory process in periodontitis has much in common with what is seen in rheumatoid arthritis, another chronic inflammatory disease. In both diseases, the inflammatory exudates will contain higher levels of inflammatory mediators such as IL-1, IL-6, and TNF-$\alpha$, enhancing the number of activated osteoclasts that could degrade the surrounding bone tissue. Furthermore, these cytokines also stimulates cells like fibroblasts to produce cytokines, prostaglandins and matrix metalloproteinase, resident molecules responsible for the degradation of the extracellular matrix.\textsuperscript{27} (For more details regarding inflammatory bone resorption, see review by Lerner).\textsuperscript{40}

**Periodontitis and circulating markers of inflammation**

C-reactive protein (CRP) is a pentameric protein synthesized in the liver mainly as a result from stimulation by IL-6. The main tasks for this protein are to activate complement and counteract infection. Recent reports have linked increased levels of high sensitive measurements of CRP (hsCRP) to both atherosclerotic disease\textsuperscript{41, 42} and periodontal disease, indicating that CRP can be a possible link between the two diseases.\textsuperscript{43-45} Other circulating markers of inflammation associated to both atherosclerotic and periodontal disease are increased levels of white blood cell count (WBC) and fibrinogen.\textsuperscript{46, 47} These findings indicate that there could be a spill over from the local inflammation in periodontal disease to the systemic circulation.

Inflammation in periodontal disease could be regarded as a double-edged sword. On one edge, the protective purpose of the local inflammatory action is to prohibit the bacteria from penetrating into the underlying tissue and cause serious infection. Once the insult is eliminated, the inflammation resolves. On the other edge, we have the more chronic types of inflammation as in periodontal disease and rheumatoid arthritis, with persistence of inflammatory mediators that in turn will lead to disturbed tissue remodeling and in some situations to tissue destruction. Therefore, the main purpose with all periodontal treatment is to resolve the inflammatory process and down regulate the host response to the bacterial challenge by trying to remove the bacterial biofilm from the affected teeth.

Hopefully, in a not too distant future we will better understand the complex immune and cytokine regulation of periodontal disease, leading to new
options for treatment of the disease and also increased knowledge regarding the systemic effects generated by the disease.

Inflammation in the atherosclerotic process (Modified from Ross)\textsuperscript{48}

"We cannot identify unknown aspects of modern life that lead to atherosclerosis until we know the true nature of the characteristic lesion of atherosclerosis"

Earl Bendit

In healthy arteries, there is an intact cell layer closest to the lumen. This layer is called the endothelium and under healthy conditions it prevents circulating lipoproteins from penetrating into the wall. The intima is the next section of the artery wall and consists of mainly connective tissue. Underlying the intima is the media which is made up of smooth muscle cells. Outside the media is the adventitia that separates the artery wall from surrounding tissues (figure 4)

Atherosclerosis is the cause for CVD. It starts rather early in life and progresses slowly, usually without symptoms until middle age. The atherosclerotic lesions occur in large and medium sized arteries, most frequently at sites where there is a change in the blood flow, with decreased shear stress and increased turbulence such as in areas with bifurcations, branches and curvatures.

The response-to-injury hypothesis is a pathophysiologic theory of the mechanisms behind atherosclerosis. Endothelial denudation was earlier suggested to be the first step of the process, but more recently has endothelial dysfunction, rather than denudation been suggested to be the initiating process. Whatever process that is ongoing in the development of atherosclerosis, different lesions represent different stages of chronic inflammation in the arteries. (figure 4)
Figure 4. Endothelial dysfunction in atherosclerosis (used with permission from New England journal of medicine). The earliest changes that precede the formation of lesions of atherosclerosis take place in the endothelium. These changes include increased endothelial permeability to lipoproteins and other plasma constituents and migration of leukocytes into the artery wall, which is mediated by oxidized low-density lipoprotein, monocyte chemotactic protein 1, interleukin-8, platelet-derived growth factor, macrophage colony stimulating factor, and osteopontin.

The earliest type of lesion is the fatty streak that can be seen in infants and young children. This is a pure inflammatory lesion consisting of macrophages and T-lymphocytes and is reversible. The endothelial dysfunction leads to compensatory response altering the normal homeostasis of the endothelium. Endothelial permeability, together with the adherence of leukocytes, is one of the earliest changes. Leukocytes adhere to and migrate across the endothelium to the intima layer under the influence of adhesion molecules like ICAM-1 and VCAM-1 expressed on the endothelial surface and chemokines. Accumulation of low density cholesterol (LDL) in the intima has an important role in the development of atherosclerosis.

The three most important cells in the vessel wall, endothelial cells, vascular smooth muscle cells (VSMC) and macrophages, produce waste products with free radical activity, e.g. superoxide anions, into the extracellular matrix, creating a pro-oxidative environment in the intima. LDL trapped in the artery wall can be oxidized and internalized (i.e. ingested) by macrophages through binding to scavenger receptors expressed on the surface of these cells. Furthermore, products from the oxidation of LDL increase the expression of adhesion molecules on the endothelial surface enhancing the recruitment of new leukocytes into the lesion. Well inside the macrophage, the LDL leads to formation of lipid peroxides and facilitates the accumulation of
cholesterol esters finally resulting in formation of foam cells when the cell cannot handle any more cholesterol.

The degree to which LDL is modified can vary greatly but once taken up by the macrophage, LDL will cause an activation of the macrophage. Modified LDL upregulates the gene expression for macrophage colony-stimulating factor (M-CSF) and monocyte chemotactic protein (MCP) in the endothelium, leading to increased inflammatory response by recruiting more monocytes into the lesion and increase the replication of macrophages. The macrophage can also present parts of the oxidized LDL to T-lymphocytes, causing them to produce cytokines and growth factors such as IL-2, granulocyte colony stimulating factor (G-CSF), platelet derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β) and fibroblast growth factor-2 (FGF-2) further amplifying the inflammatory response, leading to an asymmetrical intimal thickening by migration of smooth muscle cells from the underlying media and production of extracellular matrix. These are all steps in the first stage of atherosclerosis with fatty streak formation (figure 5).

**Figure 5. Fatty streak formation.** (used with permission from New England journal of medicine). Fatty streak initially consist of lipid-laden monocytes and macrophages (foam cells) together with T-lymphocytes. Later they are joined by various numbers of smooth-muscle cells. The steps involved in this process include smooth muscle migration, which is stimulated by platelet-derived growth factor, fibroblast growth factor 2, and transforming growth factor β; T-cell activation, which is mediated by tumor necrosis factor α (TNF-α), interleukin-2, and granulocyte-macrophage colony-stimulating factor; foam cell formation, which is mediated by oxidized low-density lipoprotein, macrophage colony-stimulating factor, TNF-α, interleukin-1; and platelet adherence and aggregation which are stimulated by in-
tegrins, P-selectin, fibrin, thromboxane A2, tissue factor, and factors responsible for adherence and migration of leucocytes.

The inflammatory response itself will enhance the inflammation in the lesion by increasing the production of inflammatory mediators such as IL-1, TNF-α and G-CSF, resulting in elevation of the number of adhesion molecules on the endothelial surface, recruitment of monocytes, T-lymphocytes and increase levels of LDL. It is therefore not surprising that one of the most important protective roles of the macrophage concerning the artery wall is to remove modified LDL, thereby minimizing the effects of oxidized LDL on the endothelium and smooth-muscle cells.

When the fatty streaks progress to more advanced lesion, a fibrous cap is developed in order to wall off the lesion which could be regarded as a healing response to the injury. This fibrous cap will encapsulate a mixture of leukocytes, lipid and degradation products which makes up a core. The development of the fibrous cap is to a large extent stimulated by cytokines like PDGF, TGF-β, IL-1, TNF-α and osteopontin (figure 6)

![Formation of an advanced, complicated lesion of atherosclerosis](image)

**Figure 6.** Formation of an advanced, complicated lesion of atherosclerosis (used with permission from New England journal of medicine). As fatty streak progress to intermediate and advanced lesions, they tend to form a fibrous cap that walls off the lesion from the lumen. This represents a type of healing or fibrous response to injury. The fibrous cap covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core. These lesions expand at their shoulders by means of continued leukocyte adhesion and entry caused by the same factors listed in figure 4 and 5. The principal factors associated with macrophage accumulation include macrophage colony-stimulating factor, monocyte chemotactic protein 1, and oxidized low-density lipoprotein. The necrotic core represents the result of apoptosis and necrosis, increased proteolytic activity and lipid accumulation. The fibrous cap forms as a
result of increased activity of platelet-derived growth factor, transforming growth factor β, interleukin-1, tumor necrosis factor α, and osteopontin and of decreased connective tissue degradation.

In the final step, the inflamed lesion becomes an unstable fibrous plaque. Rupture of the plaque usually occurs at the shoulders of the lesion as a result of thinning and erosion of the fibrous cap. Activated T-cells produce cytokines that can stimulate macrophages in the lesion to produce matrix metalloproteinase that will degrade the extracellular matrix, promoting the thinning and rupture of the fibrous cap. The lipid containing mass of the core will then come out into the lumen of the artery and initiate the coagulation process by activating platelets and the coagulation cascade, which in the worst case leads to occlusion of the artery (figure 7).

![Figure 7. Unstable fibrous plaque in atherosclerosis](image)

Although, most individuals will to some extent be exposed to plaque formation in the arteries, not everyone develops CVD. The main issue is not if we have plaque or not in our vessels, but how it is composed. A calcified plaque might considerably reduced the lumen of the vessel, but if it has a low content of cholesterol and a low degree of inflammation, the plaque will
be stable with little risk for rupture. On the other hand, the unstable plaque with high risk for rupture does not necessarily cause reduction of artery lumen, but it has a high content of cholesterol with an ongoing inflammatory process which makes the plaque vulnerable.

Mutual risk factors for periodontal and cardiovascular disease

Age

Physiological changes such as decreased turnover for cells and reduced healing capacity occur with increasing age. The most important reason for age as a risk factor for periodontitis is not the ageing process itself, but the fact that the longer we live, the longer is the possible exposure time from micro-organisms. 50-52

Age is also a dominant risk factor for cardiovascular disease and ageing is associated with a number of alterations regarding structural and functional properties of the large arteries, including diameter, wall thickness, wall stiffness and endothelial function as recently reviewed by Najjar et al. 53 Although there is mounting evidence that these age-related changes can be accelerated by cardiovascular diseases, new insights of the mechanisms involved will hopefully provide new methods to retard the arterial ageing.

Gender

There are reports concerning periodontitis indicating that men experience more clinical attachment loss than women. 54 The fact that men tend to seek help to a lesser extent can be one explanation for this difference. However, the importance of menopause concerning bone loss in women is still unclear, but there are data indicating that the estrogen reductions after menopause could increase the risk for bone loss around the teeth. 55 If that is the case, attachment loss as well as the atherosclerotic process may be increased in women after menopause and in the end become equal to that of men.

There is a significantly lower age-specific risk for women to die from CVD compared to men. Men that die of CVD are roughly 10 years younger than women. Estrogen is considered to have a major impact on this difference and intake of postmenopausal estrogen is associated with a reduced risk for CVD. 56, 57 As reviewed elsewhere, 58 there are multiple mechanisms whereby estrogen might protect against CVD, including favorable changes in lipids, lipoproteins, fibrinogen, plasminogen activator inhibitor (PAI-1) and antioxidants. There are also other gender differences that may influence the development of CVD. For instance, the distribution of fat is different in
women and men and before menopause are the levels of LDL cholesterol lower in women. Furthermore, will gender differences in how the arterial tree ages influence hemodynamic settings, control of heart rate and pulse pressure. 59

Diabetes

Individuals with diabetes mellitus (DM) have about two times higher risk for progression and development of periodontal disease and the risk is more pronounced if the blood glucose level is poorly controlled. 60, 61 Individuals with periodontitis seem to be more prone to develop complications such as retinopathy, nephropathy and CVD. 19 The biologic mechanisms underlying the increased risk for periodontitis in diabetic patients, is not fully understood, but we know that the immune system and inflammation are involved in the pathogenesis of both diabetes and periodontitis. 11, 62-64

As there are studies indicating that treatment of periodontal disease both can and cannot influence the level of glycosylated hemoglobin, we still lack convincing evidence that periodontitis directly can affect diabetes. 65, 66

Diabetes is a well known risk factor for CVD. A recent study reported an increased risk for CVD with a hazard ratio 2.5-3.0 in patients with DM. 67 There are several pathophysiologic mechanisms by which diabetes can affect the risk for CVD. For instance, increased levels of advanced glycation end products (AGEs) that bind to AGE receptors will promote atherosclerosis by increasing inflammatory reaction in the vessel. Dyslipidemia and endothelial dysfunction associated with diabetes are other important factors for the elevated risk for CVD in diabetic patients. 68, 69

Obesity

Periodontal disease has also been related to obesity in a number of studies, 70, 71 but more studies are needed to confirm this association. The mechanisms behind this association remain unclear, but it is possible that endotoxins from the biofilm and cytokines from the local inflammatory response process might enter the circulation and influence the lipid metabolism. However, there is a possibility that social-economic factors constitute the link between above mentioned association as obesity is more prevalent in lower socio-economic classes.

Obesity and especially abdominal obesity increases the risk for CVD. There can be many factors behind the higher risk for CVD in obese individuals, but the increased risk for development of insulin resistance and the low-grade inflammation that obesity leads to may be two of the most important factors as reviewed elsewhere by Luc. 72
Race

African ethnicity seems to have the highest prevalence of periodontal disease followed by Hispanics and Asians. One important factor for these differences can be related to socio-economic factors as there are disparities in periodontal health between poor and rich people. However, Borell et al. reported that high-income blacks exhibit a higher prevalence for periodontitis than low-income blacks and high-income whites. There can also be a genetic difference between races as described by Armitage et al. concerning the prevalence of polymorphism of interleukin 1α/β genotype in Chines and European populations.

As for periodontitis, there are racial differences in prevalence of CVD. Socio-economic status has also here been suggested as a key factor. However, a recent report found ethnic differences in the association between polymorphisms in the CRP gene and CVD, indicating that there might be a genetic explanation for the differences in prevalence of CVD seen between races. More research, however, is needed to elucidate the true cause for the ethnical differences.

Smoking

This is probably one of the strongest risk factors for both periodontal and cardiovascular disease. The literature pointing to smoking as a risk factor for both diseases is substantial.

A lot of biologic events are affected by smoking such as impaired phagocytosis and chemotaxis of neutrophil leukocytes, increased release of superoxide, hydrogen peroxide, proteolytic enzymes, and cytokines, all of which could contribute to the more pronounced tissue destruction seen in smokers. Although smoking has a variety of effects on the tissues, we still lack detailed knowledge about which biological effects of smoking that increases prevalence of the diseases.

In a meta-analysis, smoking increased the risk ratio for severe periodontal disease with 2.8. A 10-year prospective study revealed that periodontal health in smokers is compromised, presenting more diseased sites, and more extensive bone loss, than in non-smokers. The relative risk for tooth loss is also increased in smokers.

Smoking is also a major risk factor for CVD and in a large study including 27,089 participants from 52 countries, an increased risk for non-fatal acute myocardial infarction was reported in current smokers with an odd-ratio of 2.95. Smoking can cause impaired endothelium function, increased levels of WBC, CRP, fibrinogen, and plasma viscosity, indicating that inflammation might be an important way by which smoking could influence the development of CVD.
Psychological factors

In the last two decades, psychological factors have attracted more attention as a potential risk factor for periodontal disease and studies have reported a relationship between these factors and periodontitis. However, lack of relationship has also been reported and the pathophysiology underlying the relationship is still unclear.

Psychological stress can have many faces and has for a long time been suggested in the pathogenesis of CVD and with development of new technologies, knowledge about the biological mechanisms by which stress exerts its negative effect on vasculature is rapidly growing, as reviewed elsewhere by Rozanski.

Socio-economic factors

Socio-economic factors influence the risk for both periodontal disease and CVD. A possible common denominator for the influence of socio-economic factors on development of both diseases could be that low socio-economic status is associated with increased levels of high-risk behavior, including lack of physical activity, smoking, poor diet, obesity and alcohol consumption.

Evidence of association between oral health and cardiovascular disease

History

In the beginning of the 20th century, Miller and Hunter suggested that oral infections could cause a variety of diseases such as RA, anemia, chronic kidney diseases, fever conditions etc. This started "the focal infection theory" which was widespread during 1910-1930 and millions of teeth were extracted because of this theory. However, in 1938, R Cecil, a former supporter of the focal infection theory, published a systematic review on 200 patients suffering from RA and concluded that their condition was not improved by the extractions and tonsillectomy performed. This was the beginning of the end for the focal infection theory and in the fifties it disappeared.

As early as 1823, Rayer suggested that morbid ossification of the arteries is the result of inflammation of the fibrous layer and in 1889 Gilbert and Lion, published experimental research on infection and atherosclerosis. Osler in his Modern Medicine from 1908, pointed out four great factors in the causation of arteriosclerosis: normal wear and tear of life; acute infections; intoxication (including smoking, diabetes and obesity) and combina-
tions which kept the blood tension high, as reviewed by Nieto \(^9^4\). However, it was not until the end of 1980 that infection as a possible etiologic factor for development of CVD appeared again, this time in an article by Saiku et al in the Lancet, about the association between antibody titers against *Chlamydia pneumonia* and CHD and MI \(^9^5\). One year later, Mattila et al. published a case-control study where bad oral health increased the risk for myocardial infarction (MI) \(^9^6\). Since then there has been a lot of research done about this association, but a lot of questions still remain unanswered regarding the relationship between oral health and cardiovascular disease.

Criteria to be fulfilled before oral health can be regarded as a risk factor for CVD

At the consensus conference held in Chapell Hill 1997, it was concluded that if a suspected risk factor such as peridontitis was to be regarded as a true risk factor for CVD the following criteria should be fulfilled:

1) There should be a continuous association between the suspected risk factor and the disease.
2) The association should be strong.
3) The risk factor should be present before the debut of the disease.
4) Specificity of association.
5) Presence of a dose-response relationship between the factor and the disease.
6) A plausible biological explanation for the association.
7) Support from experimental evidence, with the strongest evidence being randomized controlled intervention studies, disclosing that removal of the potential risk factor leads to a reduced incidence of the disease.

Today, we are still far from fulfilling these criteria, but during the years as more research in this field has been concluded, more of these criteria have been achieved.

1) Evidence of association between oral health and CVD

Since 1989, at least 10 additional case-control studies have found an association between different measures of oral health and CVD and in two studies no relationship was reported (for details see table1). Furthermore, of 20 cross-sectional studies, 18 presented an association between the diseases (table 2). Of the longitudinal studies a relationship between oral health and CVD was present in 17 studies, while in 5 studies no relationship was found (table 3).

Although, no relationship was observed in some of the studies, the major portion of them did find an association. The variation of the oral health pa-
rameter used in different studies makes it very difficult and sometimes impossible to compare the results from different studies. For instance, in some studies have an old index been used such as the Russel periodontal index which was abandon about 15 years ago. Then in other studies have the oral health parameters been either gingivitis, deepened pockets (PD), bone loss, tooth loss or combinations between bone loss, PD, bleeding on probing (BOP), plaque index (PLI), apical lesions and caries (table1-3). Furthermore, in a number of studies have questionnaires with self-reported periodontal health been used, and there is a substantial risk for misclassification as periodontal disease can exist without any for the individual evident symptoms.

2) Strength of association

Case-control and cross-sectional studies reporting an association between oral health and CVD presented odd-ratios which for the major part of the studies could be regarded as weak to moderate in strength. Also for the longitudinal studies where an association between the diseases was evident, the strength of association was mainly weak to moderate (for detail see table 1-3).

As earlier mentioned it is difficult to compare studies as different methods have been used to classify periodontal disease and oral health. However, two recent meta-analyses have presented data regarding the relationship between periodontal disease and CVD. In one of these two studies, subjects with periodontal disease had an overall adjusted risk for CVD of 1.19 and if only subjects ≥65 years was included, the relative risk increased to 1.44. 97 In the other study the risk for CHD was 1.15 and for cerebrovascular disease it was 1.13. 98 Although an increased risk for CVD with only around 20% for subjects with periodontal disease seems low, it can still have a profound impact on public health as periodontitis is a rather widespread disease.

3) Risk factor present before debut of the disease

To evaluate this issue, we have to look at the longitudinal studies where periodontitis was present before any CVD was diagnosed. For most of these studies, a relationship was found between oral health and different manifestations of the CVD. However, in 5 studies no relationship was seen (See table 3).

4) Specificity of the association

Optimal for proving causality in a relationship is that the suspected risk factor only associates with the investigated disease, which in this case is CVD. However, this does not apply for periodontitis as it also has been suggested to be a risk factor for diabetes, preterm birth, respiratory diseases and
osteooporosis. While a specific association may more likely be causal, we cannot justify the rejection of a possible risk factor because it is related to other diseases. Many diseases have a multifactor etiology and a single factor may be involved in many diseases.

5) Dose-response relationship

In three studies, where periodontal disease was divided into four categories depending upon the amount of bone loss measured on radiographs, was a dose-dependent relationship to CHD present. A dose-dependent association between attachment loss and CHD was also reported by Arbes et al when ≥3mm attachment loss around 0%, 1-33%, 34-67% and >67% of sites was used to categorize the degree of periodontal disease. However, after adjustment for relevant confounders, a significant correlation remained only for the two most severe groups of attachment loss.

6) Biological theories behind association and supporting evidence

It is well known that inflammation markers such as CRP, WBC, IL-6 and fibrinogen are associated with higher risk for CVD. The biological mechanisms by which periodontal disease could influence cardiovascular disease still remain unknown. We know that the bacterial biofilm in periodontitis causes a local inflammation that leads to degradation of the tooth supporting tissues. To what extent this local inflammation could induce a systemic inflammatory reaction is unclear. However, individuals suffering from periodontitis have been shown to have increased levels of systemic inflammatory biomarkers compared to periodontal healthy subjects, which indirectly indicates that a low-grade systemic inflammation could be caused by periodontal disease (see table 4). Another indirect evidence, indicating that periodontitis might be involved in the development of atherosclerosis is that subjects with periodontitis seem to have a more atherogenic lipid profile than subjects without the disease.

7) Support from experimental evidence

Bacteria strongly associated with periodontal disease have been found in atherosclerotic plaque, and recently, also viable bacteria have been extracted from atherosclerotic plaque. There are some interesting biological properties regarding the periodontal pathogens detected in atheromas which could implicate them in the development of atherosclerosis. For instance, *Pg* has in apolipoprotein E (APO-E) deficient mice been attributed to increased size of atherosclerotic lesions, and in vitro been able to aggregate platelets, which is a crucial part in the thrombus formation. *Pg* could also in vitro induce
NADH oxidase in the endothelial cells which might increase the conversion of LDL to oxidized LDL, further implicating that this bacteria might influence the atherosclerotic process.\textsuperscript{113}

In patients with periodontitis, it is very likely that bacteria from the periodontal pockets with abilities to influence the development of atherosclerosis, such as \textit{Pg} could enter the circulation and affect the progression of cardiovascular disease.

How serum antibody levels against periodontal pathogens relates to cardiovascular disease is to a large extent unknown, but serum antibody levels against \textit{Pg} and \textit{A.a} has been associated with both coronary heart disease and stroke, indicating a possible role for periodontal pathogens in the development of CVD.\textsuperscript{115-118}

Recent treatment studies have reported that successful periodontal treatment could significantly reduce the levels of circulating CRP and improve endothelial function, further implicating that oral health could exert an effect on the vasculature.\textsuperscript{43, 119} (For more details regarding associations between oral health and CVD see review by Meurman et al.)\textsuperscript{114}

However, we still lack the strongest evidence for a causal relationship, \textit{i.e. proof that periodontal therapy lowers the risk for CVD}.\textsuperscript{116}
Studies concerning the relationship between oral health and cardiovascular disease
modified after Scannapieco et al 2003. Only studies with adjustments for confounders and with at least 50 subjects in the case group were included

Table 1: Case-control studies

<table>
<thead>
<tr>
<th>Author and year</th>
<th>Study population</th>
<th>Oral assessment</th>
<th>Cardiovascular assessment</th>
<th>Adjusted for</th>
<th>Conclusion</th>
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<tbody>
<tr>
<td>Mattila 1989</td>
<td>Cases 40 males ≤50 years old and 60 men and women &lt;65 Controls: 102 age and gender matched</td>
<td>Total dental index 0-10 (sum of scores for caries, periodontal disease, periaiapical infection and pericoronitis. Panoramic tomograph index (sum of number of residual roots, vertical bone pockets, perapical infections, furcation, caries and peri-coronitis lesions seen on radiographic pictures).</td>
<td>Evidence of MI from ECG and elevated enzyme levels</td>
<td>Age, smoking, diabetes, serum lipid concentration and social class</td>
<td>Significantly worse dental health in patients with MI.</td>
</tr>
<tr>
<td>Grau, Buggle et al. 1997</td>
<td>Cases: 69 with cerebral ischemia Controls: 60 hospitalized patients with no cerebrovascular or inflammatory disease. No alcohol derived or paraneoplastic neurological disorders.</td>
<td>Modified total dental index, range from 0-14 (sum of scores for caries, periodontal disease, periapical infection and pericoronitis built on clinical and radiological examination).</td>
<td>Diagnosed incidence of ischemic stroke or TIA with established criteria</td>
<td>Age, smoking, diabetes, pre-existing CEVD, low social class</td>
<td>Association between poor oral health and CEVD. OR=2.6</td>
</tr>
<tr>
<td>Mattila, Asikainen et al 2000</td>
<td>Cases: 85 male and female with proven CVD Controls: 53 age and gender matched</td>
<td>Indices based on sum scores from periodontal probing, furcation lesion, radiographic examination, enumerating number of caries teeth, impact teeth, periapical lesions and vertical bone loss</td>
<td>Subjects with diagnosed clinically or angiographic proven MI</td>
<td>Age, gender, smoking, lipid levels, HT and social-economic status</td>
<td>No significant correlation between MI and oral health</td>
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<td>Katz, Chaushu et al. 2001</td>
<td>1,094 Israeli army servicemen aged 25-53 years Case group 151 with CHD Control group 943 healthy subjects</td>
<td>CPITN</td>
<td>Diagnosed MI or Angiographic evidence of coronary disease</td>
<td>Age, diabetes, lipids and HT</td>
<td>No association to CHD.</td>
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<tr>
<td>Persson, Ohlsson et al. 2003</td>
<td>Cases: 80 men and women with AMI, mean age 63.4y Controls: 80 men and women, presumably healthy, mean age 61.9 years</td>
<td>Severity of periodontitis was categorised as 10 %, 20 %, 30 %, 40 %, 50 % and 60 % of teeth with a bone loss that exceeded ≥4mm measured from CEJ to the alveolar bone.</td>
<td>Patients suffering from clinical verified AMI</td>
<td>Age, sex, smoking, ethnicity, cholesterol, triglyceride, and diabetes</td>
<td>Periodontitis was significantly associated to AMI with an OR varying between 9.1-14.1, expressing the highest OR when bone loss exceeding ≥4mm was present in ≥50 % of the sites</td>
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<tr>
<td>Montebugnoli, Servidio et al. 2004</td>
<td>Cases: 63 males mean age 52.3y with CHD Controls: 50 matched for age, residence and socio-economic factors</td>
<td>Four different indices based on the number of vertical bone defects, periapical lesions, caries, pericoronitis, furcation lesions, pocket depth, BOP and pus measured clinical and on x-ray.</td>
<td>MI less than 6 month prior to the study</td>
<td>Age, smoking, HT, diabetes, education, Social class, BMI, Lipids and glucose</td>
<td>All indices showed significant correlation to CHD</td>
</tr>
<tr>
<td>Geerts, Le-grand et al. 2004</td>
<td>Cases: 108 men and women with CAD, mean age 59.2y Controls: 62 men and women randomly selected and presumably healthy, with mean age 57.7y</td>
<td>Periodontitis= (≥1 pocket with PD ≥5mm), other clinical data collected was BOP, PLI, furcation involvements and tooth mobility</td>
<td>Patients suffering from angina pectoris or MI</td>
<td>Age, sex, smoking, alcohol consumption, diet, physical activity, HT, diabetes and lipids</td>
<td>Periodontitis associated significantly with CAD, OR=6.5. The other clinical parameters were increased in CAD, and they had less remaining teeth.</td>
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<tr>
<td>Grau, Becher et al 2004 [127]</td>
<td>Cases: 303 subjects with ischemic stroke or TIA. Controls: 468 subjects of whom 300 were randomly selected from the population aged between 18-74y, and 168 were patients in the hospital with no vascular or inflammatory diseases.</td>
<td>GI, PLI, decayed surfaces, bone loss as percentage of root length (in a major part of the cases and controls), and number of teeth. Severity of periodontitis was graded according to AL and analysed as a continuous variable stratified into 1) no or mild periodontitis, defined as mean CAL ≥3mm and with steps of 1.5mm, 2) mean CAL &gt;3 to 4.5, 3) mean CAL &gt;4.5 to 6mm and 4) mean CAL &gt;6mm was defined as severe periodontitis</td>
<td>Diagnosed incidence of ischemic stroke or TIA with established criteria</td>
<td>hypertension, diabetes, smoking, previous stroke, fathers profession, and stratified by age and sex</td>
<td>Periodontitis was a risk factor for first ever stroke or TIA incidence in men and younger subjects, but not in women and older subjects (&gt;60 years). In a multivariate analyses severe bone loss and severe gingivitis (when GI &gt;1.2) was associated to cerebral ischemic OR 2.76 and 9.01, respectively.</td>
</tr>
<tr>
<td>Söder, Söder et al 2005 [128]</td>
<td>Cases: 82 randomly selected from 286 individuals with periodontitis at the basic examination 1985 Controls: 31 randomly selected from 1390 without periodontitis in 1985</td>
<td>periodontal disease was defined as presence of at least one site with a pocket depth of ≥5mm.</td>
<td>IMT and cIMA was assessed by carotid ultrasonography at the time of re-examination 2001-2003.</td>
<td>Age, gender, diabetes, smoking, education, cholesterol and BMI</td>
<td>Periodontal disease was an independent predictor for increased cIMA OR=5.2, and for increased IMT OR=4.64...</td>
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<tr>
<td>Buhlin, Gustafsson et al. 2005 [129]</td>
<td>Cases: 143 women with diagnosed CHD. Mean age 65.9 years Controls: 50 women with no CHD, mean age 64.5 years</td>
<td>Number of PD &gt;4mm, number of teeth, radiographic bone loss, assessment of oral hygiene and BOP.</td>
<td>Diagnosed CAD</td>
<td>Age, diabetes, BMI, Smoking, education and place of birth</td>
<td>Periodontitis (≥10 diseased pockets) significantly correlated with CAD with an OR=3.8</td>
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<td>Karhunen, Forss et al 2006</td>
<td>Cases: 117 males aged 33-69 years that died in sudden cardiac death. Controls: Of these 183 subjects did 120 die of unnatural causes and 63 died of non-cardiac disease</td>
<td>Number of teeth and PTI (sum of number of residual roots, vertical bone pockets, periapical infections, furcation, caries and periodontal lesions seen on radiographic pictures).</td>
<td>Sudden cardiac death</td>
<td>Age, BMI, smoking, diabetes, HT, educational level</td>
<td>Number of teeth was significantly associated to sudden cardiac death for subjects &lt;50 years of age (P=0.009) and almost significantly related for all subjects (P=0.053).</td>
</tr>
<tr>
<td>Renvert, Pettersson et al 2006</td>
<td>Cases: 161 consecutive subjects with ACS. Controls: 161 subjects matched for age, gender, socioeconomic level and smoking.</td>
<td>Oral bacterial load</td>
<td>Clinical verified diagnosis of ACS with ECG and enzyme levels</td>
<td>Age, gender, smoking and socioeconomic level</td>
<td>Patients with ACS had higher bacterial load compared to controls, significant for 26 of 40 species.</td>
</tr>
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</table>

AMI= Acute myocardial infarction, ACS=Acute coronary syndrome, BMI=Body mass index, BOP=Bleeding on probing, CAD=Coronary artery disease, CAL=Clinical attachment loss, CEJ=Cemento-enamel junction, CEVD=Cerebrovascular disease, CHD=Coronary heart disease, cIMA=Cross sectional intima, ECG=Electrocardiogram GI=Gingivitis index, HT=Hypertension, MI=Myocardial infarction, OR=Odds ratio, PD=Pocket depth, PLI=Dental plaque index, PTI=Panoramic tomography index, TIA=Transient ischemic attach.
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<tr>
<td>Paunio, Impivaara et al 1993</td>
<td>1,384 Finnish males aged 45-64 years</td>
<td>Missing teeth</td>
<td>Screening examination and interviews about previous disease, X-ray, ECG, Blood pressure. CHD defined as angina pectoris or previous MI</td>
<td>Age, HT, smoking, education and residence</td>
<td>Week, but significant association between missing teeth and ischemic heart disease.</td>
</tr>
<tr>
<td>Mattila, Valle et al. 1993</td>
<td>100 subject (88 men and 12 women) aged 28-68 years</td>
<td>Dental severity index (sum of scores for caries, periodontal disease, periapical infection and pericoronitis)</td>
<td>Extent of coronary artery occlusion by angiography</td>
<td>Age, lipids, BMI, HT, smoking and social class</td>
<td>Dental infection significantly associated to severe coronary atheromatosis in men, but not in women.</td>
</tr>
<tr>
<td>Loesche, Schork et al 1998</td>
<td>320 veterans &gt;60 years old</td>
<td>Number of teeth (0, 1-14 or 15-28), PD, attachment level, PLI and gingival bleeding and xerostomia</td>
<td>Diagnosed MI, Bypass surgery, ECG, Enzyme levels, angiography, response to heart treatment</td>
<td>Age, smoking, serum cholesterol levels, BMI and diabetes</td>
<td>Several oral health variables were risk indicators for CHD, and subjects with CHD were 2.64 times more likely found in subjects with 1-14 teeth compared to those with 0 or 15-28 teeth.</td>
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<td>Arbes et al 1999</td>
<td>5,564 subjects ≥40 years old</td>
<td>Percentage of sites with attachment loss ≥3mm categorized in 4 levels: 0 %; &gt;0-33 %; 33-67 %; and &gt;67 % based on measurements from one randomised upper and one lower quadrant in each subject.</td>
<td>Self-reported MI</td>
<td>Age, race, gender, smoking, HT, serum total cholesterol levels, BMI, poverty and diabetes</td>
<td>Unadjusted, there was a dose-dependent association between attachment loss and coronary heart disease but after adjustment, only evident for the two most severe categories with OR of 2.3 and 3.8, respectively</td>
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<tr>
<td>Beck, Elter et al. 2001 135</td>
<td>6,017 subjects form the ARIC study 1996</td>
<td>Periodontitis was defined by extent of attachment loss ≥3mm: None/mild &lt;10%, moderate 10 to &lt;30%, and severe ≥30% of the sites</td>
<td>Carotid artery intima-media thickness (IMT) ≥1mm</td>
<td>All kinds of CVD risk factors</td>
<td>Periodontitis may influence atheroma formation. There was an OR=1.31 for IMT ≥1mm in subjects with severe periodontitis.</td>
</tr>
<tr>
<td>Buhlin, Gustafsson et al. 2002 136</td>
<td>4,811 individuals received a questionnaire. 2839 answered (59%), and of them were 1,577 &gt;40 years of age</td>
<td>Self reported oral health and dentures</td>
<td>Self reported. If they had any type of CVD in the last 9 years, and if yes, specified on type (MI, stroke, angina pectoris, atherosclerosis and HT)</td>
<td>Age, gender diabetes, smoking, education and civil status</td>
<td>In subjects &gt;40 years of age was bleeding gums and wearing of dentures related to CVD with an OR= 1.6 and 1.57, respectively.</td>
</tr>
<tr>
<td>Buhlin, Gustafsson et al. 2003 137</td>
<td>723 men and women aged 20-84 years.</td>
<td>Self reported oral health and number of teeth</td>
<td>Self reported. If they had any type of CVD in the last 9 years and if yes specified on type (MI, stroke, angina pectoris, atherosclerosis and HT)</td>
<td>Age, gender diabetes, smoking, education and civil status</td>
<td>Bleeding gums was related to CVD with an OR 1.7 for the whole sample and 2.69 for the oldest group alone (75-84y)</td>
</tr>
<tr>
<td>Elter, Offenbacher et al. 2003 138</td>
<td>9,415 dentate and 1,491 edentulous subjects aged 52-75 years from the ARIC study were investigated for stroke and TIA. Periodontal examination was performed in a subset consisting of 6,436 individuals.</td>
<td>Percentage of sites with AL &gt;3mm categorised into quartiles: 0 to &lt;6.5%, 6.5 to &lt;15.4%, 15.4 to &lt;31.4%, and ≥31.4%. Number of teeth was also registered</td>
<td>Ischemic stroke and TIA</td>
<td>All kinds of CVD risk factors</td>
<td>Week association between stroke/TIA and edentulism or attachment loss in the highest quartile of.</td>
</tr>
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<tr>
<td>Desvarieux, Demmer et al. 2003</td>
<td>711 subjects aged ≥55 years (Mean age 66 y) with no baseline history of stroke or MI enrolled in INVEST study</td>
<td>Severity of periodontal disease defined as percentage of sites with PD ≥5mm and AL ≥4mm. Tooth loss was divided into 4 categories: 0-9, 10-19, 20-31 and edentulous</td>
<td>High resolution ultra sound was used to access if any plaque, defined as an area with focal wall thickening was present in the common carotid arteries, internal and external carotid arteries</td>
<td>All kinds of CVD risk factors</td>
<td>46 % of subjects with 0-9 missing teeth had plaque somewhere in the carotid artery in contrast to 60 % of those with ≥10 missing teeth. Tooth loss as a marker for past periodontal disease was related to subclinical atherosclerosis.</td>
</tr>
<tr>
<td>Desvarieux, Schwahn et al. 2004</td>
<td>1,710 randomly enrolled males and females aged 45-75 years</td>
<td>Ongoing periodontitis measured as 10 % of sites with PD ≥5mm. Long time exposure of periodontitis measured as % of sites with AL ≥4mm. Number of teeth was categorised into 0 to 8, 9 to 15 and 16 to 31 teeth</td>
<td>Ultra sound was used to access if any plaque was present in the CCA, ICA and ECA further more to measure the IMT of the fare wall in of the CCA.</td>
<td>All kinds of risk factors for CVD</td>
<td>Long-term exposure to periodontitis and tooth loss was significantly related to subclinical atherosclerosis in men, but not in women</td>
</tr>
<tr>
<td>Nakib, Pankow et al. 2004</td>
<td>Cohort of 269 from the ARIC study</td>
<td>Based on AL was periodontitis stratified with into 2 groups: no/mild &lt;10 % of the sites with AL ≥3mm, moderate/severe 10 % of the sites with AL ≥3mm.</td>
<td>CAC was estimated using CT and Agastone score calculated. Cut off levels for the score was &lt;100 and ≥100 used.</td>
<td>All kinds of risk factors for CVD</td>
<td>No significant association.</td>
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<tr>
<td>Elter, Champagné et al. 2004</td>
<td>8,363 subjects aged 52-75 years from the ARIC study</td>
<td>Four categories were formed using % of sites with attachment loss ≥ 3mm together with number of teeth: <em>low attachment and low tooth loss</em> (&lt;10% AL and ≥17 teeth left), <em>low AL and high tooth loss</em> (&lt;10 % AL and &lt;17 teeth lost), <em>high AL and low tooth loss</em>, (≥10% AL and ≥17 teeth left), and <em>high AL and high tooth loss</em> (≥10 % AL and &lt;17 teeth left).</td>
<td>CHD defined and diagnosed as MI or revascularisation procedures.</td>
<td>All kinds of CVD risk factors</td>
<td>Edentulous subjects and those with high AL and high tooth loss had significantly more CHD compared to those with low AL and low tooth loss OR 1.5 and 1.8, respectively.</td>
</tr>
<tr>
<td>Engebretson et al. 2005</td>
<td>203 stroke free subjects aged 54-94 years from the INVEST</td>
<td>Chronic periodontitis (CP) exposure was categorised depending on the amount of bone loss into no/mild/moderate periodontitis if the bone loss was &lt;50 % and severe periodontitis if it was &gt;50 %</td>
<td>High resolution ultrasound was used to identify presence or absence of plaque in the internal, common carotid artery and bifurcations.</td>
<td>Age, smoking, gender, diabetes, HT and serum Cholesterol.</td>
<td>Severe periodontitis was associated with an increased risk for presence of plaque in the carotid artery. OR=3.64</td>
</tr>
<tr>
<td>Volzke, Schwahn et al 2005</td>
<td>2,341 subjects aged ≥45 years</td>
<td>Number of teeth. Extent of AL measured as % of sites with AL ≥4mm (this was done on a subset of 1,690 individuals)</td>
<td>Two dimensional M-mode and Doppler echocardiography was used to evaluate aortic valve sclerosis.</td>
<td>All kinds of CVD risk factors</td>
<td>Number of teeth was independently associated to Aortic valve sclerosis. OR=0.98</td>
</tr>
<tr>
<td>Desvarieux, Dremmer et al. 2005</td>
<td>1,056 individuals with no history of stroke or MI was enrolled in (INVEST) and microbiologic samples were collected from 657 dentate subjects.</td>
<td>In total 4,561 subgingival microbial samples were taken on average from 7 sites/subject. These were analysed for the presence or absence of 11 known periodontal pathogens by DNA-DNA checkerboard hybridization</td>
<td>Ultra sound was used to investigate the Intima-media thickness (IMT) of carotid artery. Furthermore were values of CRP levels and white blood cell count obtained.</td>
<td>All kinds of risk factors for CVD</td>
<td>Overall bacterial burden was related to carotid IMT, but CRP levels were unrelated to periodontal microbial status. These data indicates a relationship between periodontal microbiology and subclinical atherosclerosis independent of CRP.</td>
</tr>
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<tr>
<td>Volzke, Schwahn et al 2006</td>
<td>4,185 subjects from a population based study of health of which 2,150 were women.</td>
<td>Number of teeth</td>
<td>HT was defined as a systolic blood pressure &gt;140 mmHg or a diastolic blood pressure &gt;90 mmHg or antihypertensive medication</td>
<td>Age, gender, smoking, BMI, education, diabetes, dietary pattern and antihypertensive medication</td>
<td>Men with 0–6 teeth had significantly higher systolic blood pressure compared to those with 27–28 teeth. OR 1.91. No relationship was evident for women</td>
</tr>
<tr>
<td>Holmlund et al 2006</td>
<td>4,254 subject, 3,889 referred for periodontal treatment and 907 randomly selected from same population. Mean age 53 years</td>
<td>PD, BOP, PLI and number of teeth was registered. Bone loss for each subject was measured on radiographs and depending mainly on the amount of bone loss the severity of periodontitis was stratified into: no, mild, moderate or severe periodontitis.</td>
<td>Self reported MI and medication for HT</td>
<td>Age, gender and smoking</td>
<td>There was a significant dose-response association between number of teeth (hole sample), periodontitis (in subjects aged 40–59 years) and MI. with OR 1.3–1.5. HT was associated to periodontitis and number of periodontal pockets, but not to number of teeth.</td>
</tr>
<tr>
<td>Lee et al 2006</td>
<td>5,123 subjects from the NHANES III study ≥60 years of age</td>
<td>PHS I 1) &lt;45% of the sites with CAL ≥2 mm; 2) &gt;45% of the sites with ≥2 mm CAL. PHS II 1) &lt;45% of the sites with CAL ≥3 mm; 2) &gt;45% of the sites with ≥3 mm CAL. PHS I and PHS II were also combined with tooth loss.</td>
<td>Questionnaire if a doctor ever told them that they had a stroke</td>
<td>All kinds of CVD risk factors.</td>
<td>No association between stroke and PHS</td>
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Table 2 (continued)

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<td>Gotsman, Lotan et al 2007 148</td>
<td>201 patients referred for coronary angiography</td>
<td>PLI, BOP, CAL ≥5mm, bacterial count</td>
<td>Severity of coronary disease was graded depending on number of obstructed coronary arteries and the degree of narrowing based on high-quality film angiograms</td>
<td>Age, smoking, diabetes, and family history of CAD.</td>
<td>CAL ≥5mm was significantly related to CAD and percentage of Porphyromonas gingivalis was related to ACS.</td>
</tr>
<tr>
<td>Holmlund et al 2007 149</td>
<td>Cohort study of 1,016 subjects aged 70 year</td>
<td>Self-reported number of teeth</td>
<td>The metabolic syndrome classified by NCEP/ATP III</td>
<td>Age, gender, smoking, WBC, HT, and education level</td>
<td>Number of teeth was significantly associated to the metabolic syndrome</td>
</tr>
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ACS= Acute coronary syndrome, AL= Attachment loss, ARIC= Atherosclerosis Risk in Community, BMI= Body mass index, BOP= Bleeding on probing, CAC= Coronary artery calcification, CAD= Coronary artery disease, CAL= Clinical attachment loss, CCA= Common carotid arteries, CHD= Coronary heart disease, CRP= C-reactive protein, CP= Chronic periodontitis, CT= Computer tomography, CVA= Cerebral vascular attack, CVD= Cardiovascular disease, DNA= Deoxyribonucleic acid, ECA= External carotid artery, ECG= Electrocardiogram, GI= Gingivitis index, HT= Hypertension, ICA= Internal carotid artery, IMT= Carotid artery intima-media thickness, INVEST= Oral Infections and Vascular Disease Epidemiology study, MI= Myocardial infarction, NCEP/ATP III= National Cholesterol and Education Program/Annual Treatment Planning III, OR= Odds ratio, PHS= Periodontal health status, PD= Pocket depth, PLI= Dental plaque index, TIA= Transient ischemic attack, WBC= White blood cell count.
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<td>DeStefano, Anda et al., 1993</td>
<td>9,760 subjects enrolled in the NHANES I from 1971 to 1974. Follow up until 1982.</td>
<td>Number of decayed teeth; Periodontal classification: (no disease, gingivitis, periodontitis with &gt;4mm pockets, and no teeth); Periodontal index: each tooth was given a score 0-8 depending on degree of periodontitis and the index was an average of all scored teeth. Also an oral hygiene index was registered.</td>
<td>Admission to hospital for CHD treatment. Mortality due to CHD.</td>
<td>Age, smoking (partly), alcohol, race, SBP, education, BMI, exercise, and poverty</td>
<td>Periodontal disease is associated with a small increased risk for CHD RR= 1.25 for all and 1.72 for men 25-42 years</td>
</tr>
<tr>
<td>Mattila, Valtonen et al, 1995</td>
<td>In all 214 subjects, median follow-up time of 2.2 years. 70 males aged ≥50 years with MI or admitted for angiographic examination 1983-85. 60 subjects, males ≤50 years and women ≤65 years enrolled 1985-86 with diagnosed MI. 100 enrolled for diagnostic coronary angiography and some of these already belonged to previous groups.</td>
<td>Total dental index 0-10 (sum of scores for caries, periodontal disease, periapical infection and pericoronitis.) Panoramic tomography index (sum of number of residual roots, vertical bone pockets, perialcal infections, furcation, caries and pericoronitis lesions seen on radiographic pictures).</td>
<td>Endpoints fatal and non-fatal MI and all cause morality.</td>
<td>Age, gender, previous MI, diabetes, BMI, smoking, HT, serum lipids, and socio-economics</td>
<td>Poor oral health is associated with new fatal and non-fatal cardiac events.</td>
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<tr>
<td>Joshipura, Rimm et al, 1996</td>
<td>44,119 men. (58% were dentists), 40-75 years of age. Follow up after six years.</td>
<td>Self reported number of teeth and history of periodontal disease</td>
<td>Fatal/ non-fatal MI, sudden death. Re-vascularisation procedures were excluded as endpoint</td>
<td>Age, CHD risk factors and smoking</td>
<td>No association between periodontal disease and CHD, but a small significant relationship between tooth loss and CHD. RR=1.67 for those with 1-10 teeth.</td>
</tr>
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<tr>
<td>Beck, Garcia et al 1996</td>
<td>1,147 subjects from Veteran dental longitudinal study a component of the Normative aging study</td>
<td>Alveolar bone loss measured using a Schei ruler, (0 to 20 %, &gt;20 to 40 %, &gt;40 to 60 % and &gt;60 %). Worst PD per tooth</td>
<td>Total CHD determined as cases of non-fatal MI, angina and CHD death. Stroke diagnosed with means of history and physical examination</td>
<td>Age, total cholesterol, Systolic blood pressure, BMI, and partly for smoking</td>
<td>OR=1.5 for bone loss and total CHD (this was not adjusted for smoking), OR=1.9 for fatal CHD and OR=2.8 for bone loss and stroke (both adjusted for smoking). Dose-response relationships were seen between severity of bone loss and cumulative incidence of CHD and fatal stroke.</td>
</tr>
<tr>
<td>Mendez, Scott et al 1998</td>
<td>Cases 80 subjects with PVD and 1,030 controls were enrolled from 1,231 subjects belonging to the Dental longitudinal study of the US department of veteran affairs which is a subgroup to the Normative aging study. Follow up time was 25-30 years</td>
<td>Alveolar bone loss estimate on radiographic films using a Schei ruler. A mean whole mouth alveolar bone loss of &gt;20% was regarded as significant periodontal disease.</td>
<td>PVD was defined according to following criteria: 1) Intermittent claudicatio 2) extra cranial cerebrovascular disease. 3) atherosclerosis (including aortic, renal and mesenteric disease) 4) arterial embolism and thrombosis</td>
<td>All kinds of risk factors for CVD</td>
<td>Periodontal disease was an independent risk indicator for PVD in a multivariate analysis. Subjects with clinical significant periodontal disease at baseline had a 2.27 increased risk of developing PVD after the exclusion of other vascular conditions from the control group and stroke from the case group.</td>
</tr>
<tr>
<td>Morrison, Ellison et al 1999</td>
<td>10,368 subjects from Nutrition Canada Survey. 35-84 years old.</td>
<td>Periodontitis classified as no disease, mild and severe gingivitis, obvious pockets, loose teeth, and edentulous.</td>
<td>Death from coronary heart and cerebrovascular disease</td>
<td>Age, gender, smoking, HT, serum total cholesterol and diabetes</td>
<td>Severe gingivitis and being edentulous was associated to increased risk for fatal CHD with RR =2.15 and 1.9, respectively</td>
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Table 3 (continued)

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<td>Wu, Trevi-san et al. 2000</td>
<td>9,962 subjects aged 25-74 years from NHANES-1, 1971-74 and the follow-up study 1992.</td>
<td>1) No periodontal disease if ≤1 tooth had mild gingivitis and ≥20 teeth were present, 2) Gingivitis ≥1 tooth with mild gingivitis or worse condition that did not fit 1 or 3, 3) Periodontitis ≥4 teeth with overt pockets or worse, 4) Edentulousness</td>
<td>Incident cases of CVA meeting at least one of the following criteria: death cause CVA or one or more hospital/nursing home stay during follow-up period with discharge diagnosis CVA.</td>
<td>All kinds of CVD risk factors including age and smoking</td>
<td>Compared with no periodontal disease the RR for non-hemorrhagic stroke in subjects with periodontitis was 2.11, and 1.66 for total CVA. There was no significant correlation to gingivitis or edentulousness.</td>
</tr>
<tr>
<td>Hujoel, Drangsholt et al 2000</td>
<td>8,032 dentate adults aged 25-74 years from NHANES I epidemiologic follow up study. Based on questionnaire</td>
<td>Periodontitis defined as ≥1 periodontal pocket with attachment loss, Gingivitis= inflammation with no attachment loss. Periodontal health= no inflammation or attachment loss</td>
<td>Death from CHD, hospitalization due to CHD or revascularisation procedures obtained from death certificates and medical records.</td>
<td>All kinds of CVD risk factors including age and smoking</td>
<td>No association</td>
</tr>
<tr>
<td>Jansson, Lavstedt et al. 2001</td>
<td>1,393 subjects aged 18-66 years from the County of Stockholm admitted to the study 1979. Follow up in 1997</td>
<td>Number of missing teeth, apical lesions, carious and marginal bone loss (MBL) expressed as % of the distance between apex to CEJ. Oral health score, a combination of missing teeth, MBL, number of teeth with carious and apical lesions.</td>
<td>Death due to CVD</td>
<td>Age, gender and smoking</td>
<td>Oral health was significantly related to fatal CHD in subjects &lt;45 years old with OR 2.7 Poor oral health in combination with smoking is a risk indicator for CVD.</td>
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<tr>
<td>Hujoel, Drangsholt et al 2001</td>
<td>4,027 subjects from NHANES-I based on questionnaire</td>
<td>Comparison of subjects with periodontitis= (PD ≥4mm on any teeth) and subjects with edentulism.</td>
<td>Death from CHD, hospitalization due to CHD or revascularisation procedures obtained from death certificates and medical records.</td>
<td>All kinds of CVD risk factors including Age and smoking</td>
<td>Edentulose individuals did not have lower risk for CHD compared to subjects with periodontal disease.</td>
</tr>
<tr>
<td>Howell, Riddker et al. 2001</td>
<td>22,037 male subjects aged 40-84 years from the Physician Health Study I. Based on questionnaire. Average follow-up of 12.3 years</td>
<td>Self reported presence or absence of periodontal disease at study entry</td>
<td>A CVD event was confirmed by an end point committee after examination of all information blinded to the participant’s periodontal status. Death due to CVD was confirmed by reviewing death certificates</td>
<td>All kinds of CVD risk factors including Age and smoking</td>
<td>No association</td>
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<tr>
<td>Takata, Ansu et al. 2001</td>
<td>697 octogenarians in Fukuoka. Prefecture, 277 male and 420 female aged 80 years.</td>
<td>CPI 0-4. 0=health, 1=gingival bleeding, 2=presence of calculus, 3=presence of PD 4-5mm, 4=presence of PD ≥6mm. Tooth loss</td>
<td>Abnormal ECG findings</td>
<td>All kinds of CVD risk factors including Age and smoking</td>
<td>Tooth loss may be a predictor for abnormal ECG findings in the very elderly.</td>
</tr>
<tr>
<td>Jansson, Lavstedt et al. 2002</td>
<td>1,393 subjects aged 18-66 years from the County of Stockholm admitted to the study 1979. Follow up in 1997</td>
<td>Oral health score a combination of missing teeth, MBL number of teeth with carious and apical lesions. Marginal bone loss (MBL) expressed as % of the distance between apices to CEJ.</td>
<td>Death due to CVD</td>
<td>Age, gender and smoking</td>
<td>Poor oral health is a risk indicator for all-cause mortality</td>
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<tr>
<td>Hujoel, Drangsholt et al 2002</td>
<td>636 dentate individuals initially enrolled in NHANES-I who had both medical and dental examinations and a prior history of CVD. Based on questionnaire</td>
<td>Periodontitis = increased PD of ≥4 teeth with attachment loss, Gingivitis= inflammation with no attachment loss, Periodontal health= no inflammation or attachment loss</td>
<td>Inclusion: History of CVD based on yes on 4 questions, has a doctor ever told you that you had a heart attack, heart failure or a stroke in last 6 months or do you take any drugs for a weak heart</td>
<td>All kinds of CVD risk factors including Age and smoking</td>
<td>No association. Periodontitis does not increase risk for CHD in subjects with prior heart attack or self reported CVD.</td>
</tr>
<tr>
<td>Tuominen, Reunanen et al 2003</td>
<td>6,527 men and women aged 30-69 years from the Mini-Finland Health survey. Mean follow up time 12 years</td>
<td>Periodontal disease classified as 1) no disease, 2) gingival inflammation, 3) PD 4-6mm, 4) PD &gt;6mm. Patients were categorized according to the worst quadrant. Number of teeth and PLI was also registered</td>
<td>Death in CHD</td>
<td>Age, gender, diabetes, smoking, HT, lipids and education</td>
<td>No association</td>
</tr>
<tr>
<td>Hung, Willett et al. 2003</td>
<td>Eligible was 45,136 men, aged 40-75 years that were professional health workers. Follow up time 12 years</td>
<td>Questionnaire about their oral health including: number of teeth lost and periodontal disease</td>
<td>Diagnostic and self-reported PAD</td>
<td>All kinds of CVD risk factors</td>
<td>Baseline number of teeth was not related to PAD. However, tooth loss during follow up time and periodontal disease was associated to PAD, RR 1.39 and 1.41, respectively. Tooth loss in men without periodontal disease was not related to PAD</td>
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<tr>
<td>Joshipura, Hung et al. 2003</td>
<td>Eligible was 41,380 men, aged 40-75 years that were professional health workers. Follow up time 12 years</td>
<td>Questionnaire about their oral health including: number of teeth lost and periodontal disease</td>
<td>Fatal and non-fatal stroke confirmed by medical records and sub classified into: ischemic stroke; hemorrhagic stroke; and unknown type</td>
<td>All kinds of CVD risk factors</td>
<td>Men with &lt;25 teeth at baseline were at higher risk for stroke, HR 1.57. Periodontal disease at baseline was moderately related to ischemic stroke, HR 1.33</td>
</tr>
<tr>
<td>Abnet, Qiao et al. 2005</td>
<td>29,584 healthy subject aged 44-69 years at baseline was followed for 15 years</td>
<td>Tooth loss</td>
<td>Death in heart disease</td>
<td>Age, sex, smoking, weight, height, and SBP</td>
<td>Tooth loss was related to death in heart disease with a RR of 1.28 and to stroke with a RR of 1.11</td>
</tr>
<tr>
<td>Schillinger, Kluger et al. 2006</td>
<td>411 randomly selected subjects from 1,268 participants in the Inflammation and Carotid Artery Risk for Atherosclerosis study (ICARAS).</td>
<td>DMFT, SLI; CPITN and edentulousness was calculated for each individual and compared to atherosclerosis of the carotid artery.</td>
<td>Uni or bilateral progression of carotid atherosclerosis in the extra-cranial internal carotid from baseline to follow up after 6 to 9 months</td>
<td>All kinds of CVD risk factors.</td>
<td>DMFT, SLI and CPITN was significantly related to baseline degree of carotid stenosis, but only DMFT and SLI were significant predictors for progression of the atherosclerosis in the carotid artery with OR 1.11 and 1.77, respectively</td>
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Table. 3 (continued)

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<td>Tu, Galobar-des et al. 2007</td>
<td>12,631 out of 15,322 eligible students from the University in Glasgow were included. 9,569 men and 2,654 women aged 30 years or younger at baseline. Follow-up period of 57 years.</td>
<td>Number of missing teeth, number of filled and decayed teeth.</td>
<td>Mortality in CVD, other external causes and lung cancer.</td>
<td>Age, gender, BMI, education, systolic blood pressure and socio-economic factors</td>
<td>Baseline tooth loss as continuous variable was not related to mortality. However, when tooth loss was treated as categorical variable, a HR 1.35 for mortality in CVD was seen for subjects with &gt;8 missing compared to those with &lt;5 missing teeth.</td>
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<tr>
<td>Söder, Jin et al. 2007</td>
<td>3,273 randomly selected subjects aged 30-40 years. 1,676 of these were clinically examined and followed for 16 years</td>
<td>Missing teeth, PLI, GI, PD, and Calculus. Periodontitis was defined as ≥ one tooth with PD ≥5mm</td>
<td>Cause of death according to ICD classification</td>
<td>Age, gender, smoking, socio-economic status, and education</td>
<td>Missing molar teeth in subjects with periodontitis was associated to all cause of mortality with OR 3.62</td>
</tr>
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<td>Dietrich et al. 2008</td>
<td>1,203 men in the VA Normative Aging and Dental Longitudinal Studies followed up with triennial comprehensive medical and dental examinations up to 35 years (median 24 years)</td>
<td>Bone loss was assessed on x-ray for each tooth and the amount of bone loss was divided with 20 % increments (0 = no bone loss; 1= bone loss ≤20%; 2= bone loss &gt;20% ≤40%; 3= bone loss &gt;40% ≤60%; 4= bone loss &gt;60% ≤80%; and 5= &gt;80%). Number of pockets were categorised into: 0-3mm; &gt;3mm to 5mm and &gt;5mm. Number of teeth were also assessed</td>
<td>CHD fatal or non-fatal</td>
<td>All kinds of CVD risk factors</td>
<td>Dose dependent association between periodontitis and CHD among men &lt;60 years old, HR 2.12 comparing highest versus lowest group of bone loss. Edentulous men ≥60 years tended to have a higher risk for CHD than dentate men with the lowest bone loss, HR 1.61 and lowest pocket depth, HR 1.72.</td>
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</tbody>
</table>

BMI= Body mass index, CEJ = Cemento-enamel junction, CHD= Coronary heart disease, CPI= Community periodontal index, CPTIN = Caries and periodontal index treatment of needs, CHD= Coronary heart disease, CVA= Cerebral vascular attach, CVD= Cardiovascular disease, DMFT= Decayed, missing and filled teeth, ECG= Electrocardiogram, HT= Hypertension, ICARAS= Inflammation and Carotid Artery Risk for Atherosclerosis study, MBL= Marginal bone loss, MI= Myocardial infarction, NHANES = National Health and Nutritional Survey, OR= Odds ratio, PAD= peripheral artery disease, PD= Pocket depth, PLI= Dental plaque index, PVD= Peripheral vascular disease, RR= Relative risk, SBP= Systolic blood pressure, SLI= Silness-Löe index
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Outcome</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kweider, Lowe et al. 1993</td>
<td>Case-control study 50 consecutive patients from dental hospital. 50 periodontal healthy from the hospital staff</td>
<td>Dental index (PLI, GI, CPITN) correlated significantly with fibrinogen and WBC count</td>
<td>Oral health may influence fibrinogen and WBC count</td>
</tr>
<tr>
<td>Wakai, Kawamura et al 1999</td>
<td>A cross-sectional study with 517 males and 113 females 23-83 years old</td>
<td>Poor Periodontal health measured as CPITN strongly associated with High WBC count. Total cholesterol and triglyceride were not associated with periodontal status</td>
<td>Periodontal status may influence systemic markers of inflammation</td>
</tr>
<tr>
<td>Loos, Craandijk et al. 2000</td>
<td>Case-control study 107 consecutive periodontal patients and 43 periodontal healthy controls</td>
<td>Subjects with periodontitis had higher median CRP and plasma IL-6 levels than healthy controls.</td>
<td>Periodontitis is associated with higher levels of systemic inflammation markers such as IL-6 and CRP that also have been associated with increased risk for CVD.</td>
</tr>
</tbody>
</table>
**Table 4 (continued)**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Outcome</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slade, Offenbach, et al. 2000</td>
<td>12,949 dentate subjects &gt;18 years old and 1,817 edentulous subjects &gt;18 years old from the Third NHANES</td>
<td>Periodontal health measured as percentage PD&gt;4mm categorised into: 0%, 0-10%, and &gt; 10%. CRP was dichotomised in &lt;10mg/l or ≥10mg/l. In a multivariate analysis including diabetes, arthritis, emphysema, smoking, socio-demographic factors and anti-inflammatory medication, the CRP levels were higher in subjects with extensive periodontitis.</td>
<td>Periodontal disease and edentulism were associated with higher levels of CRP most pronounced in individuals with no established risk factors for elevated CRP.</td>
</tr>
<tr>
<td>Wu, Trevisan et al. 2000</td>
<td>10,146 subjects from Third NHANES study and its follow-up</td>
<td>Significant association between indicators of poor oral health (Gingival bleeding index, calculus index, PD, and attachment loss) and increased levels of CRP and fibrinogen</td>
<td>CRP and fibrinogen levels can be a possible link for periodontal disease to increased risk for CVD</td>
</tr>
<tr>
<td>Noack, Genco et al. 2001</td>
<td>Cross-sectional study 174 subjects randomly selected from a cohort of 1,250 subjects. 50 with moderate periodontal attachment loss, 50 with severe attachment loss, and 65 periodontally healthy subjects served as controls</td>
<td>Increased CRP levels in subjects with periodontitis compared to periodontally healthy subjects. Presence of periodontal pathogens Porphyromonas Gingivalis (Pg), Prevotella Intermedia (Pi), Campylobacter recta (Cr) and Tanderella forsythus (Tf) were positively associated to CRP levels.</td>
<td>Correlation between CRP and periodontal disease may be a pathway in the association between periodontitis and CVD.</td>
</tr>
<tr>
<td>Reference</td>
<td>Study population</td>
<td>Outcome</td>
<td>Conclusion</td>
</tr>
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</tr>
<tr>
<td>D’Aiuto, Ready et al. 2004 43</td>
<td>94 Systemically healthy subjects with severe periodontal disease received periodontal treatment</td>
<td>Levels of CRP were divided into 3 groups: low = CRP &lt; 1mg/l, medium = CRP = 1-3mg/l, and high = &gt;=3mg/l. Treatment reduced the number of individuals with CRP levels belonging to the medium and high groups.</td>
<td>Periodontitis may add to the inflammatory burden of the individual that may result in increased levels of CRP and thereby higher risk for CVD</td>
</tr>
<tr>
<td>Taylor et al 2006 173</td>
<td>67 adults with advanced periodontitis in need full-mouth extraction. Blood samples were taken 1) at initial presentation, 2) 2-3 weeks later before all teeth were removed, 3) 12 weeks after full-mouth extraction.</td>
<td>CRP levels were significantly reduced after extraction. Furthermore, the extraction therapy significantly reduced the total white blood cell count and number of platelets.</td>
<td>Treatment of periodontal disease may lower cardiovascular risk</td>
</tr>
</tbody>
</table>
Aims of the thesis

Paper I.
Study local inflammatory response in periodontal disease by
- Intra-individually investigate the levels of IL-1α, IL-1β, IL-1ra and bone resorption activity in gingival crevicular fluid from healthy and diseased sites before and after treatment. Furthermore, study if changes in BRA activity and levels of IL-1 were correlated and if they could be related to probing pocket depth.

Paper II.
In a cross-sectional study based on 4,254 subjects investigate
- If oral health is related to the prevalence of myocardial infarction and hypertension in a dose-dependent manner.

Paper III.
In a cohort study investigate
- If self-reported tooth loss in 1,016 elderly subjects is related to the presence of the metabolic syndrome and to general markers of inflammation.

Paper IV.
With a prospective study based on a cohort of 7,674 subjects investigate
- If oral health in dose-dependent manner is associated to all-cause mortality, mortality in cardiovascular disease, in coronary heart disease, and in stroke.

Paper V.
In a case-control study including 100 cases and 100 age-and gender matched controls investigate
- If oral health is impaired in patients with acute myocardial infarction (MI), and if antibody levels against the periodontal pathogens Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans could be a link between oral health and MI.
Methods

Patient selection

Study I

Included were ten consecutive patients with moderate to advanced periodontitis referred to the Department of Periodontology, at the Gävle County Hospital in Sweden, for periodontal treatment. The mean age was 51 years (46-66 years). Six of the patients were smokers. To be included, each patient should have 2 healthy sites and at least 4 diseased sites (i.e. pockets >4 mm deep showing ongoing inflammation such as bleeding on probing or pus and manifestation of bone loss on X-ray). Neither did any of the patients suffer from systemic illness nor had they taken any antibiotic medication 6 months or anti-inflammatory drug 3 months, prior to the investigation. At the follow-up examination 12 month after treatment, one patient was lost because he moved to Africa and in one patient a tooth with angular bone loss was lost due to extraction.

Study II

The study included 4,254 subjects aged between 20-89 years of which 3,352 were referred to the Department of Periodontology, at Gävle County Hospital Sweden, for periodontal treatment between the years 1976-2000, and 902 individuals participated in dental health surveys (218 subjects in 1979; 376 subjects in 1989; and 308 subjects in 1999 and not 392 in 1989 or 311 in 1999 as it says in the article) from five different parts of the same geographic area.

Study III

2,025 subjects aged 70 years were invited to participate in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. 1,016 of the invited subjects were willing to participate, giving a participation rate of 50.1%.

Study IV

The study population consisted of 7,674 individuals, mean age 51.7 ±13.8 years of whom 6,788 were patients referred to the Department of Periodontology, at Gävle County Hospital Sweden, for periodontal treatment between the years 1976 and 2002. An additional 886 randomly selected individuals from the same geographic area that participated in dental health surveys (217
subjects in 1979; 359 subjects in 1989; and 310 subjects in 1999) were included in the sample to assure a certain number of orally healthy individuals. Censor data for follow-up was June 30th 2004.

Study V

100 patients admitted at Gävle County Hospital, Sweden, because of acute myocardial infarction (MI) and 100 apparently healthy, age-and sex matched controls randomly chosen from the general population of the same geographic area.

Investigations

General health

In study I, all participants were asked if they suffered from any systemic illness, if they had received periodontal treatment or antibiotics within the preceding 6-months or any anti-inflammatory drugs 3 weeks prior to the study. Smoking habits were registered as a dichotomised variable smokers (1) or non-smokers (0).

In study II, during the appointment for the clinical oral investigation the patients were interviewed about their general health. This study is based on self-reported incidence of myocardial infarction and antihypertensive medication. Smoking habits were registered in the same manner as in study I.

In study III, the participants were all asked to answer a questionnaire about their medical history, smoking habits, regular medication, and education level. Smoking was dichotomised in the same way as in study I. The educational level was divided into three different categories: six years in school or less; 7-12 years in school; and/or university studies. The medical examinations were performed in the morning after an over-night fast. No medication or smoking was allowed after midnight. After recordings of height, weight, abdominal and hip circumference, an arterial cannula was inserted in the brachial artery for blood sampling. Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to nearest mmHg after at least 30 minutes of rest and the average of three recordings was used.

The metabolic syndrome was defined according to the NECP/ATP III criteria \(^ {174} \) and three of the following five criteria should be fulfilled: Blood pressure >130/85 mmHg or antihypertensive treatment; fasting blood glucose >5.6 mmol/l; serum triglycerides >1.7 mmol/l; waist circumference >102 cm in men and >88 cm in women; and HDL-cholesterol <1.0 mmol/l in men and <1.3 in women.

In study IV, data concerning general health were not available for all subjects. However, the year and month when the oral investigation was per-
formed as well as data regarding smoking habits were accessible for all participants. Smoking habits were registered as in study I.

In study V, myocardial infarction (MI) was verified by typical changes in the electrocardiogram (ECG) readings in combination with elevation of biochemical markers in serum such as creatinine kinase isoenzyme and troponin T. All subjects answered a questionnaire about their smoking habits, education level and their medical condition prior to the infarction. The educational level was divided into the same categories as used in study III. Smoking was defined as in study I. Subjects who quit smoking in connection to the infarction were registered as current smokers. Information concerning height, weight and medication were in the MI group collected from the medical records, but in the control group by interview. Hypertension was defined by antihypertensive medication prior to infarction in the MI group, and as a systolic blood pressure >139 mmHg, and/or diastolic blood pressure >89 mmHg measured after 15 minutes rest in the control group. Diabetes was defined as fasting plasma glucose >6.9 mmol/l or if subjects were taken antidiabetic medication.

Oral health
Participants in study I, II, IV and V received a full mouth examination conducted by specialists in periodontology, including registration of number of teeth, number of sites with visible plaque, bleeding on probing, tooth mobility, furcation involvement and probing pocket depth >4 mm. Also a full mouth set of radiographs were taken. The plaque was made visible by gently scraping with a probe along the gingival margin and surfaces with bleeding on probing (BOP) were registered after gently probing the pocket depth.

Severity of periodontal disease/periodontal bone loss.
In study I, each participant should have two healthy sites and four disease sites, of which two should present angular bone loss, and two sites horizontal bone loss. A site was regarded to have bone loss if >2 mm bone loss was observed on radiographs measured from cemento-enamel junction to the alveolar crest. Sites where the bone loss presented an intrasosseous defect ≥3 mm were regarded as being of an angular type. Bone loss of a horizontal or angular type in combination with pocket depth >4 mm, bleeding on probing or pus was regarded as a site with ongoing disease. A healthy site was defined as a site with bone loss not exceeding 2 mm, and pocket depth ≤3 mm with no bleeding on probing or pus. GCF was collected from 6 sites in each individual using a filter paper technique. All subjects received periodontal treatment in terms of open flap surgery performed by the same surgeon in combination with systemic antibiotics, Doxycycline® 100 mg/day administered for 15 days.
To assess bone loss in *study II, IV and V*, a ruler with a graded scale on a transparent sheet was applied over the root on the apical x-ray, dividing the root into three parts from the enamel-cemental junction (ECJ) to the apices. Bone loss ≤2 mm was regarded as no loss. The site with the largest amount of bone loss was chosen to represent the bone loss of that particular tooth. In *study II and IV*, depending on the degree of bone loss (BL), presence or absence of bleeding on probing (BOP) and furcation involvement, a value from 0 to 4 was given to each tooth: (0) = no BOP or BL; (1) = BOP, but no BL; (2) = BL >2 mm ≤1/3 of the root length; (3) = BL >1/3 and ≤2/3 of the root length; and (4) = if the loss were > 2/3 of the root length or if it was of an angular type or if furcation involvement degree 2 and 3 was present. No pockets exceeding 4 mm were allowed for the values (0) and (1). The values for all teeth were summarised and the total sum was divided by the number of teeth giving a periodontal severity index (PDSI) for each individual. Based on the PDSI the subjects were stratified into 4 categories; no; minor; moderate; and severe periodontal disease.

In *study V*, severity of periodontal disease was calculated in almost the same way as in *paper II and IV*, with the exception that BOP and furcation involvement were not included at all. A possible value for bone loss (BL) from 0 to 3 was registered for each tooth: (0) = no BL; (1) = BL >2 mm ≤1/3 of the root length; (2) = BL >1/3 and ≤2/3 of the root length; and (3) = if the loss were > 2/3 of the root length or if it was of an angular type. The value for each tooth was summarised and divided by number of teeth giving a periodontal bone loss (PBL) score. Depending on the PBL the subjects were divided into 3 categories; no/minor; moderate; and severe bone loss.

**Number of diseased pockets**

In *study II, IV and V*, a periodontal pocket was regarded as being deepened or diseased if probing pocket depth exceeded 4 mm. The number of diseased pockets (NDP) was in *study II* used as continues variable, but in *study IV* it was stratified into four groups: 0, 1-15, 16-30, and >30 deepened pockets, and in *study V* into two groups; 0-4 pockets or >4 deepened pockets.

**Bleeding on probing**

Percentage of surfaces with BOP was in *study IV, V* as well as in the re-analysis of *study II* used as categorised variables. BOP was stratified into following groups in *study IV*: 0-19 %, 20-39 %, 40-60 %, and >60 %, and into the groups: 0-20 % and >20 % of the surfaces with bleeding on probing in *study II and V*.

**Number of teeth**

Number of teeth (NT) was also used as a categorised variable in *study II, IV and V*, but as a continuous variable in *study III*. In *study II and IV*, NT was
stratified into five groups: 0-9, 10-14, 15-19, 20-25, and >25 teeth, and in study V, into two groups: <21 and >20 teeth. NT was self-reported in study III, and 947 of the 1,016 answered the question about number of teeth.

**Laboratory analysis**

Laboratory analyses were used in study I, III and V. The GCFs fluids in study I were absorbed with the paper strips and eluted with 200 µl of sterile sodium chloride (NaCl) in combination with centrifugation and frozen at -70° C until analysed. Bone resorption was quantified by analyzing the percentage of $^{45}$Ca release from prelabelled bone5 dissected from mice that were injected at an age of 1-2 days with 1.5 µCi $^{45}$Ca. The IL-1α, IL-1β and IL-1ra concentrations in GCF eluates were assessed by using commercially available ELISA kits.

Lipid variables, leukocyte count, and fasting blood glucose were in study III measured by standard laboratory techniques and high sensitive CRP (HsCRP) was measured in human serum by an ultra sensitive particle enhanced immunoturbidimetric assay.

In study V, the plasma glucose level, fasting triglyceride levels, total cholesterol, high density lipoprotein (HDL), and HsCRP were analysed at the accredited laboratory for chemical analyses, Gävle County hospital. The levels of IL-6 and antibody titres against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were analysed using Enzyme-linked immunosorbent assay (ELISA).

**Statistical Analysis**

StatView 5.0 for windows was used in all studies for analysing data. In study I, Wilcoxon signed rank test was used to investigate differences of IL-1 levels and BRA in GCFs from diseased and healthy sites before and after treatment, with Bonferroni correction for multiple testing taken into account where appropriate. To analyse possible correlations, the nonparametric Spearman coefficient for ranked data was used.

A logistic regression analysis was used in study II to analyse if severity of periodontitis or number of remaining teeth were related to the prevalence of myocardial infarction and hypertension. Mann-Whitney test was applied to analyse how the number of diseased pockets related to the prevalence of myocardial infarction and hypertension. A multiple logistic regression analysis was then used to investigate the interaction of possible confounding variables such as age, gender and smoking.

Differences between groups in study III were evaluated with ANOVA and relationships between pairs of variables were evaluated by Pearson’s correla-
tion coefficient. A multiple regression analysis was applied to relate several independent variables to a dependent variable.

In study IV, a Cox proportional hazard analyses was performed to investigate any dose-dependent relationship between four oral health parameters and all-cause mortality, mortality in cardiovascular disease (CVD), in coronary heart disease (CHD) and in stroke. Adjustment was made for the confounders age, gender and smoking. Kaplan-Meier curves were used to visualise how the number of teeth was related to all-cause mortality and mortality in CVD.

Contingency tables and chi-square analysis were in study V used to compare proportions between nominal variables. Differences between normally distributed groups were evaluated with ANOVA and ANCOVA. A logistic regression analysis was applied to analyse associations between acute myocardial infarction and the oral health parameters with adjustment for major confounders. Finally, a logistic regression model was used to analyse if MI as dependent variable was related to the oral health parameters, and antibody levels against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, after age, gender and smoking were adjusted for. In all papers a two-tailed probability (P) ≤0.05 was regarded as statistically significant.
Results

Inflammatory response in periodontal disease (Study I)

GCF eluates harvested before treatment from sites with horizontal and vertical bone loss caused 1.81- and 1.96-fold stimulation, respectively, of $^{45}$Ca release. The stimulatory effect of eluates from the two diseased groups of pockets was significantly higher than eluates from healthy sites (P<0.001) but no difference was seen between the two diseased groups of sites. Although a majority of diseased sites showed a decrease in BRA activity after treatment (15 of 18 sites with horizontal bone loss and 13 of 17 sites with vertical bone loss), significant difference between pre- and post treatment values was only observed in sites with horizontal bone loss (p<0.01).

The levels of IL-1$\alpha$, IL-1$\beta$ and IL-1 receptor antagonist (IL-1ra) in GCFs were significantly higher from diseased sites before treatment compared to healthy ones (p<0.001 for all variables). Treatment decreased levels of IL-1$\alpha$ in GCF in all diseased sites (p<0.001) (figure 8). Also levels of IL-1$\beta$ in GCFs were significantly reduced (p<0.001 for sites with horizontal bone loss and p<0.01 for sites with vertical bone loss). Although the levels of IL-1ra in GCF were decreased in most diseased sites, significant reduction was only seen in those with horizontal bone loss (p<0.01). No difference in BRA and IL-1 levels before and after treatment was observed in GCFs from healthy sites.

With the aim to elucidate if BRA found in the GCFs due to the presence of molecules already known to stimulate osteoclastogenesis and bone resorption we analysed if there was any correlation between the amounts of IL-1$\alpha$, IL-1$\beta$ and IL-1ra and the degree of $^{45}$Ca release. In diseased sites with angular bone loss, BRA was correlated to IL-1$\alpha$ and IL-1$\beta$ before (p< 0.014, $r_s = 0.58$ and $p<0.0013$; $r_s=0.76$ respectively), and after treatment (p<0.016; $r_s=0.6$ and p< 0.008; $r_s=0.66$ respectively). However, there were no significant correlations between the individual changes in each site of BRA and the corresponding changes of IL-1$\alpha$, IL-1$\beta$, and IL-1ra.
Figure 8. The concentration of interleukin-1α (IL-1α) in gingival crevicular fluids from sites with healthy gingiva and from sites with horizontal or vertical bone loss before treatment (a). Values represent means±SEM. The IL-1α concentrations in the sites from the two diseased groups were significantly different from healthy sites (P<0.001). In (b) – (d) are shown the concentrations of IL-1α in gingival crevicular fluids before and 12 months after treatment. The decrease after treatment was statistically significant for both diseased sites (P<0.001).

Initially, mean probing pockets depth was deeper in pockets presenting angular bone loss compared to sites with horizontal loss; 8.1 ± 0.6 and 6.7 ± 0.3 mm, respectively. Treatment resulted in a significant decrease of 3.5 ± 0.5 mm (p<0.001) in sites with angular bone loss and 2.8 ± 0.3 mm (p<0.001) in sites with horizontal bone loss. There was a significant correlation between BRA and probing pocket depth (p<0.01, r_s=0.52) only in pre-treatment GCFs from sites with angular breakdown.
Association between oral health and cardiovascular disease (study II-V)

Number of teeth

Number of teeth (NT) was the only oral health parameter consistently related to CVD in this thesis. NT related to MI in a dose-dependent manner with an OR of 0.8 (p<0.03) after adjustment for age, gender and smoking (figure 9). NT was also related to HT in an unadjusted analysis, but this relationship did not remain significant after correction for confounders (study II).

Figure 9. Prevalence of myocardial infarction (MI) in relation to remaining teeth (p<0.0001 for trend).

The metabolic syndrome (MetS) was present in 23% of the subjects and they had significantly lower number of teeth compared to those without the syndrome after controlling for confounders (figure 10). Furthermore, NT was also related to the number of criteria included in the definition of MetS (study III).
Figure 10. Number of teeth in subjects with the metabolic syndrome (MetS, n=219) and in those without (n=728). Means and SEM are given.

NT was significantly associated also to all-cause mortality, mortality in CVD, and in CHD in a fairly dose-dependent manner after the adjustment for age, gender and smoking (figure 11) (for the group with 20-25 teeth $p=0.031$, $p=0.0015$ and $P=0.0099$, respectively, and for all the other strata of teeth with $p<0.0001$). The hazard ratio (HR) for mortality in coronary heart disease was 7 times higher for individuals with <10 teeth compared to those with >25 teeth (study IV).
Furthermore, in study V, we found that NT was independently related to MI (p=0.007) even after controlling for major confounders.

Severity of periodontal disease

Severity periodontal disease (SPD) was in study II found to be significantly related to myocardial infarction (MI) (figure 12) and to hypertension (HT) (figure 13) in a dose-dependent manner after adjustment for age, gender and smoking. However, the relationship to MI was significant only in the middle-age group (40-60 years), with an odds ratio (OR) of 2.7 (p<0.03) after the subjects were stratified into the age categories (<40, 40-60 and >60 years). Also in study V, using an unadjusted analysis of proportion, SPD was found to be related to MI in a dose-dependent manner, but the correlation disappeared after correction for confounders. SPD was associated to hypertension in the total sample with an OR 1.32 (p<0.005), but only in elderly subjects >60 years (p=0.0029) after stratification into above mentioned age groups (study II).

SPD related neither to all-cause mortality nor to mortality in any kind of cardiovascular disease (CVD) analysed in the study IV.
Figure 12. Prevalence of myocardial infarction (MI) in relation to periodontal disease (p<0.004 for trend)

Figure 13. Prevalence of hypertension (HT) in relation to periodontal disease (p<0.0001 for trend)

Number of periodontal pockets

Number of diseased pockets (NDP) was not related to MI in study II. However, in study V a relationship to MI was seen in subjects with >4 deepened pockets, even after adjustment for major confounders (p=0.0029).
In study II, subjects with HT had significantly more NDP than those without HT (in those with HT: median 15; range 0-89; in those without HT: median 11; range 0-89, p< 0.0001). Unfortunately, in the published article the opposite was stated (in those with HT: median 11; range 0-89; in those without HT: median 15; range 0-89). Another fault in the published abstract of study II is the statement that we have adjusted for the number of teeth, which was true only for the analyses of NDP in relation to MI and HT.

After stratification into the three age groups along with correction for age, gender, smoking and NT, a relationship was still present between NDP and HT in the two oldest age groups 40-60, and >60 years, (p<0.001 and p< 0.01, respectively).

Finally, no dose-dependent association between NDP and mortality in CVD was seen in study IV.

**Bleeding on probing**

Bleeding on probing (BOP) >20 % of the surfaces was found to be related to MI in study IV, even after major confounders were adjusted for (P=0.035). In study II, BOP in relation to MI and HT was not analysed before the article was published. After going back to the data sheet using the same logistic regression analysis, the same stratifications as in study V, with adjustment for age, gender and smoking, no association between BOP and MI was observed. However, when BOP was analysed against HT, BOP>20 % of the surfaces was associated to HT in the total sample, OR 1.6 (p=0.0001) and after age stratification, in subjects 40-60 years, OR 1.61 (p=0.0007), and >60 years, OR 1.54 (p=0.0017). As for NDP, no dose-dependent association between BOP and mortality was seen in study IV

**Oral health in relation to systemic markers of inflammation.**

Systemic markers of inflammation such as CRP, and ICAM-1, were in an unadjusted analysis significantly related to NT (p=0.0023 and p=0.0001, respectively). However, after including all parameters that were significantly related to NT, using a backward stepwise multiple regression analysis, only the leukocyte levels remained significantly related to NT (p=0.043) (Study III).

IgG antibody levels against *Porphyromonas gingivalis* (Pg IgG) was associated to MI (p=0.036) and to the oral health parameters, >4 deepened pockets (p=0.042), BOP >20% (P=0.001), and periodontal bone loss (P=0.0005), but not to NT after adjustment for age, gender and smoking.
However, when the oral parameters together with \( Pg \) IgG as covariates were included in a logistic analysis with MI as dependent variable, the relation between \( Pg \) IgG and MI disappeared (\( p=0.55 \)) (table 1). (study V)

Table 1. Logistic regression model with myocardial infarction as dependent variable versus antibody level against *porphyromonas gingivalis* (\( Pg \).IgG) adjusted for age, gender smoking and oral parameters (in the second model only). OR= Odds ratio, BOP= Bleeding on probing.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Adjusted for age, gender and smoking</th>
<th>Adjusted for age, gender, smoking and oral parameters</th>
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<tbody>
<tr>
<td></td>
<td>OR</td>
<td>p-value</td>
</tr>
<tr>
<td>( Pg ) IgG</td>
<td>1.57</td>
<td>0.033</td>
</tr>
<tr>
<td>Age</td>
<td>0.97</td>
<td>0.30</td>
</tr>
<tr>
<td>Gender</td>
<td>1.24</td>
<td>0.58</td>
</tr>
<tr>
<td>Smoking</td>
<td>4.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of teeth &lt;21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moderate periodontal bone loss</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Severe periodontal bone loss</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&gt;4 pockets &gt;4mm deep</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BOP on &gt;20% of the surfaces</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
General Discussion

Over a long period of time the oral cavity has been treated as a separate entity with no, or only marginal, influence on other tissues and organs in the body. During the two last decades however, there has been a mounting interest for oral health as a possible etiological factor for systemic conditions such as cardiovascular disease, diabetes, rheumatoid arthritis, preterm birth weight and respiratory diseases. Although reports have shown an association between oral health and CVD, the biological mechanisms responsible for the relationship remain unravelled. The aims of this thesis were to study oral inflammation and the relationship between oral health parameters and cardiovascular diseases.

Inflammatory response in periodontal disease (study I)

That BRA as well as IL-1 were enhanced in GCF from sites with periodontal disease, compared to healthy ones, is in agreement with earlier findings from our group. In the present study we investigated how periodontal treatment affects BRA and the levels of IL-1 and IL-ra. We could show that both BRA and levels of IL-1 was reduced 12 months after treatment, but that the reduction of BRA was significant only in sites with horizontal bone loss. Why BRA was not significantly reduced in sites with angular bone loss could be due to large standard deviations in combination with a too small sample size or, alternatively due to regression of the disease process.

The opinion that the periodontitis is more aggressive in sites displaying angular bone loss compared to those with horizontal loss is not supported by our findings. There was no difference in BRA or in cytokine levels when GCFs from sites with both types of bone loss were compared. Maybe the type of bone loss is more a consequence of the bone anatomy in the area than the degree of inflammation and bone loss in areas where the tooth is surrounded by thick bone will therefore be of an angular type while bone loss in diseased sites surrounded with thin bone are more likely to be horizontal.

It has previously been demonstrated that molecules causing the BRA in GCFs from patients with periodontitis has a molecular weight of 3-30 kDa, is temperature sensitive, is not due to LPS contamination and is not caused by prostaglandin E2. These observations support the view that the BRA is caused by an osteotropic cytokine. Using GCFs pooled from several patients,
antiserum specifically neutralizing IL-1β had no or only marginal effect on BRA whereas anti-IL-1α had a more profound, but still in some cases only a partial inhibitory effect. These data indicate that IL-1α, but not IL-1β, contribute to but is not fully responsible for the BRA in GCFs from parodontitis patients. In the present study, we evaluated the importance of IL-1α, IL-1β and IL-1ra by investigating, if any correlation could be found between cytokine levels and degree of BRA in GCFs from individual sites. Although BRA was correlated to IL-1α and IL-1β before as well as after treatment, there were no significant correlations between the individual changes of BRA and corresponding changes of IL-1α, IL-1β and IL-ra. These data also argue for the view that IL-1 is important but not the sole stimulant of bone resorption in patients with periodontal disease.

Although probing pocket depth was significantly reduced after treatment, the reduction was not correlated to the BRA or to IL-1 levels. An explanation could be that the BRA and IL-1 levels in GCF only reflect the degree of the inflammation at the moment when the GCF was harvested. These levels could rapidly change depending on the degree of inflammation in the surrounding tissues. Pocket depth, on the other hand, needs a longer period of time to be altered as it involves either degradation or regeneration of tooth supporting tissues. As periodontal disease does not progress continuously, but in periods with burst of activity, it is likely that levels of BRA stimulating cytokines such as IL-1 could be substantially altered without any noticeable change in pocket depth. Another explanation for the discrepancy which is supported by the findings in paper I, is that other molecules besides IL-1 are involved in the inflammatory process as no correlations were seen between the individual changes in each site of BRA and the corresponding changes of IL-1α, IL-1β, and IL-1ra.

Bone resorption is not only associated with the inflammatory process in periodontal disease but is also associated with inflammatory process in joint synovial tissues in patients with RA, osteoarthritis (OA) or loosening joint prosthesis. Enhanced levels of osteotropic cytokines have similar to GCFs, been found in synovial fluid from these patient groups. Attempts have been made to block BRA in synovial fluids from OA patients and from patients with loosened joint prosthesis by using antisera neutralizing IL-1α, IL-1β, TNF-α, IL-6 or IL-17 but such antisera have only occasionally displayed an inhibitory effect. These data show that neither in synovial fluid is IL-1 the sole stimulator of bone resorption. Ongoing studies will reveal if BRA in these two inflammatory exudates is due to the presence of known cytokines affecting osteoclast formation, or if this activity is also due to previously unknown osteotropic cytokines.

Bone resorption in mouse calvariae, induced by synovial fluids from patients with OA or loosened joint prosthesis, is associated with enhanced RANKL expression. In preliminary studies, we have observed that bone
resorption induced by GCF is also associated with a substantially increased mRNA expression of RANKL (Holmlund & Lerner, ongoing studies).

**Association between oral health and cardiovascular disease (Study II-V)**

**Number of teeth (NT)**

In *paper II*, NT related to MI in a dose-dependent manner, but not to HT. In addition, in *paper V* the group with NT <21 was associated to MI, even after adjustment for major confounders. These findings are in accordance with earlier reports. 134, 139, 142, 144, 146, 160, 166

The metabolic syndrome (MetS), a syndrome constituted of a number of cardiovascular risk factors, was an independent predictor for NT (*paper III*). To our knowledge this is the first study to report a significant relationship between the MetS and NT. Subjects with the MetS had an average of three teeth less than those without the syndrome, indicating that oral health also relates to a combination of cardiovascular risk factors, which is in line with the findings from another recent study. 178

NT was also related to all-cause mortality, mortality in CVD, and mortality in coronary heart disease (CHD) in a dose dependent manner, but not to mortality due to stroke. A subject with <10 teeth had 7-times higher risk to for mortality in CHD than a subject with >25 teeth (*paper IV*). NT has previously been associated with mortality in CVD 166, 168, 169 but a dose dependent relationship has never been reported before. As the majority of teeth are removed due to some kind of oral infection, NT could be regarded as a reflection for the cumulated amount of oral inflammation which could have influenced the progression of atherosclerosis over time. 139 This might be an explanation as to why NT predicts mortality in CVD.

Although Joshipura has reported a relationship between NT and stroke, no such relationship was observed in *paper IV*. 165 The relatively few subjects that died due to stroke could be an explanation why no association to mortality in stroke was observed in this paper.

In this thesis, NT was the only oral health parameter that consistently associated to CVD, suggestig that NT, at least for the time being, might be the best oral health parameter to use when associations between oral health and CVD are investigated.

**Severity of periodontal disease (SPD)**

In *papers II, IV* and *V* we investigated if there was a dos-dependent relationship between SPD and cardiovascular disease (CVD). SPD was dose-
dependently related to both the prevalence of self-reported myocardial infarction (MI) in the middle-aged group (40-59) and to hypertension (HT) in the total sample after adjustment for age, gender and smoking (paper II). This is in agreement with other studies, however, only a few of them have presented a dose-dependent relationship. Furthermore, the association between oral health and HT apart from paper II, has only sparsely been investigated.

In contrast to the findings in paper II, no relationship between SPD and MI was observed in paper V after the correction for confounders. An explanation for this could be that the sample size in study V was too small to disclose any dose-dependent relationship. Neither could any relationship between SPD and mortality in CVD be observed in study IV. However, other studies have also failed to find any association between periodontal disease and CVD. The inconsistency of the association indicates that there are other factors influencing the relationship. For instance, the use in different studies of different confounders such as age, gender, smoking, cholesterol, diabetes, obesity, hypertension and socio-economic factors probably contributes to this inconsistency. Other factors that also might influence the variation of the relationship between periodontal disease and CVD are the use of different oral parameters and different threshold values to define the presence of periodontal disease. Usually, we have no knowledge about the periodontal conditions around teeth that already have been removed, but we can assume that a major part of them had worse periodontal conditions than the remaining teeth. Obviously, there is a substantial risk that severity of periodontal disease could be underestimated in subjects where teeth have been removed prior to their oral examination in the studies, which also might affect the above mentioned relationship. It would therefore be desirable if future investigations concerning the relationship between periodontal disease and CVD had a more consistent way of defining the presence of periodontal disease and in some way also included the number of missing teeth into the estimation of the severity of the disease.

Number of deepened pockets (NDP)

In paper II, NDP was associated to hypertension, but not to the prevalence of MI. However, in paper V there was an association between NDP and MI in subjects with >4 deepened pockets. The discrepancy in the relationship between NDP and MI in paper II and V could to some extent be due to an underestimation of the prevalence of MI in paper II as it was self-reported. A difference concerning the time sequence between the incidence of MI and the oral investigation in the above mentioned studies might also have influenced the relationship. Although other studies have shown that NDP is related to CVD, so far no study has reported an association between NDP and mortality in CVD. This is in agreement with the result in
paper IV, where no dose-dependent association was seen between NDP and mortality in CVD.

It seems that NDP, being an indicator of ongoing periodontal disease is more related to different expressions of atherosclerosis such as MI and HT than to the accumulated result of the process, here represented by mortality in CVD.

Bleedings on probing (BOP)

BOP could also be regarded as an indicator of ongoing periodontal inflammation and, as was observed for NDP, a significant relationship between BOP and MI was seen in subjects with BOP >20% of the surfaces, even after the correction for major confounders (paper V).

Paper II, did not include an analysis of a possible association between BOP and MI or HT. After reanalysing the data in paper II, the same pattern that was seen for NDP regarding the relationship to CVD appeared, namely that BOP related to HT, but not to MI. The same reasons that were given for the diversity regarding the relationship between NDP and MI in paper II and V could also provide an explanation as to why BOP related differently to MI in these studies.

Similar to NDP has BOP previously been related to CVD 127, 137, 180 and in accordance with what was seen for NDP, no dose-dependent relationship between BOP and mortality in CVD could be observed in paper IV. So far there is no report in the literature of a dose-dependent relationship between BOP and mortality in CVD.

NDP and BOP are indicators of ongoing oral inflammation which might influence the degree of inflammation in the atherosclerotic plaque, promoting fibrous cap rupture and the acute infarction, explaining why NDP and BOP were associated to MI. However, in contrast to NT, both NDP and BOP are easily affected by periodontal treatment and therefore do not reflect the past exposure of oral inflammation. This might explain why NDP and BOP were not related to mortality in CVD.

Oral health in relation to systemic markers of inflammation.

The use of clinical parameters to investigate possible associations between oral health and CVD has been criticised because it does not include any systemic effect evoked by impaired oral health. It was only in paper III and V that we had the opportunity to study systemic effects related to impaired oral health.
Higher levels of leukocytes in subjects with periodontitis compared to healthy subjects have earlier been reported. In line with these results the levels of leukocytes in paper III, was an independent predictor for number of teeth, further emphasizing that oral health might induce a systemic low-grade inflammation.

In agreement with earlier studies, the IgG antibody levels against Porphyromonas gingivalis (Pg IgG), a bacteria considered to be a risk factor for periodontal disease, were related to MI (paper V). Also in line with other reports, the Pg IgG levels in paper V were associated to the oral health parameters. However, when MI as a dependent variable together with the oral health parameters and Pg IgG as covariates were included in a logistic regression analyses, the association disappeared between Pg IgG and MI, indicating that Pg could be a link between oral health and CVD. This is to our knowledge the first case-control study that explores how Pg relates to oral health parameters as well as to MI.

P. gingivalis have a number of qualities that could influence the atherosclerotic process such as the ability to induce the coagulation cascade, to convert Big-endothelin to endothelin, to induce cross-reactivity to human heat shock protein 60 and to invade endothelial and epithelial cells. Viable Pg have also been harvested from atherosclerotic plaque and the progression of atherosclerosis was accelerated when APO-E deficient mice were infected with Pg, further indicating that Pg could influence the atherosclerotic process.

P. gingivalis, being a risk factor for periodontal disease along with its ability to influence the atherosclerotic process, could be a possible biological link between oral health and CVD.

Limitations

The sample size in paper I might have been too small to disclose significant correlations as the standard deviations were rather large.

In paper II, a cross-sectional study was performed in a retrospective manner and suffers therefore from inherent limitations. No causality in the relationships can be concluded from this type of study. Furthermore, the prevalence for MI and hypertension was probably underestimated as it was self-reported. Adjustments for possible confounders in this study were limited to age, gender and smoking and because smoking habits were only registered as current or non-smokers, adjustment for all of the possible effects of smoking could not done.

In paper IV, the time period for inclusion of subjects was very long, with only one follow-up examination and the adjustment for smoking habits and confounders suffered from the same limitations as in study II.
**Paper III**, a cross-sectional study where the number of teeth was self-reported. Although there might be a lack of confidence in self-reported number of teeth, studies have shown that the self-estimation of this oral parameter is rather good.\(^{180}\) Other limitations in this study were the rather low participant rate (50.1%) and that data regarding number of teeth in non-participants was not available. However, this would probably only cause an underestimation of any potential relationship investigated in this study. As the study cohort consisted only of elderly subjects, the results might not be valid for other age groups.

**Paper V**, with a case-control design, is only hypothesis generating. A limitation concerning this study is that the prevalence of hypertension in the MI group may have been somewhat underestimated as only hypertensive medication prior to the infarction was used as a definition for hypertension. However, hypertension showed an odd-ratio for MI in this paper, which is similar to what is generally seen in other studies. Another limitation in this study is the sample size, which could have been too small to disclose significant relationships. Furthermore, as we lacked data on number of years the subjects had been smoking, correction for pack years could not be performed.

Finally, as the population samples in *paper I-V* mainly consist of subjects with Caucasian heritage, cautions should be taken when drawing conclusions for other ethnic groups.
Future perspectives

Although this thesis using different study designs, presents a consistent relationship between oral health and cardiovascular disease, no conclusions regarding the causality of the relationship can be drawn. More studies are needed to clarify the inflammatory response in periodontal disease and by which biological mechanisms oral health could influence the atherosclerotic process and other systemic conditions.

It seems from the results of our studies that different oral health parameters such as severity of periodontitis, number of teeth, number of deepened pockets and bleeding on probing could be related to different stages of the atherosclerotic process, however, this needs further investigation.

The use of different oral health parameters and threshold levels to define when periodontal disease is present as well as grading of the severity of periodontitis are other matters that need more attention. In order to avoid underestimation, we need to somehow involve the missing teeth in the estimate of disease severity.

To finally prove the causality of the relationship, prospective treatment studies with a randomised controlled design must show that treatment of periodontal disease could lower the prevalence of cardiovascular disease. Since these studies are time-consuming and expensive to perform, it is not likely that we will see, at least in the near future, any final evidence of the causality in the relationship.

What we can state today with rather convincing scientific evidence is that there exits an association between oral health and cardiovascular disease. As there are a number of mutual risk factors influencing both oral health and cardiovascular disease, there is a possibility that the relationship only exits because individuals prone to develop periodontal disease also are more disposed for CVD. Even though oral health might not be a risk factor, it could still be an easily obtained risk indicator for CVD and as such oral health can be an important tool in the prevention of cardiovascular disease.

In Sweden at least most people go for a dental examination on a rather regular basis. In contrast to the dental visits, most of the individuals contacts with regular health service are limited to the times when they need treatment for a serious illness.

Regardless of the reasons for the relationship between oral health and CVD or if causality does or does not exist, the results from this thesis and from other studies indicate that oral health is a risk indicator for CVD. Maybe, in a not to a distant future, individuals with severely impaired oral health will be considered for a general health examination.
Conclusions

- Diseased periodontal pockets exhibit more bone resorption activity (BRA) and higher levels of interleukin-1 (IL-1) than healthy ones. Treatment significantly reduced levels of IL-1 in diseased sites and BRA in sites with horizontal bone loss. However, the individual changes of BRA and IL-1 before and after treatment in each site were not correlated, indicating that other molecules besides IL-1 are involved in the stimulation of BRA.

- Oral health was related to myocardial infarction, hypertension, the metabolic syndrome, all-cause mortality, and to mortality in cardiovascular disease and in coronary heart disease, but not to death caused by stroke.

- The number of teeth was the only oral health parameter that consistently correlated to cardiovascular disease.

- Antibody levels against the periodontal pathogen *Porphyromonas gingivalis*, as an indication of a systemic response due to periodontal disease, was associated both to acute myocardial infarction and to the oral health parameters, suggesting the possibility that this bacteria might be a link between oral health and cardiovascular disease.
I wish to express my deepest gratitude to everyone who has contributed to thesis in different ways.
My special thanks go to

Professor Lars Lind, my principal supervisor, co-author, dear friend and tennis opponent, for guiding me into the scientific world with never lasting patience. Thanks for your inspiring enthusiasm for science, your support and for always finding time for discussions and help. Without your scientific mind, your vast knowledge of cardiovascular research together with your pedagogical skills this thesis would not have been possible. For the future, I sincerely hope, we will continue our scientific collaboration and also the meetings at our summer houses with interesting discussions, good food and good wine.

Professor Ulf. H Lerner, my co-supervisor and co-author, for helping me with the analyses of bone resorbing activity and cytokines, for all rewarding discussions regarding my scientific work and for sharing your extensive knowledge and true passion for science and inflammation. You can really make bone a living and fascinating tissue. I have enjoyed our sessions at the University of Umeå and hope that our collaboration can continue in the future.

Gunnar Holm, my co-author and former colleague, for inspiring me to become a specialist in periodontology, for all stimulating discussions and for being so far-sighted in the importance of registering data regarding oral and general health into a database. Two of the articles is thesis had not been possible without your excellent work.

Professor Lennart Hänström, co-author and former colleague, for all inspiring discussion and lectures in the field of periodontology.

Associated professor Johannes Hulte, co-author and for analysing HsCRP

Professor Pirkko Pussinen, co-author and for analysing antibodies against P gingivalis and A acinomycetemcomitans.
Associated Professor Måns Hedin, co-author and for measuring periodontal bone loss on radiographs.

Professor Marianne Högmann and her eminent staff at FoU-forum County Council of Gävleborg, for all encouragement during the ups and downs of my scientific work and for financial support. Inga-Lill Stenlund for always being service-minded and helpful, Lawrence Teeland for all valuable comments regarding the English language and finally, Hans Högberg for taking time to discuss and shear your great knowledge in statistics. You have all meant a lot to my

The department of cardiology, Gävle County Hospital, for helping me to recruit patients to the case control study.

Dental nurses Kerstin Larsson and Christina Nilsson at the department for specialist dentistry at Gävle County Hospital, for your engagement in my research work, for helping me out with the clinical investigations and for straighten out all problems that I have created by rescheduling appointments.

Laboratory assistant Kerstin Marttala at Uppsala University and Karin Hagman at Gävle County Hospital, for handling blood samples, samples with gingival crevicular fluid, sorting and arranging transportations of the samples.

Hartmut Feldmann, head of the department for specialist dentistry at Gävle County hospital and Folktandvården AB Gävleborg for helping with support and founding of this thesis.

My colleagues and friends at the clinic for specialist dentistry at Gävle County Hospital, for all support and encouragement and a special thanks to my dear colleague Catrine Isehed for taking an extended work load during my absence.

To all my friends and relatives for being there for me, you are all important in my life.

Finally, to the most precious persons in my life.

My beloved wife Annika for your endless love and support. Thank you for your patience and for shearing lives ups and downs with me. You have also given me the two most precious things in my life, my daughters Johanna and Maria. Despite my shortcomings as a father, you are always there for me with your love and support. My son in-law Johan for being like a son to me, for making the cover and some figures of this theses and for being partly
responsible for a new fantastic experience in my life, to become grandfather to Ellen.
References


71. Saito T, Shimazaki Y, Kiyohara Y, Kato I, Kubo M, Iida M, Yamashita Y. Relationship between obesity, glucose tolerance,


83. Frohlich M, Sund M, Lowel H, Imhof A, Hoffmeister A, Koenig W. Independent association of various smoking characteristics with markers of systemic inflammation in men. Re-


126. Geerts SO, Legrand V, Charpentier J, Albert A, Rompen EH. Further evidence of the association between periodontal condi-


147. Lee HJ, Garcia RI, Janket SJ, Jones JA, Mascarenhas AK, Scott TE, Nunn ME. The association between cumulative


178. Shimazaki Y, Saito T, Yonemoto K, Kiyohara Y, Iida M, Yamashita Y. Relationship of metabolic syndrome to periodontal


Acta Universitatis Upsaliensis

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Editor: The Dean of the Faculty of Medicine

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