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PERIODONTAL INFECTION
AND OBESITY—
RESULTS OF A POPULATION-
BASED SURVEY

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**PERIODONTAL INFECTION AND
OBESITY—RESULTS OF
A POPULATION-BASED SURVEY**

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Abstract

The aim of this study was to investigate the nature of the association between obesity and periodontal infection and the association of statin medication with periodontal infection.

This study was based on the nationally representative Health 2000 Survey, conducted by the National Institute for Health and Welfare (former National Public Health Institute of Finland) in 2000–2001. Article I included 396 dentate, non-diabetic subjects, aged 30–59 years, who had never smoked and who participated in the Follow-up Study on Finnish Adults' Oral Health about four years later. Article II included 2,784 dentate, non-diabetic subjects, aged 30–49 years. Article III included 425 dentate, non-diabetic, non-rheumatic subjects, aged 45–64 years, who had never smoked and who participated in the in-depth examinations of the Health 2000 Survey. Article IV included 1,297 dentate, non-diabetic subjects, aged 30–49 years, who had never smoked. Article V included 2,032 dentate, non-diabetic, non-rheumatic subjects, aged 40–69 years, who did not smoke. The data used in this study were collected via home-visit interviews, self-administered questionnaires, clinical health examinations and laboratory measurements.

In this general population of Finnish adults, high BMI was found to be associated with the incidence of new teeth with pathologically deepened periodontal pockets during a four-year follow-up. On the other hand, the presence of teeth with deepened periodontal pockets was found to be associated with obesity in an exposure-response manner. Serum IL-6 levels were found to be associated with the number of teeth with deepened periodontal pockets, but no consistent association was found between serum TNF- α , triglyceride, HDL-C or LDL-C levels and periodontal infection. Statin medication was found to be inversely associated with the number of teeth with deepened periodontal pockets among subjects with visible signs of gingival inflammation, whereas among subjects with no signs of inflammation, statin medication was associated with an increased likelihood of having periodontal infection.

The results of this study support the view that obesity could be causally related to the development of periodontal infection, but does not provide evidence that high body weight could be considered a major risk factor. The present study also suggests that a bi-directional association between obesity and periodontal infection is possible. The present study suggests that elevated serum IL-6 could mediate the association of obesity with periodontal infection. The results of this study also suggest that statins could be beneficial as a part of periodontal treatment.

Keywords: body mass index, cytokines, dyslipidaemia, obesity, periodontal infection, statins

Saxlin, Tuomas, Parodontaali-infektio ja lihavuus – tuloksia väestöpohjaisesta tutkimuksesta.

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Tiivistelmä

Tämän tutkimuksen tarkoituksena oli selvittää lihavuuden ja parodontaali-infektion välisen yhteyden luonnetta sekä statiinien käytön yhteyttä parodontaali-infektioon.

Tutkimus perustui kansalliseen Terveys 2000 -tutkimukseen, jonka toteutti Terveiden ja hyvinvoinnin laitos (entinen Kansanterveyslaitos) vuosina 2000 ja 2001. Artikkelit I perustui 396 hampaalliseen henkilöön, jotka olivat ei-diabeetikkoja, 30–59-vuotiaita, eivät koskaan olleet tupakoineet sekä olivat osallistuneet suunterveyden seurantaan neljä vuotta myöhemmin. Artikkelit II perustui 2784 hampaalliseen henkilöön, jotka olivat ei-diabeetikkoja, 30–49-vuotiaita eivätkä olleet koskaan tupakoineet. Artikkelit III perustui 425 hampaalliseen henkilöön, joilla ei ollut diabetesta tai reumaa, olivat 45–64-vuotiaita, eivät koskaan olleet tupakoineet ja olivat osallistuneet Terveys 2000 -tutkimuksen täydentäviin tutkimuksiin. Artikkelit IV perustui 1297 hampaalliseen henkilöön, jotka olivat ei-diabeetikkoja, 30–49-vuotiaita eivätkä olleet koskaan tupakoineet. Artikkelit V perustui 2032 hampaalliseen henkilöön, jotka olivat ei-diabeetikkoja, ei-reumaattikkoja, 40–69-vuotiaita, jotka olivat hampaallisia eivätkä tupakoineet. Tutkimuksen aineisto kerättiin kotihaastattelusta, kyselyistä, kliinisestä tutkimuksesta sekä laboratoriomittauksista.

Korkean painoindeksin todettiin olevan yhteydessä uusien ientaskuhampaiden ilmaantumiseen seurannan aikana. Toisaalta ientaskuhampaiden esiintymisen todettiin olevan yhteydessä lihavuuteen altistus-vastesuhteen mukaisesti. Seerumin IL-6 pitoisuuden todettiin olevan yhteydessä ientaskuhampaiden lukumäärään, mutta seerumin TNF- α -, triglyseridi-, LDL-kolesteroli- tai HDL-kolesterolipitoisuudella ei todettu yhteyttä ientaskuhampaiden lukumäärään. Statiinien käytön todettiin olevan käänteisesti yhteydessä ientaskuhampaiden lukumäärään henkilöillä, joilla oli näkyviä merkkejä ikenen inflammaatiosta. Henkilöillä, joilla ei ollut näkyviä merkkejä inflammaatiosta, statiinien käyttö oli yhteydessä suurentuneeseen todennäköisyyteen ientaskuhampaiden esiintymiseen.

Tämän tutkimuksen tulokset tukevat käsitystä, että lihavuus voi olla kausaalisesti yhteydessä parodontaali-infektion kehittymiseen, mutta ei puolla käsitystä, että sitä voitaisiin pitää merkittävänä riskitekijänä. On myös mahdollista, että lihavuuden ja parodontaali-infektion välillä on kaksisuuntainen yhteys. Tämän tutkimuksen tulosten mukaan on mahdollista, että kohonnut seerumin IL-6 pitoisuus voi välittää lihavuuden yhteyden parodontaali-infektioon. Tutkimuksen tulosten mukaan on myös mahdollista, että statiineista voi olla hyötyä osana parodontaalihoitoa.

Asiasanat: dyslipidemia, lihavuus, painoindeksi, parodontaali-infektio, statiinit, sytokiinit

To my family

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Oulu, October 2012

Tuomas Saxlin

Abbreviations

AL	attachment loss
BF%	body fat percentage
BIA	bioelectrical impedance analysis
BMI	body mass index
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular disease
GCF	gingival crevicular fluid
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
ICAM	intercellular adhesion molecule
IL	interleukin
INF	interferon
IRR	incidence rate ratio
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LPS	lipopolysaccharide
MCP	monocyte chemoattractant protein
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
NHANES	National Health and Nutrition Examination Survey
OR	odds ratio
PAI	plasminogen activator inhibitor
PAMP	pathogen-associated molecular pattern
PMN	polymorphonuclear neutrophil
RR	relative risk
TIMP	tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNF	tumour necrosis factor
VLDL	very-low-density lipoprotein cholesterol
WAT	white adipose tissue
WC	waist circumference
WHO	World Health Organization

List of original articles

This thesis is based on the following original publications, which are referred to in the text by Roman numerals:

- I Saxlin T, Ylöstalo P, Suominen-Taipale L, Aromaa A & Knuuttila M (2010) Overweight and obesity weakly predict the development of periodontal infection. *J Clin Periodontol* 37: 1059–1067.
- II Saxlin T, Ylöstalo P, Suominen-Taipale L, Männistö S & Knuuttila M (2011) Association between periodontal infection and obesity: results of the Health 2000 Survey. *J Clin Periodontol* 38: 236–242.
- III Saxlin T, Suominen-Taipale L, Leiviskä J, Jula A, Knuuttila M & Ylöstalo P (2009) Role of serum cytokines tumour necrosis factor- α and interleukin-6 in the association between body weight and periodontal infection. *J Clin Periodontol* 36: 100–105.
- IV Saxlin T, Suominen-Taipale L, Kattainen A, Marniemi J, Knuuttila M & Ylöstalo P (2008) Association between serum lipid levels and periodontal infection. *J Clin Periodontol* 35: 1040–1047.
- V Saxlin T, Suominen-Taipale L, Knuuttila M, Alha P & Ylöstalo P (2009) Dual effect of statin medication on periodontium. *J Clin Periodontol* 36: 997–1003.

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1 Introduction

Obesity is defined as a condition in which excess body fat has accumulated to such an extent that health and well-being may be adversely affected, and an obese subject is commonly defined as having a body mass index of 30.0 or more (WHO 2000). Excess body weight is considered to be one of the leading risk factors contributing to the overall worldwide disease burden and mortality (Ezzati *et al.* 2002). Obesity is associated with the development and increased incidence of several diseases, such as type II diabetes, respiratory complications and cardiovascular diseases (Kopelman 2000).

According to World Health Organization (WHO) estimates, the worldwide prevalence of obesity has more than doubled since 1980, and in 2008 more than 1.4 billion adults aged 20 years or older were overweight, out of which over 200 million men and nearly 300 million women were obese (WHO 2012). In Finland, more than 65 per cent of men and 50 per cent of women aged 25–74 years were estimated to be overweight and about 20 per cent of men and women in this age group were estimated to be obese in 2007 (Peltonen *et al.* 2008).

Periodontitis is an inflammatory disease of the tooth supporting structures; it is initiated by colonisation of periodontal tissues with harmful, mainly anaerobic, gram-negative bacteria. Activation of immunological defence mechanisms, both innate and adaptive immunity, is an integral part of the pathogenesis of periodontitis. (Pihlstrom *et al.* 2005) Risk factors for periodontitis include, among others, cigarette smoking and diabetes mellitus (Borrell & Papapanou 2005). Periodontitis is also a fairly common disease. For instance, according to the results of the third National Health and Nutrition Examination Survey (NHANES III), it has been estimated that, in the United States, approximately 35 per cent of the dentate population aged 30 years or older have periodontitis (Albandar *et al.* 1999). In Finland, according to the results of the Health 2000 Survey, periodontitis was found to be the most common oral disease among the adult population (Knuutila & Suominen-Taipale 2008).

Obesity has been characterised by a state of low-level systemic inflammation, and might thus represent a condition which is capable of influencing the onset and progression of periodontal infection. The association of obesity with periodontal infection has been investigated in several, mainly cross-sectional studies (reviewed by Chaffee & Weston 2010 and Suvan *et al.* 2011), and several possible biological explanations for this association have been suggested (Boesing *et al.* 2009, Pischon *et al.* 2007, Saito & Shimazaki 2007). However, the exact

mechanisms by which obesity may be connected to periodontal infection are still not fully understood.

Statins—inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme—have been used to prevent cardiac events in patients with high serum cholesterol levels. The use of statins has increased during the last decades, and it has been estimated that in 2010 more than 510,000 inhabitants (9.5 per cent of the population) in Finland used statins daily (Kalliokoski *et al.* 2011). Besides the well-known lipid-lowering effect, statins have also been suggested to have other effects, such as anti-inflammatory effects (Jain & Ridker 2005) and effects on bone metabolism (Horiuchi & Maeda 2006). Through these properties, statins may also have an effect on the periodontium. However, knowledge about the possible role of statin medication in periodontal tissues is scant.

This study aimed to provide evidence of the nature of the association between obesity and periodontal infection, including temporal relations of the two conditions. The possible mediating role of serum proinflammatory cytokines as well as hyperlipidaemic state in this association was investigated. The association of statin medication with periodontal infection was also investigated.

2 Review of the literature

2.1 Obesity

2.1.1 Definition, measures and classifications of obesity

Obesity is a condition of abnormal or excessive fat accumulation that represents a risk to general health. The basic underlying cause for the development of obesity is a long-term, undesirable positive imbalance between energy intake and consumption, which is affected by the interaction between genetic, environmental and psychosocial factors (Kopelman 2000). Besides the amount of adipose tissue, also the distribution of the tissue within the body is important. The adverse health consequences of obesity are related to excess fat accumulation in the intra-abdominal depots, ‘abdominal obesity’, compared with the less serious ‘gynoid obesity’, in which the distribution of fat tissue is more even and peripheral around the body (WHO 2000). Especially accumulation of adipose tissue in so-called ectopic fat depots, such as visceral adipose tissue, intrahepatic fat and pericardial fat, has been suggested to be associated with harmful effects on health (Britton & Fox 2011).

As mentioned earlier, body weight is determined primarily by the balance between energy (calorie) intake and expenditure (physical activity); to maintain weight, energy intake should equal expenditure (Palacios *et al.* 2009). Besides the amount of food ingested, also the quality of the diet has an important role in weight gain and the associated morbidities. For instance, diets rich in refined sugars, such as sugar-sweetened beverages, are associated with the risk of obesity (Malik *et al.* 2006), and also with the increased risk of type II diabetes mellitus (de Koning *et al.* 2011). On the other hand, foods with a low glycemic index—such as whole grain products, fruits and vegetables, which are low in calories but rich in dietary fibers, minerals and vitamins—are associated with lower body weight (Livesey *et al.* 2008) as well as a reduced risk of chronic diseases, such as type II diabetes and coronary heart disease (Barclay *et al.* 2008). Dietary fibers have also been suggested to possess anti-inflammatory properties (Ma *et al.* 2008).

Also high-fat diets have been suggested to be associated with obesity (Bray & Popkin 1998, Schrauwen & Westerterp 2000). However, as with carbohydrates, the quality of fat ingested is also equally or even more important than the total amount of fat consumption. For instance, replacing trans and saturated fats with

unsaturated fats has been suggested to be associated with a decreased risk of coronary heart disease (Hu *et al.* 2001).

Overweight and obesity can be measured in several ways, including body mass index (BMI), body fat percentage (BF%) and waist circumference (WC). Probably the most commonly used method of classifying underweight, overweight and obesity is BMI, which is a measure of weight in relation to height: weight/height squared (kg/m^2). BMI can be considered to provide the most useful population-level measure of overweight and obesity (WHO 2000). According to the WHO (2000), underweight among adults is defined as BMI being less than 18.5. Normal weight is defined as a BMI of 18.5–24.9, and overweight means BMI is 25.0 or more. Overweight is further classified into four subcategories: preobese state (BMI 25.0–29.9), obese class I (BMI 30.0–34.9), obese class II (BMI 35.0–39.9) and obese class III (BMI \geq 40.0). In general, obesity is defined as a BMI of 30.0 or more. (WHO 2000)

BF%, the proportion of adipose tissue in total body weight, can be determined in many ways. In field conditions, the methods commonly used to predict BF% are skinfold thickness measurements and bioelectrical impedance analysis (BIA). Prediction of BF% using BIA is based on the principle that electric current flows at different rates through the body depending upon its composition; water component of body is saturated with ions, through which an electric current can flow, whereas body fat resists the flow of electric current (Dehghan & Merchant 2008). In BIA a small alternating current is applied to the body, usually from foot to hand (total body impedance), although impedance analysers that measure the impedance of the extremities (foot to foot, hand to hand) or analysers that measure both total body impedance and the impedance of the extremities are also used (Deurenberg & Yap 1999). A limitation of bioelectrical impedance in measuring BF% is that a prediction error may be associated with the length of the arms and legs, which is supported by the finding that BF% determined from bioelectrical impedance was underestimated among subjects with shorter extremities (Snijder *et al.* 1999). The suggested cut-off values of BF% that correspond to BMI-defined obesity (30.0 or more) in young Caucasian adults are more than 25 per cent for men and more than 35 per cent for women (Deurenberg & Yap 1999).

As BMI and BF% are mainly measures of body composition and estimations of the amount of fat tissue, they do not provide any specific information about the anatomical distribution of fat. WC is a simple and useful measurement in field circumstances for assessment of upper body fat deposition; WC is measured at the

midpoint between the lower border of the rib cage and the iliac crest. WC has been found to correlate closely with BMI (Lean *et al.* 1995), and has also been found to provide a good approximation of total body fat (Lean *et al.* 1996). WHO has proposed cut-off values, for Caucasian adults, of 94.0–101.9 centimetres for men and 80.0–87.9 centimetres for women, to correspond to BMI-defined overweight (25.0–29.9), and 102.0 centimetres or more for men and 88.0 centimetres or more for women to correspond to BMI-defined obesity (30.0 or more) (WHO 2000).

2.1.2 Adipose tissue

Adipose tissue can be divided into two main types, white adipose tissue (WAT) and brown adipose tissue. WAT represents the vast majority of adipose tissue and is the site of energy storage, whereas the main function of brown adipose tissue is nonshivering thermogenesis (Fantuzzi 2005). Adipocytes constitute the majority of cell types in WAT, representing 50–85 per cent of the total amount of cellular components (Maury & Brichard 2010). Adipose tissue mass is determined by both adipocyte number and size (Spalding *et al.* 2008). It has been suggested that obese individuals have a higher total number of adipocytes than lean individuals have and that the number of adipocytes is set during childhood and stays relatively constant in adulthood, even after weight loss. Increased fat storage in fully differentiated adipocytes, adipocyte hypertrophy, is considered to be the most important determinant of increased adipose tissue depots in adults. (Spalding *et al.* 2008)

About 10 per cent of the remaining cell types in WAT, ‘stromovascular fraction’, have been estimated to consist of macrophages (Curat *et al.* 2004). Weight gain is associated with increased macrophage infiltration in WAT. Macrophage infiltration in WAT has been found to increase in proportion to adipocyte hypertrophy and BMI (Curat *et al.* 2004, Weisberg *et al.* 2003). Furthermore, a significant reduction in macrophage infiltration in WAT has been reported after surgery-induced weight loss (Cancello *et al.* 2005). Besides the increased amount of macrophages, also differences in regional distribution of macrophage infiltration in WAT during obesity has been suggested. It has been found that macrophage infiltration in omental adipose tissue is greater than in subcutaneous adipose tissue among obese subjects (Cancello *et al.* 2006, Harman-Boehm *et al.* 2007).

In addition to the increased accumulation of macrophages and preferential infiltration of macrophages into omental adipose tissue, obesity is also suggested to be associated with a switch in the activation state of WAT macrophages. Macrophages have been traditionally defined across two separate polarisation states, representing extremes of a continuum, based on their activation, namely ‘classically activated’, proinflammatory M1 macrophages and ‘alternatively activated’, anti-inflammatory M2 macrophages (Mantovani *et al.* 2004). It has been suggested that the activation state of WAT macrophages is weighted toward M1 over M2 among obese subjects, and after gastric bypass a switch to a less proinflammatory profile was found (Aron-Wisnewsky *et al.* 2009). Furthermore, a reduction in the number of WAT macrophages after surgery-induced weight loss was associated with strong staining of the remaining macrophages for the most potent anti-inflammatory cytokine interleukin 10 (IL-10), whereas before the surgery the staining was almost absent (Cancello *et al.* 2005). Murine models have suggested that this shift in the M1/M2 balance during obesity is due to migration of inflammatory monocytes from circulation into WAT, rather than conversion of resident M2 macrophages to M1 macrophages locally in adipose tissue (Lumeng *et al.* 2008). However, it has also been suggested that WAT macrophages may not exhibit strict polarisation to M1 or M2. Human WAT macrophages have been found to present M2-like surface marker expression, and are also capable of producing extensive amounts of proinflammatory cytokines (Zeyda *et al.* 2007).

One suggested mechanism for macrophage recruitment and infiltration into WAT is adipocyte death. It has been found that the preponderance of the WAT macrophages of obese subjects are localized to individual dead adipocytes, where they fuse to form syncytia that sequester and ingest the residual adipocyte lipid droplets, ultimately forming multinucleated giant cells (Cinti *et al.* 2005). Besides adipocyte death, other suggested mechanisms of macrophage infiltration include chemotactic regulation, adipose tissue hypoxia and dysregulated fatty acid flux (Sun *et al.* 2011).

2.1.3 Obesity as an inflammatory condition

Obesity has been considered a state of low-level systemic inflammation (Cancello & Clement 2006, Festa *et al.* 2001). This state of inflammation can be observed as a rise in serum inflammatory markers, such as high-sensitivity C-reactive protein (hsCRP), which is probably the best known marker of subclinical

inflammation (Guldiken *et al.* 2007, Kim *et al.* 2008). WAT is no longer thought to be merely an inactive energy reservoir, but rather an active endocrine organ that secretes proinflammatory cytokines and a total of over 50 biologically active substances, collectively known as adipokines (Trayhurn & Wood 2004). Proinflammatory cytokines secreted by WAT include, among others, tumour necrosis factor α (TNF- α) (Kern *et al.* 1995) and interleukin 6 (IL-6) (Fried *et al.* 1998, Mohamed-Ali *et al.* 1997). Elevated serum levels of TNF- α among obese subjects have been reported, as well as a fall in these levels after weight loss (Dandona *et al.* 1998, Ziccardi *et al.* 2002). Also, systemic IL-6 levels have been reported to be higher among obese individuals and lower after weight loss (Bastard *et al.* 2000, Ziccardi *et al.* 2002). Mohamed-Ali *et al.* (1997) estimated that *in vivo* release of whole-body adipose tissue could produce 15–35 per cent of systemic IL-6. Macrophages have been suggested to be a major source of TNF- α produced by WAT, and to also produce significant amounts of WAT-derived IL-6 (Weisberg *et al.* 2003). Other pro-inflammatory adipokines produced by WAT include factors such as leptin, which has a central role in the control of appetite through the central nervous system; resistin, which promotes insulin resistance and inflammation through induction of proinflammatory cytokine secretion from monocytes; plasminogen activator inhibitor-1 (PAI-1), which acts as an inhibitor of fibrinolysis and is also implicated in other biological processes including angiogenesis and atherogenesis; monocyte chemoattractant protein-1 (MCP-1), which is a potent chemoattractant agent; adiponectin, which corresponds to complement factor D and takes part in both lipid and glucose metabolism; and visfatin, which participates in glucose metabolism through insulin-mimetic effects (Fantuzzi 2005, Kershaw & Flier 2004, Ouchi *et al.* 2011).

Adipose tissue also secretes a smaller number of anti-inflammatory peptides, such as adiponectin, for instance, which acts as an insulin sensitizer and protects against obesity-related metabolic dysfunction through suppression of proinflammatory cytokine production (Ouchi *et al.* 2011).

Adipocyte size has been suggested to be an important determinant of adipokine secretion (Skurk *et al.* 2007). Skurk and co-workers (2007) reported that very large adipocytes secrete significantly more proinflammatory adipokines than do small or medium-sized adipocytes. According to one theory, this increased proinflammatory adipokine secretion may be a response to a hypoxia in the expanding fat mass (Trayhurn & Wood 2004). The expansion of the tissue leads to a situation where the vasculature is insufficient to maintain normoxia throughout the organ, resulting in an inflammatory response that is aimed to

increase blood flow and stimulate angiogenesis (Trayhurn & Wood 2004). The view that increased adipokine secretion is due to hypoxia in adipose tissue is also supported by the finding that increased proinflammatory cytokine secretion has been found in the stromovascular cells of WAT, mainly macrophages, under hypoxic conditions *in vitro* (O'Rourke *et al.* 2011). O'Rourke and colleagues (2011) also found that hypoxia upregulated phosphorylation of signal transducer molecule p38, suggesting a possible role for p38 in the regulation of hypoxia-induced WAT inflammatory responses.

Heightened systemic oxidative stress—a persistent imbalance between reactive oxygen species (ROS) and anti-oxidant defences—has also been reported to be associated with obesity (Keaney *et al.* 2003, Olusi 2002, Urakawa *et al.* 2003). WAT has been found to be a significant source of elevated plasma ROS in obese mice (Furukawa *et al.* 2004). Furthermore, compared with lean subjects, increased ROS-induced damage in lipids, proteins and amino acids has been reported among obese subjects (Dandona *et al.* 2001). Dandona and co-workers (2001) also found a decrease in ROS generation and oxidative damage after dietary restriction and weight loss among the obese.

2.1.4 Metabolic complications of obesity

Insulin resistance and atherogenic dyslipidaemia (elevated serum triglyceride and lowered high-density lipoprotein cholesterol [HDL-C] concentrations, as well as increased levels of small low-density lipoprotein [LDL] particles and very-low-density lipoprotein [VLDL] remnants) are probably the most important metabolic complications of obesity, and clustering of these metabolic disorders is often referred to as metabolic syndrome (Grundy *et al.* 2004). Insulin resistance is a critical feature of diabetes mellitus and is defined as a state in which the biological response of a given amount of insulin is lesser than normal, such as decreased insulin-mediated glucose uptake by muscle tissue, decreased suppression of hepatic glucose production and decreased inhibition of lipolysis in adipose tissue (Matthaei *et al.* 2000). TNF- α produced by adipose tissue has been suggested to have an important role in obesity-associated insulin resistance (Hotamisligil *et al.* 1993, Hotamisligil *et al.* 1995). One proposed mechanism by which TNF- α induces insulin resistance is phosphorylation of a serine residue of insulin receptor substrate-1 and subsequent inhibition of tyrosine kinase activity of the insulin receptor essential for insulin signal transduction (Hotamisligil *et al.* 1996).

Insulin resistance has been suggested to also have an important role in obesity-induced dyslipidaemia through several mechanisms, such as increased fatty acid flux to the liver and increased *de novo* lipogenesis (Chan *et al.* 2004). Besides having an essential role in atherosclerosis through lipid deposition in the vascular wall, the dyslipidaemic lipid profile has been suggested to also have inflammatory effects. Hypertriglyceridaemia has been reported to be associated with elevated levels of soluble intercellular adhesion molecule 1 (ICAM-1), soluble vascular cell adhesion molecule 1 and soluble E-selectin, possibly having an effect on increased monocyte migration from the bloodstream (Abe *et al.* 1998). In addition, hypertriglyceridaemic patients have been found to have a significantly higher capacity to produce TNF- α and IL-6, as well as higher serum levels of CRP compared with normolipidaemic controls (Jonkers *et al.* 2002). LDL has also been shown to possess proinflammatory properties. LDL is highly sensitive to oxidative modification, producing oxidised LDL, and a significant association has been reported between circulating oxidised LDL levels with serum concentrations of CRP and TNF- α (Hulthe & Fagerberg 2002). Oxidised LDL has also been found to induce production of MCP-1 from human endothelial cells *in vitro* (Mackness *et al.* 2004). Whereas triglycerides and LDL have been suggested to induce inflammation, HDL has been reported to be anti-inflammatory. HDL has been found to inhibit oxidation of LDL and oxidised LDL induced production of MCP-1 from human endothelial cells *in vitro* (Mackness *et al.* 2004). In addition, HDL has been found to attenuate TNF- α -induced IL-6 production *in vitro* (Gomaschi *et al.* 2005). Gomaschi *et al.* (2005) also reported that plasma IL-6 concentrations were higher among subjects with a low HDL-C concentration compared with those with a high HDL-C concentration.

2.2 Obesity and periodontitis

2.2.1 Periodontitis

Periodontitis: definition and pathogenesis

Periodontitis is an inflammatory disease with an infectious origin which affects the supporting structures of the tooth, *i.e.* connective tissue and alveolar bone, ultimately leading to a breakdown of these tissues and tooth loss if left untreated (Pihlstrom *et al.* 2005). Although it is obvious that subgingival accumulation of

bacterial pathogens, predominantly gram-negative bacteria, is essential for initiation and progression of periodontitis, the resulting host response primarily mediates concomitant tissue destruction (Baker 2000, Taubman *et al.* 2005). The inflammatory and immune responses in periodontitis can be considered to represent a continuum of a normal host response to infection which eventually becomes pathologic when homeostasis is lost (Van Dyke 2007). The main clinical manifestations of periodontitis are gingival bleeding, deepening of the tooth-surrounding sulcus and loss of attachment related to the destruction of tooth-supporting structures, fibrous attachment and underlying alveolar bone. Loss of alveolar bone can be detected also radiographically.

Both innate and adaptive immune responses have an integral role in the pathogenesis of periodontitis. The main functions of innate immunity include functions such as cytokine secretion, identification and removal of foreign substances by phagocytosis and activation of the adaptive immune system (Van Dyke & Kornman 2008). Gingival epithelial cells, as well as polymorphonuclear neutrophils (PMN) and monocytes/macrophages, have an essential role in the first line of defence against bacterial pathogens. They express Toll-like receptors (TLR) on their cell surfaces, which detect various bacterial components—collectively referred to as pathogen-associated molecular patterns (PAMP)—resulting in activation of innate immune response through intracellular signalling pathways (Mahanonda & Pichyangkul 2007). PAMPs include lipopolysaccharides (LPS) and bacterial DNA, for instance (Mahanonda & Pichyangkul 2007). LPS is an integral part of the outer cell membrane of gram-negative bacteria and is released as a result of the death of the bacteria, presenting an important virulence factor for these pathogens. For example, *Porphyromonas gingivalis* LPS has been found to increase IL-6, TNF- α and interferon γ (IFN- γ) secretion in oral epithelial cells through TLR2 *in vitro* (Kocgozlu *et al.* 2009), whereas bacterial DNA from *P. gingivalis* and *Tannerella forsythia* has been reported to induce IL-1 β , IL-6 and TNF- α production through TLR9 in human monocytic cells *in vitro* (Sahingur *et al.* 2010). An essential part of innate immunity is also the complement system, which has several important functions, such as interaction with pathogens, thus facilitating their removal by phagocytosis, and linkage of innate and adaptive immune responses (Gasque 2004).

Lymphocytes—T cells and B cells—are the main cell types of adaptive immunity. Whereas B cells mainly act as antibody producers, T cells are functionally divided into two classes: cytotoxic T cells (CD8⁺ T cells) and T helper cells (CD4⁺ T cells). Naïve T helper cells recognise antigens associated

with major histocompatibility complex class II (MHC-II) molecules presented by antigen-presenting cells, such as dendritic cells, which then differentiate into effector T helper cells, characterised by production of specific cytokines and functions. The best defined functional subsets of T helper cells are Th1 and Th2 cells. Th1 cells, characterised by secretion of IFN- γ , activate cell-mediated immunity, while Th2 cells, characterised by secretion of IL-4, -5 and -13, mainly regulate humoral (antibody-mediated) immunity. (Preshaw & Taylor 2011) T cells are thought to be central in the pathogenesis of periodontal infection, and Th1 cells and their cytokines are suggested to be associated with stable periodontal lesion, whereas a Th2-mediated response is considered to be associated with progression of the disease (Gemmell *et al.* 2007).

The Th1/Th2 dichotomy has been recently expanded by the identification of a novel T helper cell subset, Th17. The primary function of Th17 cells is suggested to be clearance of pathogens that are not adequately handled by Th1 or Th2 cells, and Th17 cells are potent inducers of tissue inflammation (Korn *et al.* 2009). Th17 cells have been suggested to also have a potential role in the pathogenesis of periodontitis (Gaffen & Hajishengallis 2008), and these cells have been discovered in gingiva from patients with chronic periodontitis (Cardoso *et al.* 2009). Elevated levels of a cytokine characteristic of Th17 cells, IL-17, has been found in inflamed periodontal tissues among subjects with chronic periodontitis (Lester *et al.* 2007, Vernal *et al.* 2005). IL-17 has been found to induce cytokine and matrix metalloproteinase (MMP) synthesis in gingival fibroblasts from subjects with periodontitis *in vitro* (Beklen *et al.* 2007).

Cytokines essential for the pathogenesis of periodontitis

Cytokines are a group of biologically active molecules that, once released by other cells, elicit a specific response in their effector cells. The function of cytokines is interrelated, and they form a well-orchestrated functional network. Cytokines can be roughly categorised into proinflammatory and anti-inflammatory cytokines. Proinflammatory cytokines that are considered to have an essential role in periodontal tissue destruction are IL-1 β , TNF- α and IL-6 (Graves 2008). IL-1 β in periodontal tissues is secreted by various cell types, such as monocytes/macrophages, PMN cells, fibroblasts and endothelial cells (Graves & Cochran 2003). Elevated levels of IL-1 β in gingival crevicular fluid (GCF) have been reported to be associated with periodontitis (Fitzsimmons *et al.* 2010, Orozco *et al.* 2006, Teles *et al.* 2010). It has also been found that local IL-1 β

production in periodontal tissues increased with increasing inflammation (Orozco *et al.* 2006). IL-1 β has a role in alveolar bone resorption, possibly through supporting osteoclastogenesis via its effect on human periodontal fibroblasts (Bloemen *et al.* 2011), and is also supposed to participate in connective tissue degradation through induction of MMP expression (Beklen *et al.* 2007). MMPs are a group of zinc-dependent enzymes that are able to degrade all proteinaceous components of the extracellular matrix, and thus they have a key role in periodontal tissue destruction (Hannas *et al.* 2007). Several cell types in periodontal tissues express MMPs, such as fibroblasts, epithelial cells and endothelial cells, as well as macrophages and PMN cells (Birkedal-Hansen 1993). MMPs that are suggested to be especially related to periodontal tissue destruction include collagenases 1 (MMP-1), 2 (MMP-8) and 3 (MMP-13), as well as gelatinases A (MMP-2) and B (MMP-9) (Ejeil *et al.* 2003, Kinane *et al.* 2003, Lee *et al.* 1995). Tissue inhibitors of metalloproteinases (TIMP) have an important role in the control of MMP-mediated extracellular matrix degradation through their ability to inhibit MMP activity (Verstappen & Von den Hoff 2006).

TNF- α is secreted by the same cell types as IL-1 β , and they also share many of their functions (Graves & Cochran 2003). Elevated concentrations of TNF- α in GCF among subjects with periodontitis have been reported (Kurtis *et al.* 2005). TNF- α has several important functions in the pathogenesis of periodontitis. TNF- α has been found to induce MMP expression along with IL-1 β (Beklen *et al.* 2007), and has also been suggested to have an important role in alveolar bone loss (Garlet *et al.* 2007). TNF- α may also reduce tissue repair capacity through induction of apoptosis of matrix-producing cells, such as fibroblasts (Graves *et al.* 2001).

IL-6 also has an important role in the pathogenesis of periodontitis. IL-6 is produced by several cell types, the main sources being monocytes/macrophages, endothelial cells and fibroblasts (Okada & Murakami 1998). IL-6 levels in GCF have been found to correlate with periodontal disease severity (Geivelis *et al.* 1993, Lin *et al.* 2005). In addition, significantly elevated levels of IL-6 have been found in gingival connective tissue adjacent to intrabony pockets which had not been resolved after conventional, non-surgical periodontal therapy (Guillot *et al.* 1995). IL-6 has many important functions, such as induction of osteoclastogenesis and bone resorption (Ishimi *et al.* 1990, Tamura *et al.* 1993).

Besides locally produced cytokines in gingival tissues, also systemically elevated levels of cytokines may have an effect on the pathogenesis of periodontitis. This view is supported by the findings of recent studies by

Andriankaja *et al.* (2009) and Passoja *et al.* (2011). Andriankaja and colleagues (2009) reported that an elevated level of serum IL-6 was associated with biofilm- gingival interface gingivitis among a group of non-smoking subjects with type II diabetes, whereas Passoja and co-workers (2011) found an association between elevated levels of serum IL-6 and the number of sites with bleeding and a probing depth of 4 mm or more among type I diabetic subjects. Passoja *et al.* (2011) also found that periodontal healing was poorer among subjects with high post-therapy serum IL-6 levels.

2.2.2 The role of obesity as a possible risk for periodontitis

Association between obesity and periodontitis

Obesity, which is characterised by a state of chronic, low-grade inflammation, has been recently suggested to be a condition which could predispose the subject to periodontitis. Several epidemiological studies have reported an association between high body weight and periodontitis (Al-Zahrani *et al.* 2003, Dalla Vecchia *et al.* 2005, Ekuni *et al.* 2008, Genco *et al.* 2005, Haffajee & Socransky 2009, Han *et al.* 2010, Khader *et al.* 2009, Linden *et al.* 2007, Nishida *et al.* 2005, Pataro *et al.* 2012, Reeves *et al.* 2006, Saito *et al.* 2001, Saito *et al.* 2005, Wood *et al.* 2003, Ylöstalo *et al.* 2003). Saito *et al.* (2001) studied the association of obesity with periodontitis in a population of 643 healthy Japanese subjects aged between 19 and 79 years. They assessed obesity using BMI, waist-to-hip ratio (WHR) and BF%. Periodontal status was assessed from 10 designated teeth, and six sites around each tooth, as representatives from six sextants. Periodontitis was defined as the subject having at least one tooth with a periodontal pocket of 4 millimetres or more. They found that the ratio of subjects having deeper periodontal pockets increased with higher categories of BMI, BF% and WHR. Multiple logistic regression models, adjusted for age, gender, social class, diabetes, smoking and oral hygiene, showed a significant association between each index of obesity and the increased risk of periodontitis. The adjusted odds ratio (OR) with a 95 per cent confidence interval (95% CI) for periodontitis among subjects with a high WHR (0.9 or more for males, 0.8 or more for females) and a BMI of 30.0 or more was 4.3 (95% CI 1.6–11.7). (Saito *et al.* 2001)

On the other hand, Al-Zahrani and co-workers (2003) investigated the relation of obesity to periodontitis in a sample of 13,665 subjects aged 18 years or

older who participated in the NHANES III survey, which is a nationally representative sample of the U.S. population. They used BMI and WC as measures of obesity, whereas periodontal disease was defined based on the presence of one or more sites with both an attachment loss (AL) of 3 mm or more and a probing depth of 4 mm or more. As in the study by Saito and co-workers (2001), also in this study a partial-mouth protocol was applied in the periodontal examinations, *i.e.* the examinations were conducted in one randomly assigned upper and one lower quadrant. A high BMI and a large WC were found to be associated with the prevalence of periodontal disease in multivariable logistic regression models, including confounding factors such as age, gender, race, smoking, poverty index, education, diabetes and time since the last dental visit. The adjusted OR for the association of a BMI of 30.0 or more with periodontal disease was 1.37 (95% CI 1.14–1.64), and for the association of a large WC (more than 102 cm for men, more than 88 cm for women) with periodontal disease it was 1.33 (95% CI 1.11–1.60), respectively. They further analysed these data stratified according to age, and found that obesity and periodontal disease were associated in persons aged 18 to 34 years, but no consistent association was found in older age groups. (Al-Zahrani *et al.* 2003)

The NHANES III survey data were also used in a study by Genco and colleagues (2005), where the association of BMI-defined overweight with periodontal disease was investigated in a sample of 12,367 non-diabetic subjects aged 20 to 90 years. As mentioned earlier, periodontal examinations in this survey were conducted in one randomly assigned upper and one lower quadrant, but in this study a mean AL of 1.5 mm or more was used to define periodontal disease. They reported that a BMI of 27.0 or more was statistically significantly associated with a greater level of attachment loss and an increased prevalence of periodontal disease. (Genco *et al.* 2005)

Nishida *et al.* (2005) investigated the association of lifestyle-related factors, such as smoking and obesity, with periodontitis. This study was based on a sample of 372 subjects aged 20 to 59 years. Obesity was assessed using BMI, and periodontitis using the percentage of teeth with a probing depth of more than 3.5 mm. The classification of subjects with or without periodontitis was based on subject placement above or below the upper 20th percentile of the percentage of teeth with periodontal pockets. They reported that BMI exerted the second greatest impact on periodontitis risk, right after smoking. They also found that increasingly poor periodontal status corresponded to increased BMI in an exposure-response manner, with the highest category of BMI (28.0 or more)

showing an OR of 4.40 (95% CI 1.18–16.4) for having periodontitis, adjusted for age, gender, pack-years of smoking, alcohol consumption, and frequency of toothbrushing. (Nishida *et al.* 2005)

Ylöstalo and co-workers (2008) investigated the association between body weight and periodontal infection using these same Health 2000 data as in the present study. Their study was based on a subpopulation of dentate non-diabetic subjects, aged between 30 and 49 years, yielding a sample of 2,841 subjects. Body weight was measured using BMI, and periodontal infection was measured as the number of teeth with periodontal pockets 4 mm deep or deeper and 6 mm deep or deeper. Periodontal pocket depth was measured on every tooth, excluding third molars and radices, and the highest measurement for every tooth was recorded. They found that BMI was associated with the number of teeth with periodontal pockets 4 mm deep or deeper in an exposure-response manner, with the highest quintile of BMI (29.1 or more) exhibiting a relative risk (RR) of 1.2 (95% CI 1.0–1.4). Confounding variables included factors such as gender, age, education, dental attendance pattern, toothbrushing frequency, presence of plaque and number of teeth. Additional analyses among a subpopulation of never-smokers showed an even stronger association, with the adjusted RR for the highest category of BMI being 1.5 (95% CI 1.2–1.9). (Ylöstalo *et al.* 2008)

A negative association between BMI and periodontitis has also been reported (Kongstad *et al.* 2009). They found that obesity was inversely associated with clinical AL, whereas a positive association was found between BMI and bleeding on probing. Kim and co-workers (2011) reported no association between BMI and periodontitis. A large WC, on the other hand, was found to be associated with periodontitis, suggesting an important role for abdominal obesity (Kim *et al.* 2011).

Recently, two meta-analyses of the association between high body weight and periodontitis have been conducted (Chaffee & Weston 2010, Suvan *et al.* 2011). A meta-analysis by Chaffee and Weston (2010) including 28 studies produced a summary odds ratio (sOR) of 1.35, the 95% CI being 1.23–1.47 for the association between obesity and periodontitis. They also conducted additional meta-analyses of the difference in mean BMI across groups with and without periodontitis (26 studies), and of the difference in mean clinical AL across obese and non-obese groups (five studies). The summary mean difference (sMD) in BMI among individuals with periodontitis compared with those without the disease was 0.80 BMI units (95% CI 0.70–0.95). The sMD in clinical AL was 0.58 mm (95% CI 0.40–0.74), with greater AL seen among obese individuals

(Chaffee & Weston 2010). Chaffee and Weston (2010) also reported a slight linear increase in the odds of periodontitis with increasing BMI (a meta-analysis including eight studies). On the other hand, Suvan and colleagues (2011) reported that the sOR of having periodontitis if an individual is overweight or obese was 2.13 (95% CI 1.40–3.26) compared with normal-weight subjects (a meta-analysis including six studies). Respectively, the sOR of having periodontitis if an individual was in an overweight BMI category (not obese) was 1.27 (95% CI 1.06–1.51) compared with normal-weight subjects (a meta-analysis including 12 studies). The sOR for obese individuals with periodontitis compared with normal-weight subjects was 1.81 (95% CI 1.42–2.30) (a meta-analysis including 12 studies) (Suvan *et al.* 2011).

Possible mediating mechanisms between obesity and periodontitis

There are several possible biological mechanisms that could mediate the effect of obesity on periodontitis, although the evidence is still scant. One of the suggested mechanisms that aggravate periodontal inflammation in obese subjects is the elevated systemic levels of proinflammatory cytokines, such as TNF- α and IL-6, produced by adipose tissue, which may have a direct effect on periodontal tissues (Pischon *et al.* 2007). As mentioned earlier, a high serum IL-6 concentration has been found to be associated with gingivitis (Andriankaja *et al.* 2009) and markers of periodontitis and poor periodontal healing (Passoja *et al.* 2011). In addition, a correlation has been found between BMI and GCF levels of TNF- α among individuals with a BMI of 40.0 or more (Lundin *et al.* 2004). There was also a statistically significant difference in the correlation coefficients between subjects with a BMI < 40.0 and subjects with a BMI \geq 40.0. The view that this association between BMI and GCF TNF- α is due to the systemic effect of circulating TNF- α from plasma rather than local cytokine secretion from monocytic cells in inflamed periodontal tissues is supported by the finding that the GCF level of TNF- α was significantly positively correlated with BMI in subjects with no pathological periodontal pockets (Lundin *et al.* 2004). Moreover, Khanna and Mali (2010) reported significantly higher periodontal disease index (PDI) scores and plasma TNF- α levels among obese subjects than among non-obese subjects. In addition, a significant and positive correlation was found between BMI and TNF- α , TNF- α and PDI, as well as between BMI and PDI (Khanna & Mali 2010). Of the other adipokines, serum resistin level has been found to be associated with periodontitis (Saito *et al.* 2008), whereas PAI-1 has

been detected in gingival tissues from subjects undergoing periodontal therapy (Kinnby *et al.* 1999). Interestingly, an inverse association has been found between gingival leptin concentration and the markers of periodontal inflammation; the greater the periodontal destruction, the lesser was the leptin concentration in gingival tissues (Johnson & Serio 2001, Karthikeyan & Pradeep 2007).

Another suggested mechanism is increased insulin resistance. This view is supported by the findings of studies by Genco *et al.* (2005) and Benguigui *et al.* (2010). Genco and co-workers (2005) reported that periodontal attachment loss increased proportionally with increasing insulin resistance. They also reported that overweight individuals with insulin resistance in the highest quartile exhibited an adjusted OR of 1.45 (95% CI 1.09–1.93) for the association of a high BMI with severe attachment loss, whereas this association was not statistically significant for subjects with a high BMI and low insulin resistance (Genco *et al.* 2005). Benguigui and colleagues (2010), on the other hand, reported that a high homeostasis model assessment of the insulin resistance index was associated with severe periodontitis (Benguigui *et al.* 2010). Insulin resistance contributes to a hyperinflammatory state, setting the stage for increased levels of periodontal disease triggered by oral pathogens (Genco *et al.* 2005).

A dyslipidaemic state may also be a condition which could predispose obese subjects to periodontitis (Saito & Shimazaki 2007). An association between hyperlipidaemia and periodontitis has been reported (Fentoglu *et al.* 2009, Noack *et al.* 2000). Noack *et al.* (2000) found that neutrophil (PMN) respiratory burst activity was higher in hyperlipidaemic subjects, and thus suggested that the association between hyperlipidaemia and periodontitis may be due to dysfunction of PMN cells. However, a recent study also suggested that hyperlipidaemia may be associated with periodontal disease through increased concentrations of proinflammatory cytokines IL-1 β , TNF- α and IL-6 (Fentoglu *et al.* 2011). Furthermore, LDL and oxidised LDL have been detected in GCF (Sakiyama *et al.* 2010), and oxidised LDL has been shown to induce IL-8 production in gingival epithelial cells *in vitro*, and may thus contribute to inflammatory reactions in periodontal tissues (Suzuki *et al.* 2010).

Increased oxidative stress has also been suggested to possibly mediate the association between obesity and periodontitis (Boesing *et al.* 2009). This is supported by the finding that an increased level of lipid peroxidation in gingival tissues has been found among subjects with periodontitis, which may possibly have a role in periodontal tissue destruction (Panjamurthy *et al.* 2005, Tsai *et al.* 2005). In addition, an overgrowth of *T. forsythia* has been found in subgingival

biofilm of periodontally healthy, overweight and obese individuals, which may predispose these subjects to initiation and progression of periodontitis (Haffajee & Socransky 2009).

Although several biologically plausible explanations for the association between obesity and periodontitis have been presented, it is also possible that this association is due to accumulation of poor, health-compromising behavioural habits among obese subjects. It has been shown that behavioural risk factors for oral and general health co-occur among the same individuals (Sanders *et al.* 2005), and unhealthy dental and general health habits have been found to be associated with self-reported dental diseases, such as gingival bleeding, and cardiovascular risk factors, such as a high BMI and a large WC (Ylöstalo *et al.* 2003). In addition, it has been suggested that if correlations between oral and general health habits are not properly taken into account, strong but spurious associations between oral factors and systemic conditions may arise, such as seen between a lack of flossing and obesity (Hujoel *et al.* 2006).

2.2.3 Periodontitis and weight gain

Although the direct cause of obesity is a long-term, positive imbalance between energy intake and consumption, also infections have been suggested to play a role in weight gain. Viral infections have been reported to be associated with high body weight (Atkinson *et al.* 2005), and recently also an association of periodontitis with metabolic syndrome, and with obesity as an individual component of the condition, has been reported (D'Aiuto *et al.* 2008).

The mechanism by which periodontitis could induce weight gain may involve systemically elevated level of LPS (endotoxin), also referred to as endotoxaemia. An ulcerated subgingival pocket epithelium provides a potent route for bacterial cells and their products to enter the systemic circulation (Loos 2005). The total dentogingival epithelial surface area of subjects with periodontitis has been estimated to be up to 20 cm², ranging from 1 to 44 cm² (Hujoel *et al.* 2001), and bacteraemia has been reported after chewing, toothbrushing and after various periodontal procedures, such as periodontal probing and ultrasonic scaling (Forner *et al.* 2006, Kinane *et al.* 2005). Endotoxaemia has been reported to be associated with periodontitis (Pussinen *et al.* 2007), and it has also been found to have a positive correlation with energy and fat intake (Amar *et al.* 2008). The role of endotoxaemia in weight gain is also supported by the results of a study with a murine model where a four-week continuous subcutaneous infusion of LPS into

mice that were fed a normal diet resulted in weight gain, which was similar to that of mice on a four-week high-fat diet regimen (Cani *et al.* 2007). In addition, it has been reported that mice with a loss-of-function mutation in TLR4 were protected against the development of diet-induced obesity (Tsukumo *et al.* 2007).

Besides causing endotoxaemia by the leaking of LPS into the circulation through an ulcerated area in the periodontal pocket wall, periodontitis may also facilitate metabolic endotoxaemia through ingestion of periodontal pathogens along with saliva. Metabolic endotoxaemia is caused by LPS from gram-negative bacteria of the gut microbiota, which is absorbed into intestinal capillaries (Manco *et al.* 2010). According to some estimations, about one gram of bacteria (1×10^{11} cells) is swallowed along with saliva daily (Socransky & Haffajee 2005), and thus salivary bacteria may affect gastrointestinal microbiology. In fact, *Selemonas noxia*, at levels greater than 1.05 per cent of total salivary bacteria, has been found to identify obese individuals to a sensitivity of 98.4 per cent and a specificity of 80.2 per cent (Goodson *et al.* 2009). *S. noxia* is a gram-negative pathogen found in periodontal biofilm and it has been suggested to have a role in sites converting from periodontal health to disease (Tanner *et al.* 1998). Furthermore, *S. noxia* belongs to the phylum *Firmicutes*, whose relative proportion in gut microbiota has been reported to be elevated in obese individuals (Ley *et al.* 2006).

2.3 Statin medication and periodontitis

Statins, such as simvastatin, atorvastatin, lovastatin, mevastatin, fluvastatin, pravastatin and cerivastatin, have been found to be very effective in the treatment of hyperlipidaemia and atherosclerosis (Baigent *et al.* 2005, Hunninghake 1998). Their effect on lowering cholesterol levels is based on a decrease in hepatic cholesterol synthesis. Statins act as a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Through this effect statins decrease cholesterol synthesis by preventing formation of mevalonate from 3-hydroxy-3-methylglutaryl coenzyme A. (Istvan 2003) In addition to the lipid-lowering effect, statins have been suggested to possess pleiotropic effects, such as anti-inflammatory effects (Jain & Ridker 2005) and effects on bone metabolism (Horiuchi & Maeda 2006), for instance.

2.3.1 Anti-inflammatory effects of statins

Simvastatin, lovastatin and mevastatin has been reported to inhibit the binding of lymphocyte function-associated antigen-1 (LFA-1) to its major counter receptor ICAM-1 via binding of statins to a specific site in the LFA-1 I-domain *in vitro* (Weitz-Schmidt *et al.* 2001). Through the LFA-1–ICAM-1 inhibition, statins may prevent extravasation of leucocytes from the bloodstream to the site of an inflammation. Inhibition of LFA-1 by statins was also found to result in decreased lymphocyte adhesion to ICAM-1 and impaired T cell co-stimulation (Weitz-Schmidt *et al.* 2001). On the other hand, Kwak and colleagues (2000) found that atorvastatin, lovastatin and pravastatin repress induction of MHC-II expression induced by IFN- γ in dose-dependent manner *in vitro*, meaning that statins may act as inhibitors of MHC-II-mediated T cell activation. This effect was found to be due to inhibition of the inducible promoter IV of the class II transactivator (Kwak *et al.* 2000). Atorvastatin has also been suggested to have direct suppressive effects on T cell activation (Blank *et al.* 2007).

Statins have also been suggested to inhibit secretion of various proinflammatory cytokines. Simvastatin has been found to decrease IL-1 α –induced IL-6 and IL-8 production from human oral epithelial cells (Sakoda *et al.* 2006), whereas Ikeda and Shimada (1999) found that fluvastatin and lovastatin significantly prevented LPS-induced IL-6 production from human monocytes *in vitro* (Ikeda & Shimada 1999). In addition, pravastatin has been found to be associated with lower serum IL-6 and TNF- α concentrations (Rosenson *et al.* 1999). Furthermore, atorvastatin, simvastatin and fluvastatin have been found to suppress IL-6-induced MCP-1 gene expression and protein secretion from human endothelial cells *in vitro*, as well as to inhibit monocyte chemotaxis (Jougasaki *et al.* 2010).

Also MMP expression has been suggested to be affected by statins. Fluvastatin has been found to decrease MMP-1 expression from human endothelial cells *in vitro* (Ikeda *et al.* 2000). They also found that fluvastatin had no effect on the expression of TIMP-1 expression and that the collagenolytic activity of the conditioned media of endothelial cells was reduced. Cerivastatin, on the other hand, has been found to decrease secretion of MMP-1, -3 and -9 from human vascular smooth muscle cells *in vitro* (Luan *et al.* 2003). In addition, the anti-inflammatory effects of statins are supported by the finding that decreased serum CRP levels are associated with statins, like for instance atorvastatin (van de

Ree *et al.* 2003), pravastatin (Nissen *et al.* 2005) and simvastatin (Cherfan *et al.* 2007).

2.3.2 Effects of statins on bone metabolism

In vitro studies have suggested both stimulatory and anabolic effects of statins, more precisely simvastatin, on bone-forming cells, osteoblasts (Maeda *et al.* 2001, Maeda *et al.* 2004, Mundy *et al.* 1999), as well as inhibitory effects on bone-resorbing cells, osteoclasts (Yamashita *et al.* 2010). In addition, simvastatin and lovastatin have been found to increase bone formation when injected subcutaneously and to increase cancellous bone volume when administered orally to rats (Mundy *et al.* 1999). Fluvastatin, on the other hand, has been found to increase bone formation, both new bone thickness and bone density, when injected percutaneously in the rat calvarial bone (Jinno *et al.* 2009).

Experimental studies with animal models have suggested beneficial effects of statins on bone metabolism also in the periodontium. Topically administered simvastatin has been found to protect against ligature-induced alveolar bone loss in ovariectomised rats (Vaziri *et al.* 2007), and also to enhance the recovery of alveolar bone after ligature-induced resorption (Seto *et al.* 2008). Using a bilateral mandible model, Stein and co-workers (2005) found, however, that suprapariosteally administered simvastatin significantly augmented mandibular bone growth in rats. Local application of simvastatin has also been found to maintain residual alveolar ridge height after tooth extraction and to enhance formation of new bone in the extraction socket in rats (Wu *et al.* 2008).

2.3.3 Association between statin medication and periodontitis

Several retrospective studies have suggested an association between statin medication and periodontitis. Statin medication has been found to be associated with decreased tooth loss among chronic periodontitis patients (Cunha-Cruz *et al.* 2006). However, a later study from the same group found no evidence of either increased or decreased tooth loss in periodontitis subjects with statin medication (Saver *et al.* 2007). A study by Lindy *et al.* (2008) suggested that chronic periodontitis patients taking conventional low-dose statin therapy had a significantly lower number of pathologically deepened periodontal pockets compared with subjects having no statin medication. The Periodontal Inflammatory Burden Index (PIBI)—which estimates the systemic effects of

periodontitis—was significantly lower in subjects with statins (Lindy *et al.* 2008). The PIBI is derived from the pocket depth values measured from six sites per tooth and it is calculated by adding the number of periodontal sites indicating moderate periodontitis (pocket depth 4 – 6 mm) to the weighted (2) number of periodontal sites indicating advanced periodontitis (pocket depth 6 mm or more). Thus—in a 28-tooth dentition—PIBI can reach a maximum value of 336. (Lindy *et al.* 2008)

Also clinical trials exist which have suggested beneficial effects of statins on the periodontium. Pradeep and colleagues (2010) compared conventional periodontal therapy (scaling and root planing) combined with locally administered simvastatin in the treatment of chronic periodontitis with scaling and root planing alone. Improvement in periodontal parameters (gingival bleeding, probing pocket depth, clinical attachment level) was significant in both groups, whereas significantly better results were found among patients receiving simvastatin (Pradeep & Thorat 2010). Also, a radiographically evaluated decrease in intrabony defect was greater among subjects receiving scaling and root planing in combination with simvastatin (Pradeep & Thorat 2010). On the other hand, Fajardo *et al.* (2010) investigated the effect of orally administered atorvastatin in combination with mechanical periodontal therapy. Significant improvements were found in dental mobility and the distance from the crestal alveolar bone to the cemento-enamel junction in the atorvastatin group *versus* subjects receiving only mechanical periodontal therapy (Fajardo *et al.* 2010).

3 Aims of the study

Previous studies focusing on the association between periodontal infection and obesity have been almost entirely cross-sectional studies and have concentrated mainly on investigating whether obesity is related to periodontal infection. The general aim of this thesis was to produce evidence on the nature of this association. To accomplish this, the specific aims of the studies included were:

1. To study the association of obesity with periodontal infection in a longitudinal setting (I)
2. To study whether periodontal infection is associated with obesity in a cross-sectional setting (II)
3. To study the possible mediating mechanisms of the association of obesity with periodontal infection
 - Elevated levels of serum cytokines TNF- α and IL-6 (III)
 - Abnormal serum levels of triglycerides, HDL-C and low-density lipoprotein cholesterol (LDL-C) (IV)
4. To study the association between statin medication and periodontal infection (V)

4 Material and methods

4.1 Study population

4.1.1 *The Health 2000 Survey*

The Health 2000 Survey was a nationally representative health survey conducted by the National Institute for Health and Welfare (former National Public Health Institute of Finland) in 2000 and 2001. The general aim of the Health 2000 Survey was to yield information about the health and functional capacity of the adult Finnish population. The original sample of the survey included 8,028 subjects aged 30 years or older. Of this sample, 6,986 (87 per cent) subjects were interviewed either in their homes or in an institution. Altogether 6,354 (79 per cent) subjects participated in the health examination proper (6,335 subjects participated in the oral health examination), whereas 416 (5 per cent) subjects took part in the health examination at home or at an institution. (Aromaa & Koskinen 2004)

The Health 2000 Survey had a two-stage, stratified cluster sampling design, which was planned by Statistics Finland. The sampling frame consisted of adults who were 30 years of age or older living in continental Finland. The sampling frame was regionally stratified according to the five university hospital regions, each containing about one million inhabitants. Sixteen health centre districts were sampled as clusters from each university hospital region, producing a total of 80 health centre districts in the whole country, including 160 municipalities. The 15 largest health centre districts in the country were selected with a probability of 1, whereas the remaining 65 health centre districts were selected by systematic PPS (Probability-Proportional-to-Size) sampling in each stratum. The 80 health centre districts were the primary sampling units, whereas the ultimate sampling units were persons who were selected by systematic sampling from these health centre districts. The sample sizes for the 15 largest health centre districts were proportional to population size. In the 65 PPS-sampled clusters, the sample sizes were equal within each university hospital region, so that the total number of persons drawn from a university hospital region was proportional to the corresponding population size. The Social Insurance Institution selected the sample which was comprised of 8,028 persons aged 30 years or over, the smallest sample size in the 65 small health centre districts being 50 and the largest being

100. (Aromaa & Koskinen 2004) The basic characteristics of the subjects of the Health 2000 Survey aged 30–49 years are presented in Table 1.

Data for the Health 2000 Survey were obtained from a home-visit interview, questionnaires (questionnaires 1, 2, 3 and a dietary questionnaire), clinical health and oral health examinations, and laboratory measurements. Questionnaire 1 (basic questionnaire) included topics such as functional capacity, symptoms, use of time and leisure activities and alcohol use. Questionnaire 2 (infection questionnaire) dealt with infectious diseases, and it included topics such as acute gastrointestinal symptoms and respiratory infections. Questionnaire 3 (complementary questionnaire) included questions about sleeping and living habits, and oral health and quality of life, among others. (Heistaro 2008) Information from questionnaires 2 and 3 was not used in this study.

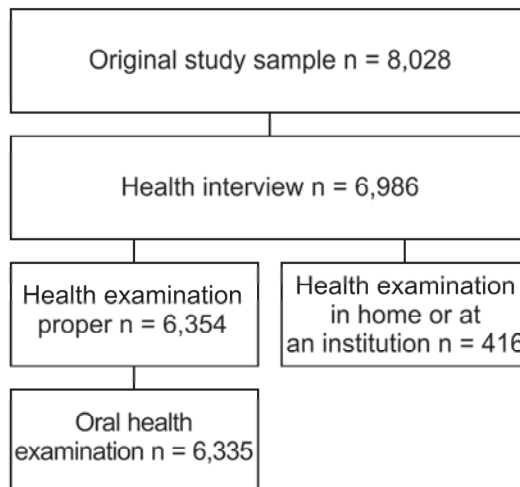


Fig. 1. Participation in the Health 2000 Survey.

4.1.2 The Follow-up Study on Finnish Adults' Oral Health

The National Institute for Health and Welfare launched a series of population studies in 2000 in collaboration with the Social Insurance Institution of Finland (KELA) to investigate the effects of dental care reform that had been implemented in Finland in 2001 and 2002. The data for these studies were collected using specially designed postal questionnaires about self-rated oral health, need for dental care and use of dental care. The first of the surveys took

place before the reform in the spring of 2001 and was followed by similar ones after the first phase of the reform (autumn 2002) and again later, when the reform had been fully implemented (spring 2004). (Kiiskinen *et al.* 2005) To assess the short-term effects of the reform also in clinically determined oral health, the Follow-up Study on Finnish Adults' Oral Health was conducted in 2004–2005. For this survey, 2,000 subjects were randomly selected from participants who had attended the clinical oral health examinations in the Health 2000 Survey (Suominen-Taipale 2005). From this sample, edentulous subjects and persons from public dental service units, which included less than 15 participants, were excluded. The final sample of the survey was comprised of 1,248 subjects who were invited to a clinical oral health re-examination similar to the one conducted in the Health 2000 Survey. Of these subjects, 1,049 (84 per cent) eventually participated in the oral health examination.

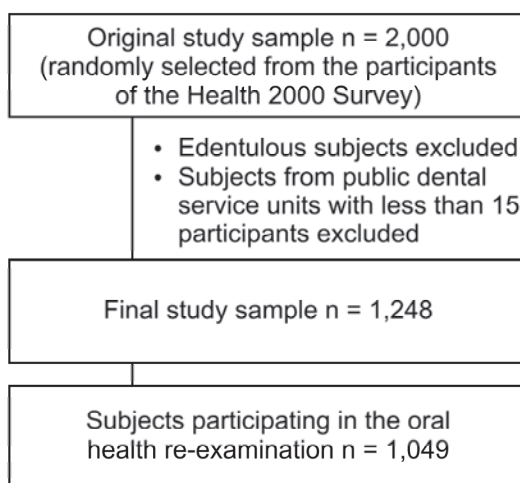


Fig. 2. Participation in the Follow-up Study on Finnish Adults' Oral Health.

4.1.3 Subjects

Article I was based on a subpopulation of subjects who had participated in the periodontal examinations in both the Health 2000 Survey and the Follow-up Study on Finnish Adults' Oral Health approximately four years later (mean 1,504 days; minimum 1,327 days, maximum 1,696 days), and who were dentate, non-diabetic, had never smoked and were aged less than 60 years (n = 396). The study

population of article II consisted of dentate, non-diabetic subjects who were 30–49 years old (n = 2,784). Article III was based on a subsample of the Health 2000 Survey, which was invited to in-depth examinations, including laboratory analyses of serum cytokines which were not included in the original survey in 2001 and 2002 (the average time span since the original survey was 481 days). Of this sample, subjects who were dentate, 45–64 years of age, had never smoked and were non-diabetic and non-rheumatic were included in the study population (n = 430). Article IV was based on a subpopulation of the Health 2000 Survey, which consisted of dentate subjects who were 30–49 years old, had never smoked and were non-diabetic (n = 1,297). Finally, the study population of article V was comprised of dentate subjects, aged between 40 and 69 years, who did not smoke and were non-diabetic and non-rheumatic (n = 2,032).

Subjects were considered to be dentate if they had at least one natural tooth observed during the clinical oral examination. Information about smoking (daily, occasional, quit, never) was obtained from the health interview. Diabetes was determined on the basis of information obtained from a health interview and a health examination. Subjects were considered to be non-diabetic if they had not been diagnosed with diabetes previously and had no indications of the disease based on the health interview and the health examination (fasting glucose less than 7.0 mmol/l, and/or the result of the glucose tolerance test [conducted in the in-depth examinations] less than 11.1 mmol/l). Rheumatoid arthritis was determined on the basis of information obtained from the health interview. The question asked was “Do you have rheumatoid arthritis diagnosed by a physician?” with the answer options being yes/no.

4.2 Variables

4.2.1 Oral health variables

Periodontal infection

The clinical oral health examinations were conducted identically in both the Health 2000 Survey and the Follow-up Study on Finnish Adults’ Oral Health. The clinical oral health examinations included an assessment of the condition of the periodontium and teeth. Subjects requiring antibiotic prophylaxis were excluded from the periodontal examinations. The oral health examinations in the Health

2000 Survey were conducted by five calibrated dentists, whereas in the Follow-up Study on Finnish Adults' Oral Health the clinical oral examinations were conducted by one dentist who was also one of the five dentists conducting the examinations in the Health 2000 Survey. The oral health examinations were conducted in a portable dental treatment unit (Dentronic Mini-Dent®, Planmeca Oy) with a portable patient chair, using a fibre optic light (Novar), a fibre optic headlamp (Tekmala Oy) and a letter scale. The examinations were conducted using a dental mirror and a WHO periodontal probe in line with the WHO instructions. (Suominen-Taipale & Vehkalahti 2008)

Periodontal pocket depth on probing was measured on four surfaces of each tooth (distobuccal, mid-buccal, mesio-oral, mid-oral), apart from the third molars and radices, always starting from the last tooth on the upper right side and finishing at the last tooth on the lower right side. Periodontal pocket depth was measured in millimetres, but was recorded categorised into three categories: no deepened periodontal pocket, a periodontal pocket 4–5 mm deep or a periodontal pocket 6 mm deep or deeper. The deepest measurement for each tooth was recorded. (Knuuttila & Suominen-Taipale 2008) For this study, two variables were formed to describe periodontal infection: the number of teeth with deepened (4 mm deep or deeper) periodontal pockets and the number of teeth with deep (6 mm deep or deeper) periodontal pockets. Periodontal infection was assessed in this study based on the presence of teeth with deepened periodontal pockets, and was used as the outcome variable in articles III, IV and V, and as the explanatory variable in article II.

In article I, the subjects were classified as periodontally healthy at the baseline if they had no teeth with deepened periodontal pockets, whereas periodontal infection at the baseline was defined as the presence of at least one tooth with deepened periodontal pocket at the baseline. The incidence of teeth with deepened periodontal pockets (the number of new teeth with deepened periodontal pockets at the follow-up examination) was used as the outcome variable to assess the development of periodontal infection among subjects who were periodontally healthy at the baseline and the progression of periodontal infection among subjects who had periodontal infection at the baseline. Also the change in pocket depth among subjects who had periodontal infection at the baseline was used as the outcome variable in article I. The change in pocket depth consisted of deepening or shallowing of existing deepened periodontal pockets, shallowing of existing deep periodontal pockets and formation of new periodontal pockets ('deepening of the pocket depth of teeth with no periodontal pockets at

the baseline'). Each tooth was given a score (no change in pocket depth was given a value of 0, any deepening of pocket depth was given a value of 1 and any reduction in pocket depth was given a value of -1) and these scores were then added together to produce one score for each subject.

In article I, the incidence of teeth with deepened periodontal pockets was used as a continuous variable in the statistical analyses. In article II, the number of teeth with deepened periodontal pockets was categorised into four categories in the statistical analyses: 0, 1–3, 4–6 and 7 or more. In articles III, IV and V, the number of teeth with deepened periodontal pockets was used as a continuous variable in the statistical analyses.

Gingival bleeding

Bleeding on probing was observed immediately after probing pocket depth measurements and was recorded by sextant. The presence of gingival bleeding was used as a secondary outcome variable in article V, and the number of bleeding sextants was used as a continuous variable in the statistical analyses.

4.2.2 Obesity

Obesity was measured primarily using BMI. Information about height and weight was primarily obtained from the clinical health examination. In some instances this was not possible, in which case information from a questionnaire was used (Heistaro 2008). BMI at the time of the Health 2000 Survey was used as an explanatory variable in article I and as an outcome variable in article II. In articles III and IV BMI was used as a confounding variable in the main analyses as well as an explanatory variable in the secondary analyses. In article V BMI was used as a confounding variable. BF% and WC were used as secondary measurements of obesity in article II. BF% was measured using a high-precision body composition analyser, an eight-polar tactile-electrode impedance meter InBody 3.0 (Biospace Co., Ltd., Söul, Korea). Subjects with pacemakers were not tested (Heistaro 2008). WC was measured in a standing position from the mid-point between the lowest rib bones and the high point of the iliac crest (Heistaro 2008). BF% and WC-defined obesity were used as outcome variables in article II.

In article I, BMI was categorised into three categories according to the WHO criteria of overweight: less than 25.0 (normal weight), 25.0–29.9 (overweight) and 30.0 or more (obesity). BMI was also used as a continuous variable in article

I. In article II, BMI was categorised dichotomously according to the WHO criteria of obesity: a BMI of less than 30.0 vs. 30.0 or over. Different cut-off values of BF%-defined obesity and WC-defined obesity were used for men and women; men were defined to be obese if their BF% was more than 25 per cent or their WC was 102 cm or more, and women if their BF% was more than 35 per cent or their WC was 88 cm or more. BMI was used as a continuous variable in articles III and V. In article IV, BMI was used as a continuous variable in the main analyses. In the secondary analyses, as well as being used as a continuous variable, body weight was categorised into five categories: less than 22.4, 22.4–23.9, 24.0–25.8, 25.9–28.6 and 28.7 or more. For the stratified analyses in article IV, BMI was categorised into three categories, less than 25.0, 25.0–29.9 and 30.0 or more.

4.2.3 Serum markers

Serum TNF- α and IL-6

During the in-depth examinations examinations of the Health 2000 Survey approximately one year later, blood samples including serum cytokines TNF- α and IL-6 were collected. Serum levels of TNF- α and IL-6 were analysed with a solid phase, enzyme-labelled chemiluminescent immunometric assay using an analyser Immulite (Siemens Healthcare Diagnostics, Deerfield, IL, USA) in the laboratory of the National Institute for Health and Welfare (THL, former National Public Health Institute [KTL]). The detection limits of the assays were 1,5 ng/l for TNF- α and 0.5 ng/l for IL-6. The inter-assay coefficient of variation (CV) of TNF- α varied from 2.5 per cent (high-level control, 500 ng/l) to 11.0 per cent (low-level control, 7 ng/l). Respectively, the inter-assay CV of IL-6 varied from 5.0 per cent (high-level control, 500 ng/l) to 7.0 per cent (low-level control, 21 ng/l).

Serum levels of TNF- α and IL-6 (as continuous variables) were used as explanatory variables in the main analyses and as confounding variables in the secondary analyses of article III. Logarithmic transformation of serum TNF- α and IL-6 levels were done before the multivariate analyses to normalise the skewed distributions of serum TNF- α and IL-6.

Serum triglycerides, HDL-C and LDL-C

Serum triglycerides were analysed enzymatically (Olympus System Reagent, Olympus Life Science Research Europa GmbH, Munich, Germany). The inter-assay CV was 2.1 per cent. Serum levels of HDL-C and LDL-C were analysed using direct methods based on immunocomplex separation followed by enzymatic cholesterol determination (Roche Diagnostics, Mannheim, Germany). The inter-assay CV of HDL-C analyses was 4.8 per cent, and of LDL-C analyses, 4.5 per cent. The analyses were done on an Olympus AU400 (Olympus Diagnostica GmbH, Hamburg, Germany) clinical chemistry autoanalyser in the Research and Development Centre of the Social Insurance Institution of Finland (KELA). (Heistaro 2008)

In article IV, serum levels of triglycerides, HDL-C and LDL-C were used as explanatory variables in the main analyses, and as confounding variables in the secondary analyses. Serum triglyceride, HDL-C and LDL-C levels were used as categorised variables and also as continuous variables. Serum triglyceride level was categorised into quintiles as follows: less than 0.80 mmol/l, 0.80–0.99 mmol/l, 1.00–1.29 mmol/l, 1.30–1.79 mmol/l and 1.80 mmol/l or more. Serum HDL-C level was categorised in the same manner: less than 1.09 mmol/l, 1.09–1.25 mmol/l, 1.26–1.44 mmol/l, 1.45–1.69 mmol/l and 1.70 mmol/l or more. Serum LDL-C level was also categorised into quintiles: less than 2.70 mmol/l, 2.70–3.16 mmol/l, 3.17–3.63 mmol/l, 3.64–4.27 mmol/l and 4.28 mmol/l or more.

4.2.4 Statin medication

Information about statin medication was obtained from the interview. Statin medication was used as an explanatory variable in article V. Statin medication was categorised in two ways for the statistical analyses: first, subjects with statin medication of some sort ($n = 134$) vs. those with none, and second, subjects who use either simvastatin ($n = 58$), atorvastatin ($n = 38$), some other statin ($n = 38$), or no statin medication.

In article IV, lipid-lowering medication was used as a confounding variable. This variable incorporated also other lipid medications in addition to statins, and was categorised into three categories: no lipid medication, lipid medication of some sort and no information.

4.2.5 Socio-economic factors

The socio-economic factors used in this study included gender, age and education. Age was used as a continuous variable in the statistical analyses. Information about education was obtained from the interview and was categorised into three categories. The lowest level of education included those who had less than a high school education and did not have formal vocational qualifications. The intermediate level of education included those who had graduated from high school or a vocational school. The highest level of education included those who had a university degree or who had graduated from polytechnics.

4.2.6 Number of teeth

The total number of teeth was counted during the clinical oral health examinations. The number of teeth was used as a confounding variable in all of the original articles. The number of teeth (continuous variable) was treated as the offset variable in the statistical analyses in articles I, III, IV and V. In article II, the number of teeth was categorised into three categories: 1–20, 21–25 and 26 or more.

4.2.7 Level of oral hygiene, oral health behavioural factors and periodontal treatment during the follow-up

The presence of dental plaque was used to assess the level of oral hygiene. It was measured during the clinical oral examination using a modified version of the method described by Sillness & Løe (1964). The presence of plaque was measured from three indicator teeth on one surface each as follows: the buccal surface of the most posterior tooth on the upper right side, the lingual surface of the most posterior tooth on the lower left side and the buccal surface of the left lower canine. Each indicator teeth was given a score ranging from zero to two, zero indicating no visible dental plaque, one indicating visible plaque on gingival margins only and two indicating visible plaque also elsewhere. The highest score of any of the indicator teeth described the subject's plaque status. The presence of dental plaque was used as a secondary explanatory variable in article II and as a confounding variable in articles I, III, IV and V.

The oral health behavioural factors used as variables in this study were dental attendance pattern and toothbrushing frequency. They were both based on self-

reported information obtained during the interview. Dental attendance pattern was measured with the question: “Do you usually go to a dentist?” The answer options were: “1. Regularly for a check-up; 2. Only when you have toothache or some other trouble; and 3. Never.” Answer options two and three were combined for the data analyses. Toothbrushing frequency was measured with the question “How often do you usually brush your teeth?” The answer options were: “1. More often than twice a day; 2. Twice a day; 3. Once a day; 4. Less frequently than every day; and 5. Never.” Answer options one and two, and also four and five were combined to yield a three-class variable for the statistical analyses. Dental attendance pattern and toothbrushing frequency were used as confounding variables in articles I, IV and V.

Information about periodontal treatment during the follow up-period was obtained from a postal questionnaire. Periodontal treatment (scaling and root planing) (no/yes/missing information) during the last 12 months of the follow-up period was used as a confounding variable in article I.

4.2.8 Self-reported health

Information about perceived health was obtained from the interview. The question posed was “Is your present state of health 1. Good; 2. Fairly good; 3. Moderate; 4. Fairly poor; or 5. Poor?” For the statistical analyses, answer options good and fairly good as well as moderate, fairly poor and poor were combined to get a dichotomous variable. Self-reported health was used as a confounding variable in article II.

4.2.9 Frequency of physical exercise

Information about physical exercise was obtained from the questionnaire and was measured with a question “How often do you exercise in your leisure time so that you are at least slightly out of breath and sweating?”, with the answer options being: “1. Daily; 2. Four to six times a week; 3. Two to three times a week; 4. Once a week; 5. Two to three times a month; 6. Few times a year or even more rarely.” Answer options one and two as well as five and six were combined for the statistical analyses. Frequency of physical exercise was used as a confounding variable in article II.

4.2.10 Diet

Information about diet was obtained from a validated (Männistö *et al.* 1996, Paalanen *et al.* 2006) food frequency questionnaire. The aim of the food frequency questionnaire was to assess the subjects' whole diet over the previous 12 months, and it consisted of 128 food items. The items were grouped under 12 subheadings. There were nine response options for each item which ranged from "never or rarely" to "six or more times per day". The portion sizes were fixed and, if possible, specified using natural units such as cups of coffee, for instance. Daily food consumption was then divided into components using the Finnish national food composition database. Daily energy intake (in kcal/day) and the proportions of fats, carbohydrates and proteins in daily energy intake were used as confounding variables in article II. They were used as continuous variables in the statistical analyses.

4.2.11 Smoking and alcohol consumption

Information about cigarette smoking was obtained from the interview. In articles I, III and IV the study population was restricted to never-smokers, and in article V, to non-smokers. In article II, in addition to analyses among never-smokers, smoking was used as a confounding variable in analyses among the total population, and it was categorised into four categories: daily, occasional, quitted and never.

Information about alcohol consumption was obtained from the questionnaire. The estimated amount of alcohol consumption was used as a confounding variable in article IV, and it was used as a continuous variable (g/week) in the statistical analyses.

4.3 Statistical methods

Due to the two-stage cluster sampling design used in the Health 2000 Survey, weights based on post-stratification with gender, age and region were used for the data sample of articles II, IV and V. Weights were not used for the data sample of article I, because this was not necessary due to the sampling design of the Follow-up Study on Finnish Adults' Oral Health. Weights were neither used for the data sample of article III, because this study was based on a sample of subjects

participating in the supplemental examinations of the Health 2000 Survey, and the use of the weights was therefore not necessary.

Results in this thesis are presented mainly as risk estimates with 95% confidence intervals (CI), which is in accordance with the recommendations of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (ICMJE 2010). Incidence rate ratios (IRR) with 95% CIs were estimated using Poisson regression models with the DSCALE option in article I. Linear regression analyses of the association between body weight and the change in pocket depth were done in article I using generalised linear models with an identity link function and normal distribution. In article II, where there were dichotomous outcome variables, odds ratios (OR) with 95% CIs were estimated using logistic regression models. Relative risks (RR) with 95% CIs were estimated using Poisson regression models in articles III and IV. In article V, negative binomial and Poisson regression models were used to estimate RRs with 95% CIs in the main analyses among the total study population, whereas Poisson regression models alone were used in the secondary analyses where periodontally healthy subjects were excluded.

In article I, analyses were conducted among two subpopulations, namely subjects who were periodontally healthy at the baseline *vs.* subjects who had periodontal infection at the baseline. Stratified analyses by gender and additional analyses among never-smokers were conducted in article II. Stratified analyses by BMI were conducted in article IV. In article V, analyses were conducted among the total study population and, in addition, among a subpopulation where subjects with no teeth with pathologically deepened periodontal pockets were excluded to also assess the association of statin medication with the extent of periodontal infection.

Statistical analyses were done using SUDAAN statistical package version 9.0.1 (Research Triangle Institute, Raleigh, NC, USA) in articles II, III, IV and V to take into account the two-stage cluster sampling design of the Health 2000 Survey. Data analyses for article I were done using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

4.4 Ethical considerations

Participation in the Health 2000 Survey and the Follow-up Study on Finnish Adults' Oral Health was voluntary, and written, informed consent was obtained

from the participants. Both the Health 2000 Survey and the Follow-up Study on Finnish Adults' Oral Health were approved by the Ethical Committee for Epidemiology and Public Health of the Hospital District of Helsinki and Uusimaa.

Table 1. Basic characteristics of the subjects¹ of the Health 2000 Survey aged 30–49 years; proportions/means and their standard errors (in parentheses) in the total population and in the categories of the number of teeth with deepened (4 mm deep or deeper) periodontal pockets.

Variable	Teeth with periodontal pockets ≥ 4 mm				
	Total (n = 2,784)	0 (n = 1,143)	1–3 (n = 708)	4–6 (n = 351)	≥ 7 (n = 582)
Age (mean) (SE) (n = 2,784)	39.8 (0.1)	38.8 (0.2)	40.2 (0.2)	40.3 (0.3)	40.9 (0.3)
Gender (%) (SE) (n = 2,784)					
Male	49.9 (0.9)	40.4 (1.5)	49.2 (1.9)	55.4 (2.5)	65.4 (1.9)
Female	50.1 (0.9)	59.6 (1.5)	50.8 (1.9)	44.6 (2.5)	34.6 (1.9)
Education (%) (SE) (n = 2,773)					
Basic	18.3 (0.8)	15.4 (1.0)	16.8 (1.4)	18.5 (2.1)	25.2 (2.0)
Intermediate	41.9 (1.0)	38.8 (1.4)	40.6 (1.9)	42.2 (2.5)	49.1 (1.9)
Higher	39.9 (0.9)	45.8 (1.5)	42.6 (1.7)	39.3 (2.5)	25.7 (1.8)
Number of teeth (%) (SE) (n = 2,784)					
1–20	7.5 (0.5)	7.5 (0.7)	7.4 (1.0)	7.7 (1.4)	7.6 (1.0)
21–25	9.8 (0.6)	8.3 (0.9)	10.9 (1.2)	9.3 (1.6)	11.7 (1.5)
≥ 26	82.7 (0.8)	84.2 (1.1)	81.7 (1.5)	83.0 (2.0)	80.8 (1.8)
Smoking (%) (SE) (n = 2,773)					
Daily	29.6 (0.9)	22.5 (1.2)	24.6 (1.8)	34.7 (2.7)	45.8 (2.4)
Occasional	6.6 (0.5)	6.1 (0.7)	7.1 (0.9)	7.5 (1.4)	6.3 (1.0)
Quitted	17.6 (0.7)	18.3 (1.2)	16.1 (1.4)	17.8 (1.9)	17.6 (1.6)
Never	46.3 (0.9)	53.1 (1.5)	52.1 (1.9)	40.0 (2.6)	30.2 (2.3)
Body mass index (%) (SE) (n = 2,783)					
Less than 25.0	47.1 (1.0)	51.5 (1.6)	47.2 (1.7)	44.6 (2.8)	40.3 (2.0)
25.0–29.9	37.5 (0.9)	35.8 (1.5)	38.5 (1.7)	40.1 (2.6)	38.0 (1.9)
30.0 or more	15.4 (0.7)	12.7 (1.0)	14.3 (1.3)	15.3 (2.0)	21.7 (1.9)

¹ Dentate subjects, periodontal examination conducted.

5 Results

5.1 Association of obesity with the development of periodontal infection (I)

The incidence of teeth with deepened (4 mm deep or deeper) periodontal pockets over the about-four-year follow-up period among subjects who were periodontally healthy (no teeth with deepened periodontal pockets) in the baseline examinations was 3.0 for normal-weight, 3.6 for overweight and 3.7 for obese subjects, respectively. The IRRs with 95% CIs are presented in Table 2.

Table 2. Association between body mass index (BMI) and incidence of teeth with deepened (4 mm deep or deeper) periodontal pockets during a four-year follow-up among subjects who were periodontally healthy (no teeth with deepened periodontal pockets) in the baseline examinations. Unadjusted and adjusted¹ incidence rate ratios (IRR) with 95% confidence intervals (CI).

BMI	Teeth with periodontal pockets \geq 4 mm	
	Unadjusted IRR (95% CI) n = 151	Adjusted IRR (95% CI) n = 145
Less than 25.0	1.0	1.0
25.0–29.9	1.3 (0.9–2.0)	1.2 (0.7–1.8)
30.0 or more	1.4 (0.8–2.3)	1.3 (0.7–2.1)
Continuous variable	1.05 (1.01–1.09)	1.04 (0.99–1.08)

¹ Adjusted for gender, age (continuous variable), education, presence of dental plaque, dental attendance pattern, toothbrushing frequency, periodontal treatment and number of teeth (offset variable).

Among subjects who had periodontal infection (teeth with deepened periodontal pockets) at the baseline, the incidences of teeth with deepened periodontal pockets over the four-year follow-up for normal weight, overweight and obese subjects were 3.3, 3.6 and 3.5, respectively. The corresponding IRRs with 95% CIs are presented in Table 3. There were no statistically significant associations of overweight and obesity with the change in periodontal pocket depth at the site level (For more detailed information, see Article I, Table 7).

Table 3. Association between body mass index (BMI) and incidence of teeth with deepened (4 mm deep or deeper) periodontal pockets during a four-year follow-up among subjects who had periodontal infection (teeth with deepened periodontal pockets) in the baseline examinations. Unadjusted and adjusted¹ incidence rate ratios (IRR) with 95% confidence intervals (CI).

BMI	Teeth with periodontal pockets \geq 4 mm	
	Unadjusted IRR (95% CI) n = 228	Adjusted IRR (95% CI) n = 207
Less than 25.0	1.0	1.0
25.0–29.9	1.1 (0.8–1.5)	1.0 (0.7–1.4)
30.0 or more	1.2 (0.8–1.7)	1.1 (0.8–1.7)
Continuous variable	1.01 (0.98–1.04)	1.01 (0.97–1.04)

¹ Adjusted for gender, age (continuous variable), education, presence of dental plaque, dental attendance pattern, toothbrushing frequency, periodontal treatment and number of teeth (offset variable).

5.2 Association of periodontal infection with obesity (II)

Having periodontal infection (teeth with deepened [4 mm deep or deeper] periodontal pockets) was found to be associated with obesity (BMI 30.0 or more) after adjusting for confounding factors such as gender, age, education, number of teeth, smoking, frequency of physical exercise, self-reported health, daily energy intake and the proportions of fats, carbohydrates and proteins in daily energy intake. The association was found also among a subpopulation of never-smokers (Table 4).

Table 4. Association between the number of teeth with deepened (4 mm deep or deeper) periodontal pockets and obesity (body mass index 30 or more). Unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (CI).

Number of teeth with deepened periodontal pockets	Obesity	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Total population ¹	n = 2,783	n = 2,571
0	1.0	1.0
1–3	1.1 (0.9–1.5)	1.2 (0.9–1.6)
4–6	1.2 (0.9–1.8)	1.3 (0.9–1.9)
7 or more	1.9 (1.4–2.5)	1.8 (1.3–2.4)
Never-smokers ²	n = 1,296	n = 1,217
0	1.0	1.0
1–3	1.5 (1.0–2.3)	1.5 (1.0–2.3)
4–6	1.4 (0.8–2.4)	1.3 (0.7–2.4)
7 or more	2.5 (1.7–3.8)	2.4 (1.5–3.8)

¹Adjusted for gender, age (continuous variable), education, number of teeth, smoking, frequency of physical exercise, self-reported health, daily energy intake (continuous variable) and proportions of fats, carbohydrates and proteins in daily energy intake (continuous variables), ²Adjusted for gender, age (continuous variable), education, number of teeth, frequency of physical exercise, self-reported health, daily energy intake (continuous variable) and proportions of fats, carbohydrates and proteins in daily energy intake (continuous variables).

An association between the number of teeth with deepened periodontal pockets and BMI was found among both men and women in the stratified analyses according to gender, although the association was weaker among women. The association was also found among both genders when the stratified analyses were conducted among a subpopulation of never-smokers (Table 5).

Table 5. Association between the number of teeth with deepened (4 mm deep or deeper) periodontal pockets and obesity (body mass index 30 or more), stratified according to gender. Adjusted odds ratios (OR) with 95% confidence intervals (CI).

Number of teeth with deepened periodontal pockets	Obesity	Obesity
	OR (95% CI)	OR 95% CI
Total population ¹		
	Among men (effective n = 1,176)	Among women (effective n = 1,395)
0	1.0	1.0
1–3	1.2 (0.8–2.0)	1.2 (0.8–1.9)
4–6	1.5 (0.9–2.5)	1.2 (0.7–2.0)
7 or more	2.2 (1.4–3.4)	1.3 (0.8–2.2)
Never-smokers ²		
	Among men (effective n = 468)	Among women (effective n = 749)
0	1.0	1.0
1–3	1.3 (0.6–2.6)	1.7 (0.9–3.1)
4–6	1.4 (0.6–3.3)	1.3 (0.5–3.0)
7 or more	2.8 (1.4–5.4)	2.0 (0.9–4.2)

¹ Adjusted for age (continuous variable), education, number of teeth, smoking, frequency of physical exercise, self-reported health, daily energy intake (continuous variable) and proportions of fats, carbohydrates and proteins in daily energy intake (continuous variables), ² Adjusted for age (continuous variable), education, number of teeth, frequency of physical exercise, self-reported health, daily energy intake (continuous variable) and proportions of fats, carbohydrates and proteins in daily energy intake (continuous variables).

The presence of dental plaque was found to be associated with BMI weakly and inconsistently. A similar pattern was also found when this association was studied in stratified analyses according to gender, and also among a subpopulation of never-smokers (Table 6).

Table 6. Association between the presence of dental plaque and obesity (body mass index 30 or more). Adjusted odds ratios (OR) with 95% confidence intervals (95% CI).

Presence of dental plaque	Obesity OR (95% CI)	Obesity OR (95% CI)	Obesity OR (95% CI)
Total population ¹			
	Total (effective n = 2,565)	Among men (effective n = 1,171)	Among women (effective n = 1,394)
None	1.0	1.0	1.0
On gingival margins only	1.3 (1.0–1.7)	1.3 (0.9–1.9)	1.2 (0.9–1.7)
Also elsewhere	1.4 (0.9–2.2)	1.5 (0.9–2.6)	1.0 (0.5–2.1)
Never-smokers ²			
	Total (effective n = 1,214)	Among men (effective n = 466)	Among women (effective n = 748)
None	1.0	1.0	1.0
On gingival margins only	1.4 (1.0–2.0)	1.5 (0.7–3.1)	1.3 (0.8–2.1)
Also elsewhere	1.2 (0.6–2.5)	1.1 (0.4–3.0)	1.5 (0.6–3.9)

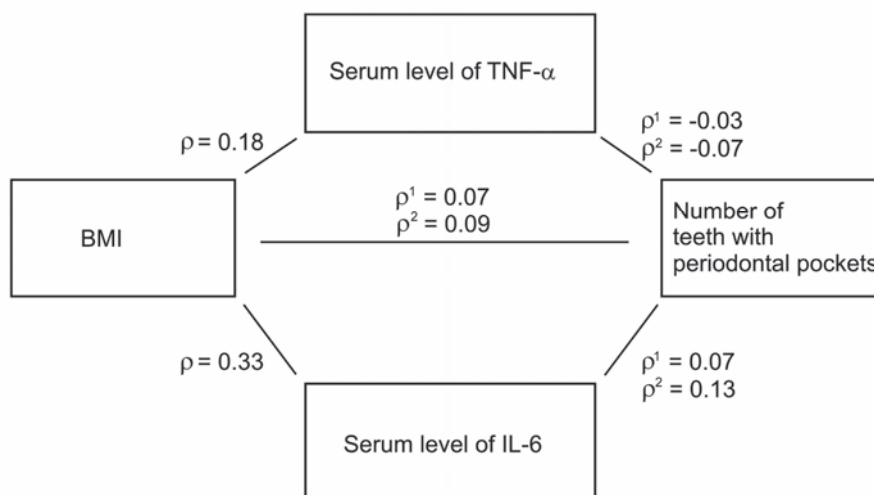
¹ Adjusted for gender (not in stratified analyses according to gender), age (continuous variable), education, number of teeth, smoking, frequency of physical exercise, self-reported health, daily energy intake (continuous variable) and proportions of fats, carbohydrates and proteins in daily energy intake (continuous variables), ² Adjusted for gender (not in stratified analyses according to gender), age (continuous variable), education, number of teeth, frequency of physical exercise, self-reported health, daily energy intake (continuous variable) and proportions of fats, carbohydrates and proteins in daily energy intake (continuous variables).

The number of teeth with deepened periodontal pockets was found to be associated with a high BF% and a large WC among never-smokers. This association was found among both genders, although it was more inconsistent among women (For more detailed information, see Article II, Tables 4 & 5).

5.3 Serum cytokines and serum lipids

5.3.1 Serum TNF- α and IL-6 in the association between body weight and periodontal infection (III)

The mean serum concentrations of TNF- α and IL-6 with their standard deviations (in parentheses) were 5.7 ng/l (6.1) and 1.5 ng/l (1.2), respectively. Spearman's correlation coefficients between the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets, BMI and the serum levels of TNF- α and IL-6 are presented in Figure 1.



¹Number of teeth with periodontal pockets of 4 mm deep or deeper.

²Number of teeth with periodontal pockets of 6 mm deep or deeper.

Fig. 3. Spearman's correlation coefficients (ρ) between the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets, body mass index (BMI) and serum levels of tumour necrosis factor α (TNF- α) and interleukin 6 (IL-6) (reprinted with permission from John Wiley & Sons A/S).

The serum level of IL-6 was found to be associated with the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets after adjusting for potential confounders such as gender, age, education and the presence of dental plaque. No consistent association between the serum level of TNF- α and the number of teeth with deepened or deep periodontal pockets was found (For more detailed information, see Article III, Table 2).

BMI was associated with the number of teeth with both deepened and deep periodontal pockets. Adding the serum level of TNF- α to the regression model did not have an effect on the strength of these associations (Table 7). When the serum level of IL-6 was added to the regression model, the strength of the association between BMI and the number of teeth with deep periodontal pockets was slightly weakened, but it had no essential effect on the strength of the association between BMI and the number of teeth with deepened periodontal pockets (Table 7).

Table 7. Association between body mass index (BMI) and the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets. Adjusted relative risks (RR) with 95% confidence intervals (CI).

BMI	Teeth with periodontal pockets	Teeth with periodontal pockets
	≥ 4 mm RR (95% CI)	≥ 6 mm RR (95% CI)
Model 1 ^a (effective n = 425)	1.03 (1.00–1.06)	1.04 (0.94–1.14)
Model 2 ^b (effective n = 425)	1.03 (1.00–1.06)	1.04 (0.94–1.14)
Model 3 ^c (effective n = 425)	1.03 (1.00–1.06)	1.02 (0.93–1.13)

^a Adjusted for gender, age (continuous variable), education, presence of dental plaque and number of teeth (offset variable), ^b Adjusted for gender, age (continuous variable), education, presence of dental plaque, number of teeth (offset variable) and serum tumour necrosis factor α level (log-transformed value, continuous variable), ^c Adjusted for gender, age (continuous variable), education, presence of dental plaque, number of teeth (offset variable) and serum interleukin 6 level (log-transformed value, continuous variable).

5.3.2 Serum lipids in the association between body weight and periodontal infection (IV)

The mean serum concentrations of triglycerides, HDL-C and LDL-C with their standard errors (in parentheses) were 1.4 mmol/l (0.03), 1.4 mmol/l (0.01) and 3.5 mmol/l (0.03), respectively. There were no consistent associations between serum levels of triglycerides, HDL-C or LDL-C and the number of teeth with deepened (4 mm deep or deeper) or deep (6 mm deep or deeper) periodontal pockets after adjusting for confounding factors such as gender, age, education, toothbrushing frequency, dental attendance patterns, presence of dental plaque, lipid medication, alcohol consumption and BMI. Omitting BMI from the regression model had only a slight impact on the estimates (For more detailed information, see Article IV, Table 3).

Stratified analyses according to body weight were conducted. No clear associations were found between serum levels of triglycerides, HDL-C or LDL-C and the number of teeth with deepened periodontal pockets among normal-weight subjects (BMI less than 25.0). There were elevated estimates with high triglyceride levels and low HDL-C levels among overweight (BMI 25.0–29.9) and obese (BMI 30.0 or more) subjects, but the associations were not consistent.

An association was found between BMI and the number of teeth with deepened periodontal pockets also in this subpopulation of dentate, non-diabetic

subjects aged 30–49 years who had never smoked. Adding the serum levels of triglycerides and HDL-C to the regression model slightly weakened the strength of the association. On the other hand, adding the serum level of LDL-C had virtually no effect on the strength of this association (Table 8).

Table 8. Association between body mass index (BMI) and the number of teeth with deepened (4 mm deep or deeper) periodontal pockets, adjusted relative risks (RR) with 95% confidence intervals (CI).

BMI	Teeth with periodontal pockets \geq 4 mm RR (95% CI)
Model 1^a (effective n = 1,239)	
I quintile (lowest)	1.0
II quintile	1.1 (0.8–1.5)
III quintile	1.1 (0.9–1.4)
IV quintile	1.3 (1.0–1.7)
V quintile (highest)	1.4 (1.1–1.8)
Continuous variable	1.03 (1.01–1.04)
Model 2^b (effective n = 1,236)	
I quintile	1.0
II quintile	1.1 (0.8–1.5)
III quintile	1.1 (0.9–1.4)
IV quintile	1.3 (0.9–1.6)
V quintile	1.3 (1.0–1.5)
Continuous variable	1.02 (1.00–1.04)
Model 3^c (effective n = 1,236)	
I quintile	1.0
II quintile	1.1 (0.8–1.4)
III quintile	1.1 (0.8–1.4)
IV quintile	1.3 (1.0–1.7)
V quintile	1.3 (1.0–1.7)
Continuous variable	1.02 (1.00–1.04)
Model 4^d (effective n = 1,230)	
I quintile	1.0
II quintile	1.1 (0.9–1.5)
III quintile	1.1 (0.9–1.4)
IV quintile	1.3 (1.0–1.7)
V quintile	1.4 (1.1–1.8)
Continuous variable	1.03 (1.01–1.04)

^a Adjusted for gender, age (continuous variable), education, toothbrushing frequency, dental attendance pattern, presence of dental plaque, lipid medication, alcohol consumption (continuous variable) and number of teeth (offset variable), ^b Adjusted for gender, age (continuous variable), education, toothbrushing frequency, dental attendance pattern, presence of dental plaque, lipid medication, alcohol consumption (continuous variable), number of teeth (offset variable) and serum level of triglycerides (continuous variable), ^c Adjusted for gender, age (continuous variable), education, toothbrushing frequency, dental attendance pattern, presence of dental plaque, lipid medication, alcohol consumption (continuous variable) and serum level of HDL-C (continuous variable), ^d Adjusted for gender, age (continuous variable), education, toothbrushing frequency, dental attendance pattern, presence of dental plaque, lipid medication, alcohol consumption (continuous variable), number of teeth (offset variable) and serum level of LDL-C (continuous variable).

5.4 Association between statin medication and periodontal infection (V)

Use of statin medication was found to be inversely associated with the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets after controlling for potential confounding factors such as gender, age, education, presence of dental plaque, dental attendance pattern, toothbrushing frequency, BMI and the number of teeth (offset variable), the inverse association being stronger between statin medication and the number of teeth with deep periodontal pockets (Table 9). A similar inverse association was also found in the secondary analyses, where subjects with no teeth with deepened periodontal pockets were excluded (Table 9). Of the specific statin medications, simvastatin was found to be most strongly inversely associated with the number of teeth with deepened and deep periodontal pockets (Table 9). Statin medication was also found to be weakly inversely associated with gingival bleeding (the number of bleeding sextants) (adjusted RR 0.8 [95% CI 0.7–1.0]).

Table 9. Association between statin medication and the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets. Unadjusted and adjusted¹ relative risks (RR) with 95% confidence intervals (CI).

Statin medication	Teeth with periodontal pockets \geq 4 mm		Teeth with periodontal pockets \geq 6 mm	
	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)
Total population ²				
	n = 2,032	n = 1,949	n = 2,032	n = 1,949
No	1.0	1.0	1.0	1.0
Yes	1.0 (0.8–1.3)	0.9 (0.7–1.2)	0.9 (0.5–1.5)	0.7 (0.4–1.1)
Subjects with number of teeth with periodontal pockets > 0 ^a				
	n = 1,294	n = 1,248	n = 1,294	n = 1,248
No	1.0	1.0	1.0	1.0
Yes	1.0 (0.8–1.2)	0.9 (0.7–1.1)	0.8 (0.5–1.3)	0.6 (0.3–1.0)
Total population ²				
	n = 2,032	n = 1,949	n = 2,032	n = 1,949
No statin	1.0	1.0	1.0	1.0
Simvastatin	1.0 (0.7–1.4)	0.8 (0.6–1.2)	0.6 (0.3–1.3)	0.4 (0.2–1.0)
Atorvastatin	1.1 (0.8–1.6)	1.1 (0.7–1.6)	0.7 (0.3–1.8)	0.8 (0.3–1.8)
Other statin	1.0 (0.6–1.6)	0.8 (0.5–1.3)	1.5 (0.6–3.9)	1.1 (0.5–2.4)
Subjects with number of teeth with periodontal pockets > 0 ^a				
	n = 1,294	n = 1,248	n = 1,294	n = 1,248
No statin	1.0	1.0	1.0	1.0
Simvastatin	1.0 (0.8–1.3)	0.9 (0.7–1.1)	0.7 (0.3–1.5)	0.4 (0.2–0.9)
Atorvastatin	1.0 (0.7–1.4)	0.9 (0.6–1.3)	0.7 (0.3–1.7)	0.6 (0.2–1.4)
Other statin	1.0 (0.7–1.5)	0.9 (0.6–1.3)	1.1 (0.5–2.4)	0.9 (0.4–2.1)

¹ Adjusted for gender, age (continuous variable), education, presence of dental plaque, dental attendance pattern, toothbrushing frequency, body mass index (continuous variable) and number of teeth (offset variable), ² Negative binomial regression models used, ^a Poisson regression models used.

Possible interaction between statin medication and the other covariates was studied by adding the product terms to the regression model one by one. An interaction between statin medication and the presence of dental plaque was found (statistically significant product term for the number of teeth with deepened periodontal pockets [$p = 0.004$]). Due to the perceived interaction, stratified analyses according to the presence of dental plaque were conducted. The inverse association between statin medication and the number of teeth with deepened and deep periodontal pockets was stronger among subjects with plaque, whereas among subjects with no dental plaque, statin medication was found to be weakly

associated with an increased likelihood of having teeth with deepened and deep periodontal pockets (Table 10).

Table 10. Association between statin medication (no/yes) and the number of teeth with deepened (4 mm deep or deeper) periodontal pockets, stratified according to the presence of dental plaque. Adjusted¹ relative risks (RR) with 95% confidence intervals (CI).

Statin medication	Teeth with periodontal pockets \geq 4 mm		
	No plaque RR (95% CI)	Plaque on gingival margins only RR (95% CI)	Plaque also elsewhere RR (95% CI)
Total population ²	effective n = 821	effective n = 944	effective n = 184
No	1.0	1.0	1.0
Yes	1.2 (0.8–1.8)	0.6 (0.5–0.9)	0.8 (0.5–1.2)
Subjects with number of teeth with periodontal pockets $>$ 0 ^a	effective n = 430	effective n = 666	effective n = 152
No	1.0	1.0	1.0
Yes	1.2 (0.9–1.7)	0.7 (0.6–0.9)	0.8 (0.6–1.1)

¹ Adjusted for gender, age (continuous variable), education, dental attendance pattern, toothbrushing frequency, body mass index (continuous variable) and number of teeth (offset variable), ² Negative binomial regression models used, ^a Poisson regression models used.

Stratified analyses were also conducted according to the presence of gingival bleeding, based on the findings of the stratified analyses according to the presence of dental plaque. Statin medication was found to be inversely associated with the number of teeth with deepened (4 mm deep or deeper) periodontal pockets among subjects with gingival bleeding in one or more sextants (Table 11). Among subjects with no gingival bleeding in any sextants, statin medication was found to be associated with an increased likelihood of having teeth with deepened periodontal pockets (Table 11). Statin medication was found to be associated similarly, although more strongly, with the number of teeth with deep (6 mm deep or deeper) periodontal pockets.

Table 11. Association between statin medication (no/yes) and the number of teeth with deepened (4 mm deep or deeper) periodontal pockets, stratified according to the presence of gingival bleeding. Adjusted¹ relative risks (RR) with 95% confidence intervals (CI).

Statin medication	Teeth with periodontal pockets \geq 4 mm	
	Among subjects with no gingival bleeding in any sextants	Among subjects with gingival bleeding in one or more sextants
	RR (95% CI)	RR (95% CI)
Total population ²	effective n = 494	effective n = 1,453
No	1.0	1.0
Yes	1.7 (1.0–2.8)	0.9 (0.7–1.1)
Subjects with number of teeth with periodontal pockets > 0 ^a	effective n = 175	effective n = 1,071
No	1.0	1.0
Yes	1.7 (1.2–2.5)	0.8 (0.7–1.0)

¹ Adjusted for gender, age (continuous variable), education, presence of dental plaque, dental attendance pattern, toothbrushing frequency, body mass index (continuous variable) and number of teeth (offset variable), ² Negative binomial regression models used, ^a Poisson regression models used.

6 Discussion

6.1 The nature of the association between obesity and periodontal infection

Periodontitis is an infectious disease in which immune responses play an important role. Therefore it is not surprising that many systemic conditions affecting inflammatory status have been suggested to be related to the development of periodontitis. These include overweight and obesity, which, based on the results of many recent cross-sectional studies, have been found to be associated with periodontal infection (Al-Zahrani *et al.* 2003, Dalla Vecchia *et al.* 2005, Ekuni *et al.* 2008, Genco *et al.* 2005, Haffajee & Socransky 2009, Han *et al.* 2010, Kim *et al.* 2011, Khader *et al.* 2009, Linden *et al.* 2007, Nishida *et al.* 2005, Pataro *et al.* 2011, Reeves *et al.* 2006, Saito *et al.* 2001, Saito *et al.* 2005, Wood *et al.* 2003, Ylöstalo *et al.* 2003). In this study, the association between obesity and periodontal infection was investigated in a longitudinal setting in order to obtain evidence about the nature of this association. As expected, in this study overweight and obesity were associated with the incidence of deepened (4 mm deep or deeper) periodontal pockets. These results are in line with the findings of previous cross-sectional studies which have suggested that obesity is a risk factor for periodontal infection, although the strength of the association was slightly weaker than that presented in two recent meta-analyses (Chaffee & Weston 2010, Suvan *et al.* 2011).

The results of the present study are also in accordance with another longitudinal study on this association (Morita *et al.* 2011), which was published shortly after the publication of the article I of this thesis. That study included a total of 2,787 men and 803 women, and periodontal disease was assessed using the Community Periodontal Index, where the cut-off value for pathological pocket depth was 4 mm and pocket depths were examined around 10 indicator teeth. Morita and colleagues (2011) reported an exposure-response association between BMI and the development of periodontal pockets 4 mm deep or deeper after a five-year follow-up. The exposure-response pattern of the association found in both studies lends further support to the role of high body weight as a risk for periodontal infection. However, compared with commonly accepted risk factors for periodontal infection, such as smoking and type II diabetes mellitus,

for instance, it seems unlikely that obesity plays a major role in the development of periodontal infection.

Until this study, evidence of the relation of obesity to periodontal infection has been based on cross-sectional studies. However, the cross-sectional study design prevents making inferences about causality, because these studies are carried out at one time point, and in many cases they give no indication of the temporal sequence of the events, *i.e.* whether exposure occurred before, after or during the onset of the disease outcome. This means the direction of the relation between obesity and periodontitis may also be opposite to what is expected. It must be added that a biologically plausible explanation for the possible role of periodontal infection in obesity exists, which is the reason why in this study the relation of periodontal infection to obesity was examined. The results of this study showed that the number of teeth with deepened periodontal pockets was associated with BMI-defined obesity in an exposure-response manner. A similar association was also found when obesity was defined using BF% or WC. The presence of dental plaque was not found to be essentially associated with periodontal infection, which suggests that the association found between periodontal infection and obesity may be specifically related to infection in periodontal tissues, not to overall poor oral hygiene or its possible consequences. Although not proving causality, these findings support the conception that also periodontal infection might have a role in weight gain. Despite the fact that only few studies on the association of periodontal infection to obesity exist, the results of the present study are in accordance with the findings of a study by D'Aiuto *et al.* (2008), in which moderate and severe periodontitis was associated with metabolic syndrome and central obesity as an individual component of the condition. In addition, the results of this study are somewhat analogous with the findings of a study suggesting that human viral infections are associated with increased body weight (Atkinson *et al.* 2005).

However, in this context, two aspects must be noted. Firstly, it must be emphasised that the main cause of obesity is a long-term, positive, undesirable imbalance between energy intake and expenditure. Secondly, the results of this study must be interpreted cautiously due to the slight crudeness of the diet variable used; it presents the proportions of macronutrients of daily energy intake, but does not discriminate between the quality of carbohydrates, for instance, which has been found to have a role in obesity (Livesey *et al.* 2008, Malik *et al.* 2006). Besides the biological explanation for the association of periodontal infection to obesity, it is possible that the observed association is due to

confounding related to inaccuracies in the measurement of diet or physical activity.

Periodontal infection has earlier been suggested to be associated with cardiovascular disease (CVD) (Mattila *et al.* 2005, Meurman *et al.* 2004, Mustapha *et al.* 2007). In light of the findings of the present study, it is also possible that the association of periodontal infection with CVD may be more complex than previously assumed, with part of the effect of periodontal infection on CVD mediated through the effect of periodontal infection on weight gain. However, since obesity seems to be associated with a risk of both periodontal infection and CVD, it is also possible that the association of periodontal infection with CVD is confounded by obesity.

6.2 Serum inflammatory cytokines and lipoproteins in the association between obesity and periodontal infection

6.2.1 Serum IL-6 and TNF- α

Systemically elevated levels of proinflammatory cytokines, such as IL-6 and TNF- α , produced by adipose tissue, have been suggested to be one possible mechanism mediating the effects of obesity on the periodontium. In this study, a correlation was found between BMI and serum IL-6 levels. In addition, serum IL-6 levels were found to be associated with the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets. This means that an elevated serum IL-6 level could indeed be a mediating mechanism in the association between obesity and periodontitis. Furthermore, an elevated serum IL-6 level as a possible mediating mechanism is supported by the finding that adding serum IL-6 level to the multivariate model slightly attenuated the association between BMI and the number of teeth with deep periodontal pockets. However, due to the cross-sectional study design, the association found between serum IL-6 and the number of teeth with deepened and deep periodontal pockets could also mean that periodontal infection causes systemic elevation of IL-6 levels. Altogether, the findings of the present study are in accordance with previous studies that have reported an association between obesity and elevated serum IL-6 level (Fried *et al.* 1998, Mohamed-Ali *et al.* 1997), as well as with the finding of the role of an elevated systemic IL-6 level in periodontal inflammation (Andriankaja *et al.* 2009, Passoja *et al.* 2011). Since elevated IL-6 levels have

also been suggested to be associated with CVD risk (Danesh *et al.* 2008), these results also support the view of a possibly more complex interrelationship between obesity, periodontal infection and CVD than previously assumed, as discussed earlier.

In contrast to IL-6, no consistent association was found between serum TNF- α level and the number of teeth with deepened or deep periodontal pockets, and there was only a weak correlation between BMI and serum TNF- α level. Therefore it was not surprising that adding serum TNF- α level to the multivariate model had practically no effect on the strength of the association between BMI and periodontal infection. This implies that serum TNF- α has no role as a mediating factor in the association between obesity and periodontal infection. These results are in disagreement with the findings of previous studies reporting an association between obesity and elevated serum levels of TNF- α (Dandona *et al.* 1998, Ziccardi *et al.* 2002), and also with the results of studies suggesting that adipose-tissue-derived TNF- α may have an effect on periodontal tissues (Khanna & Mali 2010, Lundin *et al.* 2004). The weak correlation between BMI and serum TNF- α level and also between serum TNF- α level and periodontal infection in this study may be related to the fact that this study was based on a low-risk population, where the number of morbidly obese subjects was low.

Serum samples of IL-6 and TNF- α were collected about one year (mean 481 days) after the health examination. However, since the main aim was to study the possible mediating effects of systemic IL-6 and TNF- α on the relation of body weight to periodontal infection, this time span is not considered to be a major obstacle due to the study design and the stability of body weight; mean BMI in this study at the time of the health examination was 26.68, and at the time of the in-depth examinations, including the serum samples of IL-6 and TNF- α , it was 26.71. In addition, intra-individual variation in IL-6 and TNF- α levels have been reported to be fairly low, suggesting that a single serum sample could be used as a surrogate for serum cytokine levels over a longer period (Hoffman *et al.* 2011, Lee *et al.* 2007).

6.2.2 Serum triglycerides, HDL-C and LDL-C

Hyperlipidaemia—high serum concentrations of triglycerides, total cholesterol and LDL-C and a low concentration of HDL-C—is often associated with obesity. Besides the effects on atherogenesis, serum lipids, more precisely triglycerides and LDL-C, have also been suggested to possess proinflammatory properties

(Abe *et al.* 1998, Hulthe & Fagerberg 2002, Mackness *et al.* 2004). HDL-C, on the other hand, has been suggested to be anti-inflammatory (Gomaschi *et al.* 2005). Therefore, hyperlipidaemia could possibly act as a mediating mechanism in the association between obesity and periodontal infection. The aim of this study was to investigate the association of serum levels of triglycerides, HDL-C and LDL-C with periodontal infection, as well as the role of serum lipids in the association between obesity and periodontal infection. The results of this study showed no consistent associations between serum triglyceride, HDL-C or LDL-C levels and the number of teeth with deepened (4 mm deep or deeper) or deep (6 mm deep) periodontal pockets. The role of serum lipids in the association between obesity and periodontal infection was studied by adding serum levels of triglycerides, HDL-C and LDL-C to the multivariate model one by one. Serum triglyceride and HDL-C levels slightly attenuated the strength of the association between obesity and periodontal infection, whereas serum LDL-C level had no effect on this association. These results suggest that the association between obesity and periodontal infection is mainly mediated through some other mechanism than hyperlipidaemia.

The results of this study are in disagreement with the findings of previous studies by Noack *et al.* (2000) and Fentoglu *et al.* (2009), in which impaired lipid metabolism was found to be associated with periodontal disease. The lack of an association between hyperlipidaemia and periodontal infection in this study may be related to differences in the study populations. In contrast to the above-mentioned studies, this study was a population-based study. Several restrictions rendered this study sample to a low-risk population in which the prevalence of both hyperlipidaemia and periodontal infection were low, thus possibly preventing the association from manifesting itself. However, it is possible that in certain subgroups of this study sample—such as among the morbidly obese—subjects with hyperlipidaemia could run a greater risk of developing periodontal infection. This view is supported by the findings of the stratified analyses according to BMI, in which a high serum triglyceride concentration and a low HDL-C concentration were found to be associated with teeth having deepened periodontal pockets among subjects with a BMI of 30.0 or more.

6.3 Other possible explanations for the association of obesity with periodontal infection

This study investigated the possible role of adipose-tissue-derived elevated serum levels of cytokines TNF- α and IL-6, as well as hyperlipidaemia as an explanation for the association of obesity with periodontal infection. However, it is possible that this association is mediated through some other mechanisms. These include some other adipokines, such as resistin (Saito *et al.* 2008), and insulin resistance (Benguigui *et al.* 2010, Genco *et al.* 2005). Insulin resistance has been found to be weakly associated with periodontal infection also in these Health 2000 data (Timonen *et al.* 2011). However, due to the cross-sectional nature of these studies, it is also possible that periodontal infection has an effect on glucose metabolism. This is supported by the findings that periodontal treatment resulted in a decrease in the level of insulin resistance among type II diabetic patients (Sun *et al.* 2011), and also that deep periodontal pockets were associated with the development of glucose intolerance (Saito *et al.* 2004).

6.4 Statin medication and periodontal infection

Besides being highly effective in the treatment of hyperlipidaemia and in preventing cardiac events, statins have also been suggested to possess many pleiotropic effects, such as anti-inflammatory effects and effects on bone metabolism. Since many of the ‘target’ functions of statins are also crucial in the pathogenesis of periodontal infection, such as effects on leucocyte chemotaxis (Weitz-Schmidt *et al.* 2001), T cell activation (Kwak *et al.* 2000) and inhibition of cytokine production (Sakoda *et al.* 2006) and MMP expression (Ikeda *et al.* 2000), as well as osteoclast function (Yamashita *et al.* 2010), statins have been thought to possibly have a protective effect against periodontitis. In this study, statin medication and the number of teeth with deepened (4 mm deep or deeper) and deep (6 mm deep or deeper) periodontal pockets were found to be weakly negatively associated. In stratified analyses this negative association was found only among subjects with dental plaque or gingival bleeding; in subjects with no dental plaque or gingival bleeding statin medication was associated with an increased likelihood of having teeth with deepened periodontal pockets. This effect is most likely mediated through a mechanism other than the lipid lowering effect, possibly through the anti-inflammatory effect, since no consistent

association between serum lipid levels and periodontal infection was found in these data.

The overall inverse association of statins with periodontal infection found among the total population in this study, although fairly weak, is in line with the results of previous studies suggesting a protective role against periodontal infection for statins (Cunha-Cruz *et al.* 2006, Lindy *et al.* 2008). An interesting new finding in this study was that the effect of statins on the periodontium seems to be dependent on the inflammatory condition of the periodontium. This result could be interpreted in a way that statins suppress the immune response in a situation where the aetiological load in a form of dental plaque is substantial, thus protecting against periodontal tissue destruction, which is in congruence with the findings of studies suggesting that statins could be advantageous as a part of periodontal therapy (Fajardo *et al.* 2010, Pradeep & Thorat 2010). On the other hand, in a situation where the aetiological burden (no visible dental plaque) and the visible inflammation are minimal (no gingival bleeding), this suppression of immune response by statins may lead to disruption of immune homeostasis in periodontal tissues, which could predispose to periodontal tissue breakdown.

6.5 Methodological considerations

6.5.1 Study design

Sample

This study was based on a nationally representative sample of the Finnish adult population, including over 8,000 participants out of which over 6,300 subjects had an oral health examination done. Full-mouth probing pocket depth measurements were conducted for over 5,200 subjects, which is quite exceptional in a study of this size. The large data set offers several advantages. These include the possibility to increase validity through several restrictions, such as age restrictions and restriction of the study subjects to non-diabetics, non-rheumatics (original articles III and V) and subjects who had never smoked. Restrictions were implemented to obtain as homogeneous a study population as possible. Age restrictions were used to reduce age-related confounding, which may be difficult to control otherwise. By restricting elderly subjects, the biological effects of ageing could be reduced, as well as the effects of other diseases and medications

often accompanied with ageing. Tooth loss and edentulousness also increase with age, and inclusion of older age groups in the analyses could cause inconsistencies when studying associations with periodontal infection due to the cohort effect, related to for example extraction-oriented treatment protocols and attitudes in Finland in the past. In this study, diabetic and pre-diabetic subjects were excluded due to the complex association of diabetes with periodontitis (Mealey & Ocampo 2007), as well as the close association of obesity with diabetes (Kopelman 2000). Likewise, rheumatic subjects were excluded due to the association between rheumatoid arthritis and periodontal infection (Bartold *et al.* 2005). Rheumatic subjects also often have anti-inflammatory and immunomodulatory medications, whose effect would have otherwise been difficult to control. However, the possibility that some undiagnosed diseases existed cannot be excluded.

Restriction to never-smokers was done for several reasons. Cigarette smoking is strongly related to factors like health awareness, lifestyle and education, for instance, and is a well-established risk factor for periodontitis with a two- to eight-fold increased risk for periodontal attachment and/or bone loss, depending on the definition of disease severity and smoking dose (Johnson & Guthmiller 2007). In addition to being a strong risk factor for periodontitis, smoking has been suggested to be associated with lower body weight (Albanes *et al.* 1987), and cessation of smoking with increased body weight (Flegal *et al.* 1995). Furthermore, it has been recommended that periodontitis-systemic disease associations should be studied among subjects who have never smoked, since controlling the effect of smoking may otherwise be inadequate (Hujoel *et al.* 2002).

Although advantageous, the restrictions implemented in this study also have limitations. The exclusion of several known risk factors, especially in relation to periodontal infection, render this study sample to a low-risk population, *i.e.* the subjects on average have a relatively low number of teeth with pathologically deepened periodontal pockets, especially deep (6 mm deep or deeper) periodontal pockets. This increases the role of random occurrence, and thus can lead to inconsistencies in risk estimates. Another limitation is that the effects of co-actions of different risk factors cannot be investigated.

Power calculations were not done in this study for several reasons. One obvious reason is that the Health 2000 data set, as well as the data set of the Follow-up Study on Finnish Adults' Oral Health, were designed and collected for other purposes than this study and before this study was conducted. Therefore, even if such calculations would have been done, showing that this study is

underpowered, it would have been impossible to increase the number of study subjects. Retrospective sample size calculations are sometimes done to statistically assess the probability of rejecting a null hypothesis, given that the null hypothesis is false and some alternative hypothesis is true (Thomas 1997). However, because inferences in this study are not based on hypothesis testing, but rather on overall judgement, as suggested in epidemiological literature (Rothman 1998), the lack of retrospective power analyses in this study cannot be considered an essential limitation.

Possible sources of biases

Epidemiological studies are subject to several biases. In general, these can be divided into three main categories: selection bias, information bias and confounding (Rothman *et al.* 2008). Selection biases are distortions that result from procedures used to select subjects and from factors that influence study participation (Rothman *et al.* 2008). In the Health 2000 Survey, a two-staged cluster sampling design was used to obtain as representative a sample of the Finnish adult population as possible. The participation rate in the survey was fairly high; 79 per cent of the subjects participated in both the interview and the health examination, including the oral examination, which decreased the role of selection bias (Aromaa & Koskinen 2004). In addition, post-stratum weights based on gender, age and region were used to correct for non-response. These also increased the external validity of the study.

Bias in estimating an effect can be caused by measurement errors in the needed information, which is often referred to as information bias (Rothman *et al.* 2008). In this study, such bias may have arisen from subjects giving erroneous information in the interview or in the questionnaires, for instance due to obliviousness. In addition, social desirability, a tendency to give answers more according to a social norm than to the actual situation, may have had an effect on the subjects' answers (Sjöström & Holst 2002). However, the possible bias related to self-reported data should not be decisive in this study, since the main variables in this study were based on a clinical examination. In clinical examinations, a possible source of information bias is misclassification due to measurement errors. To reduce this, classification of subjects into healthy or diseased based on some arbitrary cut-off points was avoided. Instead, the number of teeth with deepened periodontal pockets, for instance, as a measurement of periodontal infection was used a continuous variable. Besides reducing the risk of misclassification, it

reflects the true-pattern of periodontal infection better than the dichotomised outcome variable.

The third main category of bias is confounding. Rothman and colleagues (2008) define confounders as extraneous factors that are responsible for a difference in disease frequency between the exposed and unexposed. They present three criteria for a confounding factor. First, a confounding factor must be an extraneous predictive factor for the disease under study. The second criterion is that a confounding factor must be associated with the exposure under study in the source population. Thirdly, a confounding factor must not be affected by the exposure or the disease; especially it cannot be an intermediate step in the causal path between the exposure and the disease. (Rothman *et al.* 2008) In this study, the effect of confounding minimised by using the above-mentioned restrictions and stratifications and also by using multivariate models. The selection of covariates was based on current knowledge about the potential risk factors for periodontal infection (articles I, III, IV and V) and obesity (article II). However, despite the fairly thorough handling of confounding in this study, the possibility of some residual confounding related to unknown extraneous factors, such as behavioural factors, that are difficult to adjust for, naturally cannot be excluded.

6.5.2 Variables

Oral health variables

The variability in the results of studies on periodontitis-systemic disease associations may be partly due to the lack of a uniform definition of periodontitis; case definitions vary depending on the parameters of the disease that are measured in each study. In the present study, the presence and extent of periodontal infection was measured as the number of teeth with deepened (4 mm deep or deeper) periodontal pockets, which is a widely used method in both research and clinical work. The boundary value of 4 mm is a commonly applied cut-off value for pathological pocket depth (Page & Eke 2007). The use of this cut-off value is supported by a recent study where it was found that relatively shallow pockets best reflect the whole mouth exposure to bacterial burden (Demmer *et al.* 2010). The cut-off value of 6 mm was used for deep periodontal pockets. However, the number of teeth with deep periodontal pockets was low and thus the risk estimates are subject to large random variation, *i.e.* chance. The

numbers of teeth with deepened and deep periodontal pockets were used as continuous outcome variables in the statistical analyses, which is advantageous in reducing misclassification, as mentioned before.

The number of teeth with deepened periodontal pockets as an outcome variable has some limitations, also. It represents only one clinical parameter of periodontal disease, which means it does not contain any information about clinical attachment level or bleeding pockets, for instance. This is one reason why the use of the term ‘periodontitis’ was avoided when referring to the results of this study.

In the follow-up study (article I), the outcome variable—the incidence of teeth with deepened periodontal pockets—underestimates the progression of periodontal infection, because it does not take into account deepening of periodontal pockets or formation of a new deepened periodontal pocket on a tooth that already had a deepened periodontal pocket at the baseline.

To enhance the analysis of the progression of periodontal infection among subjects who had periodontal infection at the baseline, the change in periodontal pocket depth was used as an outcome variable. However, this variable also has certain limitations. It does not discriminate whether deepening of pocket depth is a result of deepening of an existing pocket or formation of a new one. Neither does it take into account deepening of deep (6 mm deep or deeper) periodontal pockets. One might also consider the relatively short time span of the follow-up, about four years, to be a limitation in relation to worsening of periodontal condition. Periodontal disease generally progresses slowly (Albandar 1990), and thus a longer follow-up period would be advantageous to more thoroughly analyse the effect of obesity on the progression of periodontal infection.

Of the 6,335 subjects participating in the oral health examination of the Health 2000 Survey, 5,401 dentate subjects participated in the periodontal examination. Of these subjects, 137 were excluded due to a need for antibiotic prophylaxis. Regrettably, the effect of this exclusion could not be studied since there were no alternative methods for assessing the presence of periodontal infection among the subjects requiring antibiotic prophylaxis. However, it is worth noting that the number of these subjects was low, and the conditions requiring antibiotic prophylaxis are to a large extent unrelated to the outcome and explanatory variables of this study. This means that this exclusion most likely did not have an essential effect on the results.

Clinical examinations in the Health 2000 Survey were conducted by five dentists who were calibrated in advance. Clinical examinations in the Follow-up

Study on Finnish Adults' Oral Health were done by one dentist who was also one of the five dentists conducting the measurements in the Health 2000 Survey. In the Health 2000 Survey, the percentual agreement on deepened periodontal pockets was 77% (kappa value 0.41) in the parallel measurements where the assessments of the field examiners were individually compared with those of the reference examiner in field circumstances (Vehkalahti *et al.* 2004). The results for intra-examiner reliability assessments concerning periodontal pocket measurements in the Health 2000 Survey showed a kappa value of 0.83 (Vehkalahti *et al.* 2004). The results of McNemar tests for skewness showed that the field examiners reported fewer findings than the reference examiner in registering periodontal pockets (Vehkalahti *et al.* 2004). This under-registration of deepened periodontal pockets may lead to a situation where the perceived associations with periodontal infection in this study are somewhat conservative.

The presence of dental plaque was used as a secondary explanatory variable in article II. However, this variable has some limitations. Since it was measured from only three indicator teeth, it is a somewhat crude estimate of the presence of dental plaque, which may cause some inconsistency in the results. The percentual agreement on the presence of dental plaque was 58% (kappa value 0.36) in the parallel measurements between the five field examiners and the reference examiner in field circumstances (Vehkalahti *et al.* 2004). The results for intra-examiner reliability assessments related to the presence of dental plaque showed a kappa value of 0.79 (Vehkalahti *et al.* 2004).

Also the presence of gingival bleeding, used as a secondary outcome variable in article V, has some limitations due to its slight indistinctness. Although it was measured on every tooth, it was recorded by sextant. The percentual agreement in the parallel measurements between the field examiners and the reference examiner on the presence of gingival bleeding in field circumstances was 66% (kappa value 0.36) (Vehkalahti *et al.* 2004). The kappa value for intra-examiner reliability assessments concerning the presence of gingival bleeding was 0.66 (Vehkalahti *et al.* 2004).

The quality assurance of the clinical measurements in the Health 2000 Survey was fairly successful. The level of agreement was high for areas that are fairly easy to measure, such as determination of the condition of teeth. For areas that are more difficult to measure, such as measurement of the periodontal pocket depth, the levels of agreement were somewhat lower, which is consistent with earlier experiences from similar surveys. (Vehkalahti *et al.* 2004)

Measures of obesity

The main measure used to assess overweight and obesity in this study was BMI, which is probably the most widely used measure in research. Although it provides a fairly simple and accurate method for assessing body fatness, BMI has certain limitations. BMI does not distinguish between fat mass and fat-free mass. Romero-Corral *et al.* (2008) found that BMI-defined obesity had high specificity, but poor sensitivity to detect BF%-defined obesity among an adult general population, and that the diagnostic performance of BMI diminished as age increased. Among men, BMI correlated better with lean mass than with BF%, whereas among women BMI had a better correlation with BF% compared with lean mass. Among subjects with a BMI of 25.0–29.9, BMI discriminated poorly between BF% and lean mass among both genders (Romero-Corral *et al.* 2008). However, in the Health 2000 data, among subjects aged between 30–49 years, BMI correlated fairly well with other measures of obesity, namely BF% and WC, the correlations being 0.90 and 0.87, respectively (Ylöstalo *et al.* 2008).

In the longitudinal analyses, BMI measured at the baseline was used as the explanatory variable. Changes in the body weight of some of the subjects may have occurred during the follow-up, which could have an effect on the results. However, this is not considered to be a major obstacle. The reason for this is that the changes in body weight are normally quite moderate, especially since the time span of the follow-up was fairly short. In addition, BMI is a relative measure of body weight, so the number of subjects shifting from one BMI category to another, *i.e.* from normal weight to overweight, for instance, owing to age-related habitual weight gain during the follow-up, is most likely small.

6.5.3 Statistical methods

In this study, periodontal infection was measured as the number of teeth with deepened periodontal pockets. This produced count data, which means that regression models, such as Poisson regression models, could be used. These models yield prevalence rate ratios (cross-sectional settings) and incidence rate ratios (longitudinal settings), which are rough estimates of relative risk and can be thus interpreted in a probabilistic manner.

The number of subjects with no teeth with deepened periodontal pockets in this study population was relatively large. This slight overdispersion or ‘zero-inflation’ means the assumptions of Poisson distribution, namely that the mean

and variance of the outcome variable distribution are equal, may not have been fully satisfied, which may have some effect on the results, mainly on the confidence intervals. However, this overdispersion should not have an overwhelming effect. In article I, the statistical analyses were done using Poisson regression models with and without the DSCALE option, as well as using negative binomial regression models. The DSCALE option is a property of the statistical software that forces the scale parameter of the regression model to equal 1, and it is used to correct for overdispersion of the outcome variable. Poisson regression models with the DSCALE option were chosen based on the goodness-of-fit statistics, although there were no essential differences in the estimates between these regression models (data not shown). Furthermore, the statistical analyses in article V were done using both negative binomial regression and Poisson regression models. The negative binomial regression models were finally used, although there were no essential differences when compared with the risk estimates of the Poisson regression models (data not shown). Furthermore, additional analyses in article V were conducted where subjects with no teeth with deepened periodontal pockets were excluded. This is one suggested method for handling overdispersion (Lewsey *et al.* 2000). Poisson regression models were used, and no essential difference in the estimates between these secondary analyses and the main analyses was found, which also increases the credibility of the main analyses.

In article II, where the outcome variable was dichotomised, statistical analyses were conducted using logistic regression models. Logistic regression models yield odds ratios which have some limitations; they have no meaningful interpretations except in case-control studies, where controls are sampled from the total study base, or if the outcome is rare (Miettinen 1976). If the incidence of the disease under study is rare (less than 10 per cent), odds ratios approximate relative risk (McNutt *et al.* 2003). Therefore, odds ratios in this study can be interpreted as a rough estimate of relative risk, since the prevalence of obesity in this study population was low, about 15 per cent.

7 Summary and conclusions

This study was based on a general adult population and the aim was to provide evidence on the nature of the association between obesity and periodontal infection. This study used Health 2000 data, and the strengths of the Health 2000 data were a large study sample and a fairly profound amount of data collected, whereas the limitations were mainly cross-sectional study design, the crudeness of certain variables and the time gap between clinical and certain laboratory measurements.

The findings of this study showed that body weight was associated with the incidence of new teeth with deepened (4 mm deep or deeper) periodontal pockets during a four-year follow-up. These results support the view that overweight and obesity could be causally related to the development and progression of periodontal infection, but, on the other hand, they do not provide evidence that high body weight could be considered a major risk factor for periodontal infection.

The results of this study also showed that periodontal infection was associated with obesity in an exposure-response manner, which means periodontal infection might have a role in weight gain, although the cross-sectional study design prevents making any causal inferences. Longitudinal and intervention studies on this subject are needed. Nevertheless, the results of this study suggest that a bi-directional association between body weight and periodontal infection is possible.

Another aim of this study was to investigate the role of serum proinflammatory cytokines TNF- α and IL-6 as well as lipids as possible mediating mechanisms in the relation of obesity to periodontal infection. In this low-risk population, an elevated serum IL-6 level was found to be a possible mediating factor, whereas the mediating role of elevated levels of serum TNF- α , triglycerides and LDL-C and a lowered level of serum HDL-C was not supported by the results of this study. However, certain reservations must be made when interpreting these results; the disease mechanisms leading to periodontal infection may differ in subgroups such as smokers, diabetics and rheumatics and among morbidly obese subjects, for instance, which were not investigated in this study.

A new finding in this study was that statin medication seemed to have an effect on the periodontium, which is dependent on the inflammatory condition of the periodontium; among subjects with clinical signs of inflammation statins were negatively associated with the number of teeth with deepened periodontal pockets, whereas among subjects with no signs of inflammation statins increased the

likelihood of having teeth with deepened periodontal pockets. These results suggest that statins could be beneficial as a part of periodontal treatment, but further studies on the effects of statins on the periodontium are needed.

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Original articles

This thesis is based on the following original publications:

- I Saxlin T, Ylöstalo P, Suominen-Taipale L, Aromaa A & Knuuttila M (2010) Overweight and obesity weakly predict the development of periodontal infection. *J Clin Periodontol* 37: 1059–1067.
- II Saxlin T, Ylöstalo P, Suominen-Taipale L, Männistö S & Knuuttila M (2011) Association between periodontal infection and obesity: results of the Health 2000 Survey. *J Clin Periodontol* 38: 236–242.
- III Saxlin T, Suominen-Taipale L, Leiviskä J, Jula A, Knuuttila M & Ylöstalo P (2009) Role of serum cytokines tumour necrosis factor- α and interleukin-6 in the association between body weight and periodontal infection. *J Clin Periodontol* 36: 100–105.
- IV Saxlin T, Suominen-Taipale L, Kattainen A, Marniemi J, Knuuttila M & Ylöstalo P (2008) Association between serum lipid levels and periodontal infection. *J Clin Periodontol* 35: 1040–1047.
- V Saxlin T, Suominen-Taipale L, Knuuttila M, Alha P & Ylöstalo P (2009) Dual effect of statin medication on periodontium. *J Clin Periodontol* 36: 997–1003.

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1158. Takala, Heikki (2012) Biomarkers in esophageal cancer
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