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ASSOCIATIONS BETWEEN OBESITY, PERIODONTAL INFLAMMATION, SUBGINGIVAL MICROFLORA AND SALIVARY FLOW RATE.

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ASSOCIATIONS BETWEEN OBESITY, PERIODONTAL INFLAMMATION, SUBGINGIVAL MICROFLORA AND SALIVARY FLOW RATE.

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When I went to school, they asked me what I wanted to be when I grew up. I wrote down 'happy'. They told me I didn't understand the assignment, so I told them they didn't understand life.

-John Lennon

To my family

ABSTRACT

Obesity is a large health problem and is associated with increased risk of diabetes type II, cardiovascular disease as well as implicated in a wide range of gastrointestinal disorders such as Crohn's disease and subclinical bowel inflammation. Obesity is also associated with enhanced prevalence of chronic periodontitis in adults.

The overall aim of this thesis was to investigate if and how the low grade chronic inflammation accompanying obesity affects oral health in adolescents. More specifically: **Study I** was designed to investigate whether obesity in adolescence is associated with periodontal risk indicators or disease. **Study II** tests the hypothesis whether subgingival microbiological colonization is linked with obesity in adolescents**. Study III** was designed to investigate whether periodontal disease in terms of pathological periodontal pockets is associated with raised blood pressure and other risk markers for cardiovascular disease (CVD) in obese adolescents. **Study IV** investigates the level of inflammatory markers in saliva and their association to salivary flow rate in obese adolescents.

Obese adolescents (**Studies I-IV**) and normal weight subjects (**Studies I, II and IV**) within the age range 11-18 years were examined with respect to visible plaque index (VPI%), gingival inflammation (bleeding on probing (BOP%)), occurrence of pathological pockets exceeding or equal to 4mm (PD≥ 4mm).

The subjects answered a questionnaire concerning medical conditions, oral hygiene habits, smoking habits and sociodemographic background. Body mass index (BMI) was calculated and adjusted for age and gender (BMI-sds)

Samples of gingival crevicular fluid (GCF), subgingival plaque, blood serum and stimulated whole saliva were collected and analyzed. Systolic and diastolic blood pressures were measured and registered. The flow rate of stimulated whole saliva (ml/min) was determined.

Obese subjects exhibited more BOP> 25% ($P< 0.001$) and more PD≥ 4mm ($P<0.001$) than the normal weight subjects.

Higher levels of Interleukin (IL)-1β ($P< 0.001$) and IL-8 ($P= 0.002$) were measured in GCF from obese subjects compared with the controls. In a multivariate logistic regression analysis, BMI-sds ($P= 0.03$; Odds Ratio (OR) = 1.87) was significantly associated with the occurrence of PD≥ 4mm after adjusting for BOP> 25% and subgingival calculus.

The sum of bacterial cells in subgingival biofilm was significantly associated with obesity ($P< 0.001$). The link between sum of bacterial cells and obesity was not confounded by any of the studied variables (chronic disease, education, VPI%, BOP%, flow rate of whole saliva, or meal frequency). Totally 23 bacterial species were present in approximately three-fold higher amounts, on average, in obese subjects compared with normal weight controls. Of the Proteobacteria phylum, *Campylobacter rectus* and *Neisseria mucosa* were present in six-fold higher amounts among obese subjects.

Obese adolescents with PD≥ 4mm had higher diastolic blood pressure (P= 0.008), higher levels of IL-6 (P< 0.001), Leptin (P= 0.018), Macrophage Chemoattractant Protein-1 (MCP-1) (P= 0.049) and thyroid stimulating hormone (TSH) (P= 0.004) in blood serum compared with subjects without PD≥ 4mm. The bivariate linear regression analysis demonstrated that PD≥ 4mm (P= 0.008) and systolic blood pressure (P< 0.001) were significantly associated with the dependent variable "diastolic blood pressure". The association between PD≥ 4mm and diastolic blood pressure remained significant (P= 0.006) even after adjusting for the potential confounders BMI-sds, age, gender, mother's country of birth, BOP> 25%, IL-6, IL-8, Leptin, MCP-1, TSH and total cholesterol in the multiple regression analysis.

The obese subjects exhibited lower mean value of flow rate of stimulated whole saliva, 1.3 vs. 2.0 ml/min (P< 0.001), compared to their normal weight counterparts. Obese adolescents had higher levels of salivary Insulin (p< 0.001), Leptin (p= 0.005), C-reactive protein (CRP) (p= 0.002) and Interferon (INF)-γ (p= 0.011). Low stimulated whole saliva flow rate (< 1.5ml/min) was significantly associated with BMI-sds (p< 0.001), as well as the biomarkers IL-1β (p= 0.026) and IL-8 (p= 0.013) and the associations were not confounded by age, gender, chronic disease, drugs affecting salivary flow, PD≥ 4mm and protein content in saliva.

In conclusion, obesity in adolescents is associated with increased periodontal inflammation, increased subgingival microbiological colonization and low flow rate of whole saliva. In addition, the presence of pathological periodontal pockets in obese adolescents is associated with raised diastolic blood pressure. The results call for collaboration between pediatric dentists and medical physicians in preventing obesity development and its associated disorders.

LIST OF SCIENTIFIC PAPERS

T. Modéer, **C. Blomberg**, B Wondimu, T Yucel-Lindberg, C. Marcus: Association between obesity and periodontal risk indicators in adolescents. *Int J Pediatr Obes* 2011, 6: 264-270.

C. Zeigler, R. Persson, B. Wondimu, C. Marcus, T. Sobko, T. Modéer: Microbiota in the oral subgingival biofilm is associated with obesity in adolescence. *Obesity (Silver Spring)* 2012, 20: 157-164.

C. Zeigler, B. Wondimu, C. Marcus, T. Modéer: Pathological periodontal pockets are associated with diastolic blood pressure in obese adolescents. *BMC Oral Health* 2015, 15: 41.

C. Zeigler, A. Kats, B. Wondimu, C. Marcus, T. Modéer: The levels of inflammatory biomarkers in saliva are enhanced in obese adolescents.

- manuscript

CONTENTS

List of abbreviations

AC	Alveolar bone crest
BMI	Body Mass Index
BMI-sds	Body mass index standard deviation score
BOP	Bleeding on probing
CEJ	Cemento-enamel junction
CI	Confidence interval
CNS	Central nervous system
CVD	Cardiovascular disease
CRP	C-reactive protein
GCF	Gingival Crevicular Fluid
Hba1c	Hemoglobin, adult, 1c
HDL /-C	High density lipoprotein / cholesterol
HPA	Hypothalamic pituitary adrenal
IL	Interleukin
INF	Interferon
ISO-BMI	Sex and age adjusted body mass index
LDL	Low density lipoprotein
LPS	Lipopolysaccharides
MCP-1	Monocyte chemotactic protein-1
MMPs	Matrix metalloproteinase
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor-1
PD≥ 4mm	Periodontal Pocket ≥ 4mm
PGE2	Prostaglandin-E2
Th	T helper cell
TNF	Tumor necrosis factor
TSH	Thyroid stimulating hormone
VPI	Visible plaque index
WHO	World Health Organization

INTRODUCTION

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height (in meters). A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 is considered overweight.

Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings [1].

OBESITY AND OVERWEIGHT IN THE WORLD

Obesity is the sixth most important risk factor in terms of the number of deaths in the world. In 1997, the WHO officially declared obesity to be a chronic condition which requires treatment, fosters the development of other diseases, and is connected with increased mortality [1].

Worldwide, the proportion of adults with BMI of 25 kg/m2 or greater increased between 1980 and 2013 from 29% to 37% in men, and from 30% to 38% in women. Prevalence has increased substantially in children and adolescents in developed countries; 23% of boys and 23% of girls under 16 years of age were overweight or obese in 2013. The prevalence of overweight and obesity has also increased in children and adolescents in developing countries, from 8% to 13% in 2013 for boys and from 8% to 13% in girls. In adults, estimated prevalence of obesity exceeded 50% in men in Tonga and in women in Kuwait, Kiribati, Federated States of Micronesia, Libya, Qatar, Tonga and Samoa. Since 2006, the increase in adult obesity in developed countries has started to level out [2].

ETIOLOGY OF OBESITY

The rising epidemic of overweight and obesity reflects the profound changes in society and in behavioral patterns of communities in recent decades. Incomes rise, populations become more urban and their diet changes to include more ready-prepared meals, fast foods, increased snacking and high sugar-containing drinks [3, 4] such a diet is higher in fats, saturated fats and sugars. At the same time, the population worldwide is shifting towards less physically demanding work, mostly due to the increase in the number of computers and machines at work. Also on the increase is the use of automated transport (cars, buses, trains, escalators and elevators) [5], technology in the home (television, computers and computer games, etc.) and more passive leisure pursuits [1, 6-8]. Less physical activity is in general the modern style of

life. In more academic terms these changes could be described as: economic growth, modernization, urbanization and globalization of the food market [1].

GENETIC INFLUENCES ON OBESITY

Genes are important in determining a person's susceptibility to weight gain, but the genetic factors predisposing to obesity are poorly understood. Early studies indicate that obesity runs along family lines [9-14], this seems logical if you presume that a family would share the same diet and physical activity interests. However, a Danish study showed that adopted children had a BMI closer related to their biological parents that to the adopted parents [15].

One gene in particular has been associated with obesity, *the FTO-gene.* This gene is situated on chromosome 16 and was first mentioned in connection to obesity in 2007 [16]. There is especially a positive association between FTO gene polymorphism and overweight/obesity risk among children and adolescents [17]. In addition, it appears that FTO-gene variants are associated with a preference for energy-dense foods, greater food intake, less sensitivity to satiety cues and loss-of-control eating episodes[18].

There are also several genetic syndromes connected with obesity. Many have a characteristic presentation and several, an overlapping phenotype indicating there might be a shared common underlying mechanism or pathway. Most common amongst obesity syndromes is Prader-Willi, caused by the loss of imprinted genes on 15q11-13. There are several other syndromes as well: Bardet-Biedl syndrome, Alstrom syndrome, Cohen syndrome, Carpenter syndrome, MOMO syndrome Rubinstein-Taybi syndrome, cases with deletions of 6q16, 1p36, 2q37 and 9q34, [19]. All obesity related syndromes are rare and always connected with further symptoms. A German specialized clinic for endocrinology and obesity screened all new patients for genetic syndromes disorders and less than 1% of 1405 patients were diagnosed [20].

OBESITY AND MICROBIOLOGY

The healthy human microbiota is comprised of 10^{14} microorganisms, at least 10 times the amount of all human cells in the body. Most indigenous microbes reside in the colon [21, 22]. Collectively, the human microbiota encodes 150 times more genes than the human genome (3-4 million) [23]. This enables the microbiota to perform diverse metabolic activities not encoded in the human genome[24]. Approximately 500–1000 different bacterial species comprise this busy ecosystem. Factors influencing the microbiota composition include age, diet, antibiotics and most of the elements of a modern lifestyle in addition to certain disease states. From birth, the microbiome and host immune system co-develop and are mutually inter-dependent [25]. Thus, the microbiota shapes the development of the immune system, and in turn the immune system shapes the composition of the microbiota. Disruption or sustained changes to the gut microbiota may be associated with obesity [26] and insulin resistance [27].

COMPOSITION OF GUT MICROBIOTA

The phylum Firmicutes (including *Clostridium, Enterococcus*, *Lactobacillus* and *Ruminococcus*) and Bacteroidetes (including *Prevotella* and *Bacteroides* genera) constitute over 90% of known phylogenetic categories and dominate the distal gut microbiota in humans [28]. Obese individuals, however, demonstrate differences in gut microbiota at the phylum level, with less overall diversity [29] and greater proportion of Firmicutes than Bacteroidetes, compared with normal weigh individuals [29, 30]. However these findings are not universal, a number of other studies have demonstrated variable patterns in phylum level changes measured in the composition of the microbiota of obese subjects [31, 32].

ENERGY HARVEST

The gut microbiota significantly affects energy yield from diet. Hydrolysis and fermentation of otherwise indigestible dietary polysaccharides by the gut microbiota enhances host energy harvest. The proximal small intestine and stomach are responsible for most nutrient digestion and absorption. Normally, almost all fats, ~85% of carbohydrates and 65–95% of proteins are absorbed prior to entering the colon[33]. The remaining indigestible nutrients would be generally excreted without absorption if not for colonic microbiota, which enhance energy uptake or harvest from ingested food. Bacteroidetes are reported to encode higher levels of carbohydrate active enzymes than Firmicutes [34].

Over nutrition is associated with raised Firmicutes versus Bacteroidetes ratio. Interestingly, in lean subjects phylum level changes in the fecal microbiota were significantly associated with stool energy loss, positively associated with proportional increase in Bacteroidetes, the opposite for Firmicutes [21, 35].

OBESITY IS CHRONIC INFLAMMATION

Adipose (fat) tissue is where the body stores the excess energy consumed as lipids. It is also a tissue that produces a wide range of inflammatory cytokines, often called adipocytokines or adipokines [36-40]. More than a hundred adipose tissue secretion products have been described including fatty acids, prostaglandins and steroids, as well as complex proteins [41].

Cytokines have been found to be central to the pathogenesis of an ever-increasing number of diseases. They are inter-cellular messengers and, as such, represent a key mechanism by which cells involved in immune response communicate.

People who are overweight and obese have larger quantities of adipose tissue which leads to an increased production and secretion of adipokines [42]. Fain observed in 2006 that the total release of Tumor necrosis factor (TNF)-α, IL-6 and IL-8 by adipose tissue was markedly elevated in obese patients [39]. It has also been reported that adipose tissue in an obese person is infiltrated by macrophages to a higher concentration than in a normal weight person, which may be a major source of secreted adipokines [43]. In addition, it has been reported that other inflammatory cell

types, such as T-lymphocytes, also infiltrate the obese adipose tissue and contribute to adipose tissue inflammation [44]. This overproduction of adipokines has effects on both local and distant sites, influencing important biological processes including lipid homeostasis, immune function, insulin sensitivity, control of blood pressure, appetite and energy balance [37, 45].

Leptin was the first adipocyte hormone discovered. Its most known effect is that it increases a person's appetite. It is also believed to be involved in a variety of biological processes, for example to regulate adipose tissue mass. Elevated leptin concentrations result in increased energy expenditure, decreased food intake and a negative energy balance [46]. Most overweight and obese persons show resistance to leptin at the receptor level, leading to higher leptin levels than normal weight individuals [47]. In addition, several studies have suggested that elevated levels of leptin can be found during infection and inflammation [48].

Plasminogen activator inhibitor-1 (PAI-1) is the principal inhibitor of tissue plasminogen activator and urokinase, the activators of plasminogen and hence fibrinolysis.

Resistin in rodents has been shown to induce insulin resistance, while in humans its implication in the control of insulin sensitivity is still a matter of debate. Current evidence suggests that, in humans, resistin is more closely related to inflammatory processes than to insulin resistance [49, 50].

Adiponectin, an insulin-sensitizing effector, is down-regulated during obesity. Circulating adiponectin levels are also decreased in subjects with obesity-related insulin resistance, type-2 diabetes and coronary heart disease. In addition, adiponectin counteracts the pro-inflammatory effects of TNF-α on the arterial wall and probably protects against the development of arteriosclerosis.

TNF-α is believed to have an important role in the pathogenesis of insulin resistance and IL-6 induces hepatic CRP synthesis and may promote the onset of cardiovascular complications. Both TNF-α and IL-6 can alter insulin sensitivity by triggering different key steps in the insulin signaling pathway [42, 51, 52].

The full effects of these pro-inflammatory factors and how exactly they trigger or inhibit different physiological reactions are not yet properly understood but research on mainly rodents is starting to indicate how and why these changes in human physiology occur [44, 53].

OBESITY AND HEALTH

Obesity is currently considered as a chronic disease which must be treated like any other medical condition. It has an epidemic-like nature and is not only one of the main causes of morbidity and mortality, in particular in developed countries, but also causes huge social and economic burdens [54, 55]. Overweight and obesity increases the risk of type 2 diabetes, cardiovascular diseases and several forms of cancer. In addition, excess bodyweight contributes to disabling disorders such as osteoarthritis,

infertility, asthma and sleep apnea. Maternal obesity has also been linked to an increased risk of congenital anomalies [56].

MEDICAL CONSEQUENCES OF CHILDHOOD OBESITY

Childhood obesity can adversely affect nearly every organ system **(Figure 1)** and often causes serious consequences including hypertension, dyslipidemia, insulin resistance/diabetes, fatty liver disease and psychosocial complications. In addition, atherosclerotic process appears to be accelerated in obese children. BMI in childhood and adolescence is associated with increased risk of cardiovascular disease in adulthood [57].

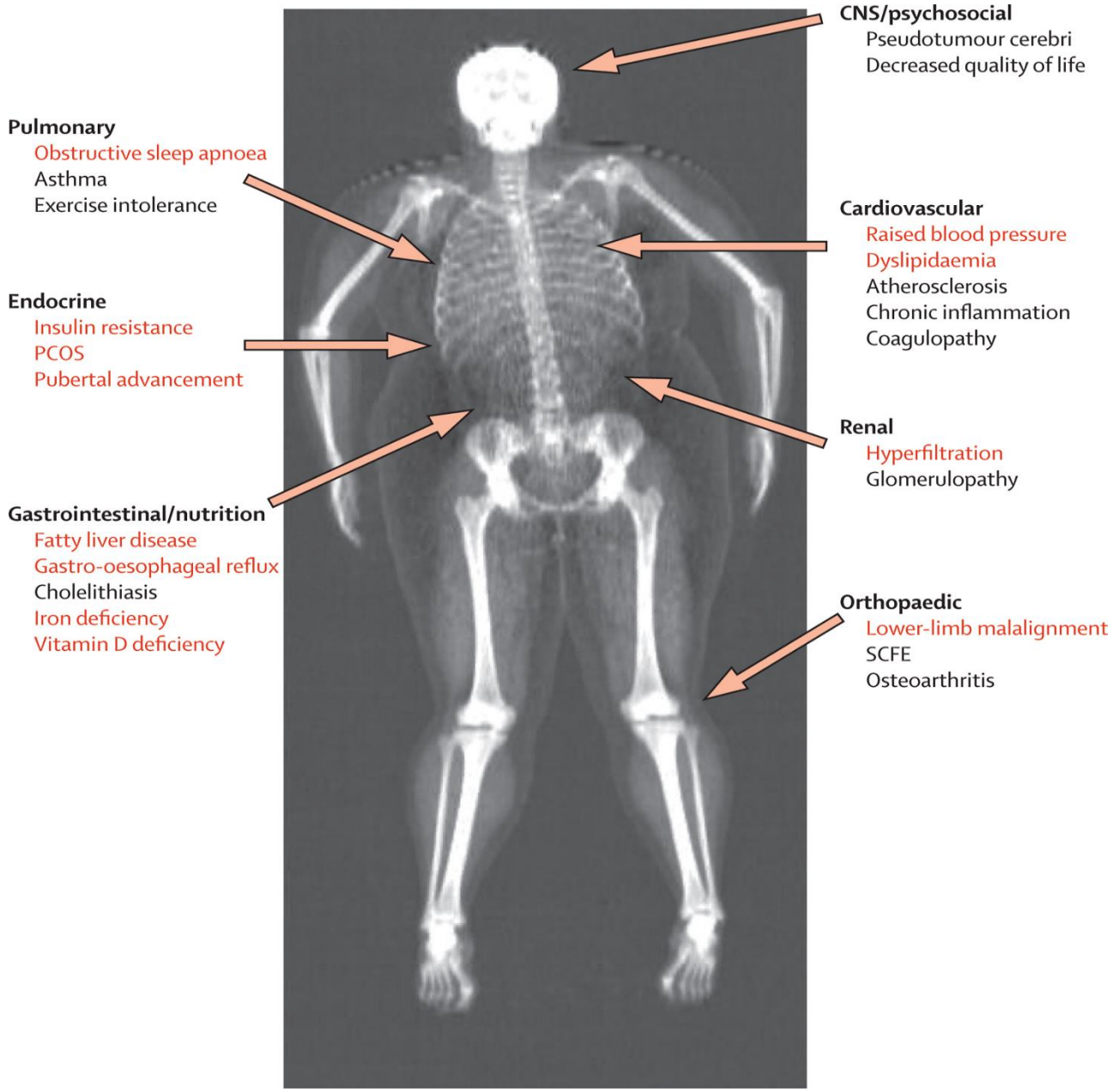


Figure 1. Medical complications associated with childhood obesity
Image obtained by dual energy x-ray absorptiometry from a teenage girl with BMI 38 kg/m^2. Disorders that are of high prevalence and are well established in their association with childhood obesity are shown in pink. CNS=central nervous system. PCOS=polycystic ovary syndrome. SCFE=slipped capital femoral epiphysis. Figure courtesy of Han et al. *Lancet* 2010.

PSYCHOSOCIAL COMORBIDITIES

- Anxiety, depression, stress, low self-esteem, bullying, social withdrawal and lower quality of life have been reported to be more common in obese adolescents [58, 59].
- Poor school performance, including difficulty with concentration and missed school days, are 4 times more likely in an obese adolescent population when compared with their normal weight counterparts [58, 59].
- Overweight/obese adolescents show higher lifetime rates of eating disorders, especially bulimia nervosa, than population-based samples [58, 59].

METABOLIC SYNDROME

In adults, the metabolic syndrome is defined as a clustering of features including insulin resistance/elevated glucose, hypertension, abdominal obesity and dyslipidemia that portends risk for type 2 diabetes and CVD [60]. The metabolic syndrome is also prevalent in other conditions linked to insulin resistance, such as polycystic ovarian syndrome and nonalcoholic fatty liver disease [61, 62].

International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity joint statement of diagnosis guidelines for metabolic syndrome[60]:

Central obesity

- Waist circumference—ethnicity specific

Plus any two:

- Raised triglycerides
  - >150 mg/dL (1·7 mmol/L)
- Specific treatment for this lipid abnormality
- Reduced HDL-cholesterol
  - <40 mg/dL (1·03 mmol/L) in men
  - <50 mg/dL (1·29 mmol/L) in women
- Specific treatment for this lipid abnormality
- Raised blood pressure
  - Systolic ⩾130 mm Hg
  - Diastolic ⩾85 mm Hg
- Treatment of previously diagnosed hypertension
- Fasting plasma glucose
  - ⩾100 mg/dL (5·6 mmol/L)
- Previously diagnosed type 2 diabetes

In children and adolescents, the metabolic syndrome is defined in a similar cluster but there is no single generally accepted definition [63]. A systematic review of studies performed in the pediatric age range indicates a prevalence of metabolic syndrome in population-based studies of 3.3%, 11.9%, and 29.2% of normal-weight, overweight, and obese children, respectively [62, 64]. Almost half of children with BMI ≥ 97th percentile have one or more of the conditions which comprise the metabolic syndrome[57].

OBESITY AND ORAL HEALTH

OBESITY, CRANIOFACIAL AND DENTAL DEVELOPMENT

Obese adolescents have been shown to develop an increased mandibular length, prognatic jaws and a reduced upper anterior face height [65]. They also have an accelerated dental development and skeletal maturity [66, 67]. These are important factors to consider in pediatric and orthodontic treatment planning where timing is crucial.

OBESITY AND DENTAL CARIES

Both dental caries and obesity are diseases with multifactor etiology related to dietary habits but also closely correlated with sociodemographic background of the individuals. National data from Sweden suggest a positive correlation between dental caries and BMI, as well as, demonstrated that obesogenic behavior such as snacking in between meals predicted caries development in adolescence [68]. In addition, recent studies link obesity, dental caries and high sugar intake [69-71].

The majority of studies regarding association between obesity and dental caries are based on clinical data expressing caries experience that reflect not only the actual caries situation but also previous accumulation of caries and filled surfaces. Several clinical studies demonstrates a relationship between obesity and dental caries [68, 69, 72-75], but contradictory results are also present [76, 77]. Longitudinal studies present evidence of the association between overweight/obesity and dental caries as conflicting and inconclusive [78]. Major confounders for association between dental caries and obesity are the population's access to oral health services, use of fluorinated substances, socioeconomic and dietary conditions. Generally European studies showed an association between obesity and dental caries whereas Latin-American studies did not. Both continents have similar access to fluoridated products/fluoridated water supply but vary in socioeconomic and dietary conditions [79].

In addition, obese adolescents exhibit a lower flow rate of stimulated whole saliva compared to normal-weight controls and this may explain part of the possible link between obesity and dental caries [80].

OBESITY AND SALIVA

Saliva secretion is essential for adequate functions such as chewing and swallowing and hypo-salivation may trigger many oral problems [81]. Normal salivation and salivary components initiate digestion, enhance healing of mucosa and maintain oral microflora in balance [82]. Several studies demonstrate an association between obesity and reduced flow rate of saliva in adults and adolescents [80, 81], but not in younger children [83, 84].

Adipose tissue is well known to secrete and affect the regulation of a number of pro- and anti-inflammatory cytokines, so-called adipokines, including TNF-α, Il-1β, IL-6, IL-8, leptin, plasminogen activator inhibitor-1, resistin and adiponectin [36, 85]. In turn, this dysregulation of adipokines may increase both the risk and the development of chronic and autoimmune disease [86].

Both human and animal studies indicate that levels of inflammatory cytokines in saliva are associated with decreased salivary flow [87-89]. In addition, during the last few years, several studies concerning inflammatory markers in saliva from obese subjects have been published. In obese populations of adults and children decreased levels of adiponectin [83, 90] and in children increased levels of CRP, salivary insulin and leptin have been reported [83]. Interestingly, obese subjects have also been reported to exhibit a significant enlargement of parotid glands probably by an enhanced storage of adipocytes in the parotid parenchyma whereas the submandibular glands seem to remain unaffected [91, 92].

There are, as well, several reports indicating that saliva from obese patients suppresses some taste aromas and that salivary composition, sensitivity to dietary fat intake and oral postprandial lipid metabolism is affected in obese subjects [93, 94]. All these indicate that salivary composition and function may be altered in obese patients [83, 93-96].

OBESITY AND PERIODONTAL DISEASE

Periodontal disease is a bacterial infection involving the dental biofilm. Some of the periodontal pathogens in subgingival microbiome have been identified to have virulence potential and are associated with the etiology and the pathogenesis of periodontal disease. Biofilms that cause gingivitis and periodontitis are site-specific, complex poly-microbial communities that are resistant to host-defense mechanisms and difficult to treat with antimicrobial agent, especially with long term results. However, the rate of progression, age at onset and severity of periodontal disease in an individual are determined by systemic risk factors in the specific individual (**figure 2**) [97].

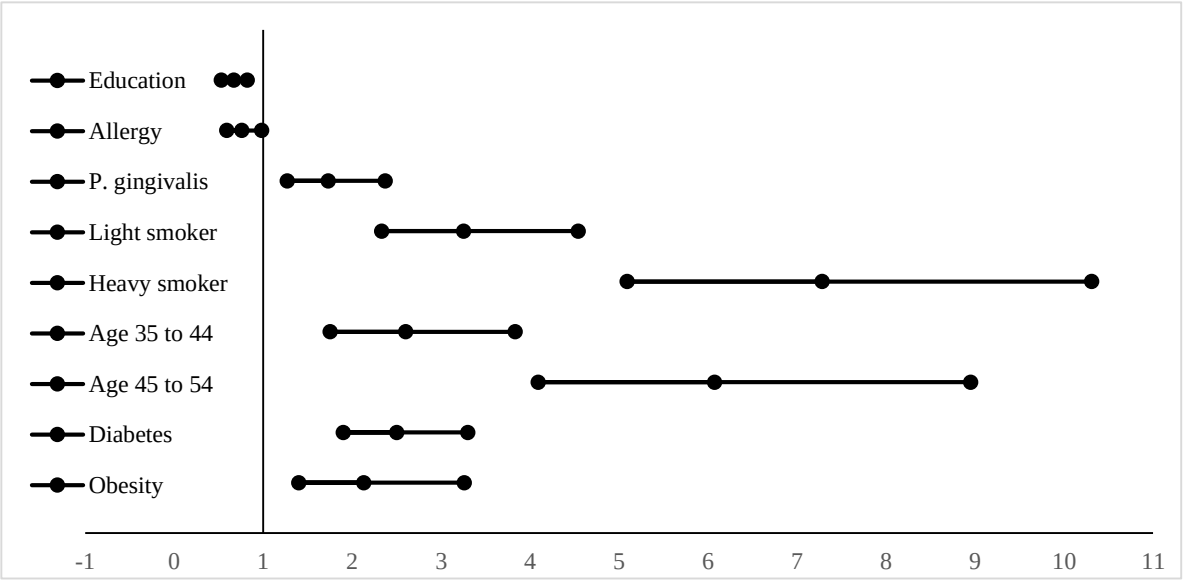


Figure 2. Potential risk factors for periodontal disease. The error bars represent the 95% confidence intervals. If 95% confidence intervals do not cross the odds ratio 1.0 they are statistically significant at $P \leq 0.05$. Odds ratios >1 can be considered to increase risk for periodontal clinical attachment loss, and may be risk factors; odds ratios <1 are associated with less attachment loss, and may be protective factors. Data from Genco et al 2013 and Gossi et al 1995 [97, 98].

The first report on the relationship between obesity and periodontal disease was published in 1977, when a research group observed histopathologic changes in the periodontium of hereditary obese Zucker rats [99] but not until 1998 was an association between obesity and periodontal disease shown in humans [100].

Several cross-sectional studies have since reported that obesity is associated with enhanced prevalence of chronic periodontitis in adults over 17 years of age [100-102]. More recently, large longitudinal studies, from 5 to 40 years duration, demonstrate that both overall obesity and central adiposity are associated with an increased risk and faster progression of periodontal disease [97].

Before our first study there were no published studies reporting an association between obesity and periodontal disease in children and adolescents under 17 years of age. At present, the available literature on a possible association between obesity and periodontal disease is inconclusive. In addition, published studies use different

measurements and cut off values for gingival inflammation and pathological periodontal pockets, making results difficult to compare [103-105].

The mechanism(s) whereby obesity may affect periodontal health is so far unclear. In an animal model, however, obesity interferes with the ability of the immune system to appropriately respond to the periodontal pathogen Porphyromonas gingivalis infection in terms of TNF-α, IL-6 and serum amyloid response, suggesting that this immune dysregulation participates in the increased alveolar bone loss after bacterial infection [106].

In adolescents 18 years of age with severe obesity, it has previously been reported that the level of TNF-α in GCF is positively correlated with BMI, indicating that the level of TNF-α in GCF was affected by the obese condition [107].

Circulating adipokines might also influence the immune response at the mucosal level both in the oral cavity and in the gut, thereby affecting the microbial colonization. Whether there is a relationship between obesity and the oral microbiota is so far unclear. In an animal model, however, obesity has been reported to interfere with the ability of the immune system to appropriately respond to infection by the periodontal pathogen *Porphyromonas gingivalis* [106]. Furthermore, enhanced colonization of *Tannerella forsythia* in subgingival biofilm has been reported in obese subjects [108].

OBESITY, METABOLIC DISORDERS, CVD AND PERIODONTAL DISEASE

The current epidemiological data indicate that there is an association between periodontal disease and hypertension in adults [109, 110]. Systemic inflammatory response associated with periodontal disease has been proposed to have adverse effects on blood pressure [109]. Periodontitis may also be capable of inducing vascular inflammation, which leads to endothelial dysfunction, an initial step for CVD [109]. In fact, this association may be multidirectional. For example, it has been well-established that inflammation is an essential component in the development of atherosclerosis, and observational studies showed that periodontitis is associated with a higher risk of coronary heart disease [111-113]. There are several studies presenting evidence of the oral infection theory of atherogenesis [114]. Periodontal pathogens are able to cause transient bacteremia, invading the arterial wall and possibly lead to vascular inflammation and atherosclerosis [109]. Inflammatory diseases like periodontitis induce the production of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 [115]. It has been suggested that the secretion of TNF-α by adipose tissue triggered by lipopolysaccharide (LPS) from periodontal gram-negative bacteria promotes hepatic dyslipidemia and decreases insulin sensitivity [116, 117]. There are studies reporting an association between periodontitis, impaired fasting glucose and diabetes type 2 [118]. Type 2 diabetes and decreased insulin sensitivity are associated with the production of advanced glycation end-products, which trigger inflammatory cytokine production, thus predisposing for inflammatory diseases such as periodontitis [117].

Interestingly, periodontal therapy has been demonstrated, in clinical intervention studies, to decrease levels of biomarkers CRP and IL-6 in serum as well as improving endothelial function [109, 119]. Periodontal treatment also seems to reduce levels of cholesterol and triglycerides both in adults [120, 121] and children [122]. In addition, periodontal treatment may reduce circulating TNF-α and serum levels of glycosylated hemoglobin, and has beneficial effects on the control of type 2 diabetes [117]. There is, as well, emerging evidence that successful periodontal treatment might help reduce blood pressure in patients with hypertension [123].

Periodontitis, once it exists, may promote systemic inflammation and thereby increase the risk and progression of obesity co-morbities [111, 115].

GINGIVAL CREVICULAR FLUID

GCF is mainly an inflammatory exudate that is collected in the gingival crevices surrounding the teeth [124-126]. GCF components have a variety of sources and contain substances originating from the host as well as from micro-organisms in subgingival and supragingival plaque. Substances from the host include molecules from the blood as well as contributions from periodontal cells and tissues. When micro-organisms in the dental biofilm initiate an inflammatory response, various inflammatory mediators are produced. These mediators: cytokines, prostaglandins, and enzymes like matrix metalloproteinase (MMPs) can be detected in GCF [127-134].

The collection and analysis of GCF is a non-invasive method for evaluating the host response to periodontal disease. The volume of GCF present at a site may be directly related to tissue inflammation as well as permeability and ulceration of the crevicular epithelium. As inflammation deteriorates gingival health, the volume of GCF increases [135].

One of the methods of collecting GCF is with paper strips (**Figure 3**). The advantages of using a paper strip are that the method is quick, easy, and individual sites can be sampled separately. Furthermore, using paper strips correctly is, most likely, the least traumatic method available to collect GCF from the gingival crevice [130].

Figure 3. GCF collecting with paper strip.
(Courtesy of Prof. Modéer and Dr Tsilingaridis)

AIM OF THE THESIS

The overall aim of this thesis was to investigate if and how the low grade chronic inflammation accompanying obesity affects oral health in adolescents.

SPECIFIC AIMS

STUDY I

In a cross-sectional study design we test the hypothesis whether obesity in adolescence is associated with periodontal risk indicators.

STUDY II

In this study we investigate whether microbiota in subgingival plaque is linked with obesity in adolescents.

STUDY III

This study in obese adolescents was designed to investigate whether periodontal disease in terms of pathological periodontal pockets is associated with raised blood pressure and other risk markers for CVD.

STUDY IV

We designed this cross-sectional study to investigate the levels of inflammatory markers in saliva and their association to salivary flow rate in obese adolescents.

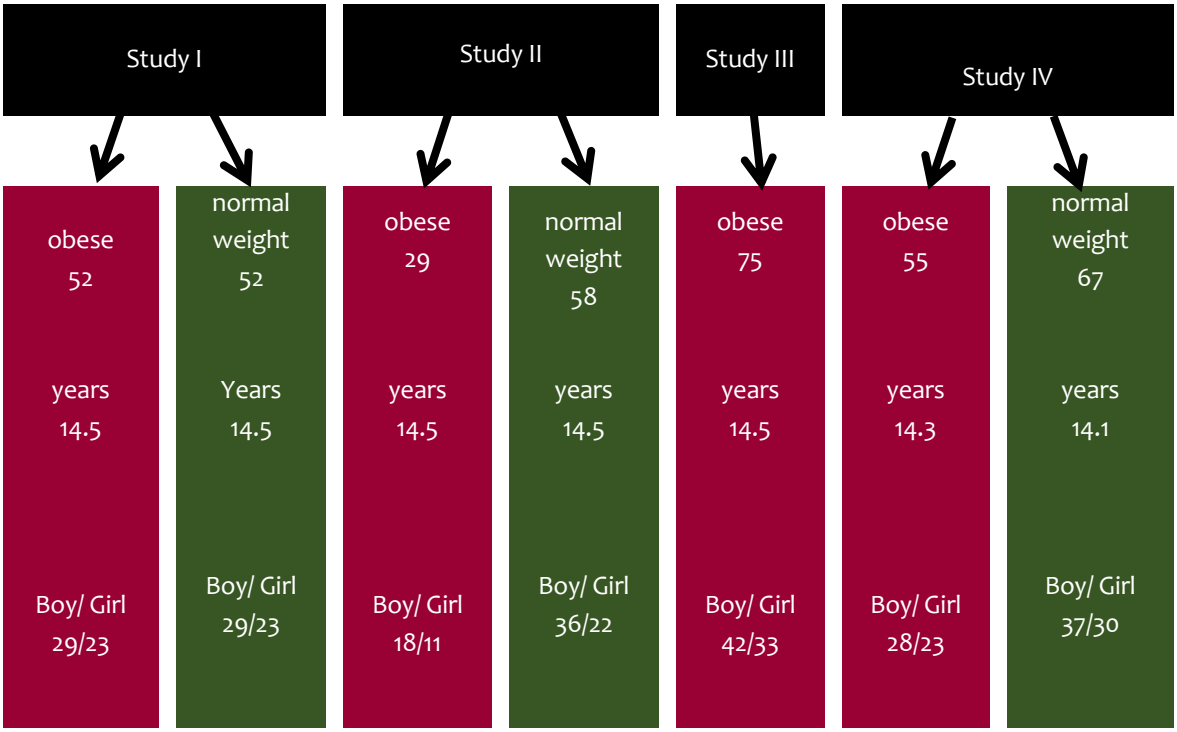
MATERIALS AND METHODS

This section gives a brief overview of the methods used to obtain the results presented in this thesis. The study design was cross-sectional and the Ethics Committee at Karolinska University Hospital, Karolinska Institutet, Huddinge, Sweden, approved the study protocol, methods and selection of subjects. Subjects and/or their parents received verbal as well as written information, and all subjects and/or their parents gave their informed consent for participation in the studies.

STUDY POPULATION

Obese:
Referred from
National Childhood Obesity Center,
Karolinska University Hospital

Normal weight:
Recruited from
Division of Pediatric Dentistry, Department of Dental Medicine, Karolinska Institutet



Inclusion criteria:

Obese subjects: ISO-BMI> 30kg/m^2

Normal weight subjects: ISO-BMI< 25kg/m^2 [136]

Exclusion criteria:

Studies I-IV

Antibiotic treatment during the last 3 months.

Ongoing orthodontic treatment.

Current daily smoking

Study III

Under 12 years of age.

Incomplete sample sets.

QUESTIONNAIRE

All the subjects answered a questionnaire that covered topics of their medical condition, medication, dietary habits and oral hygiene habits as well as their parents' educational level and country of birth. In case subjects did not understand the Swedish language, an interpreter assisted. Parents' country of birth was categorized into "born in Sweden" or "born abroad".

Participants under 18 years were assisted by a parent when answering the questionnaire.

CLINICAL EXAMINATION

DENTAL PLAQUE AND GINGIVAL INFLAMMATION (STUDIES I-IV)

The presence of dental plaque on tooth surfaces were recorded when clearly visible and expressed using VPI%. Gingival inflammation was based on BOP% of the gingival sulcus of all teeth (wisdom teeth excluded) at six sites of each tooth [137]. The proportion of surfaces (%) with visible dental plaque and gingival inflammation, respectively, was calculated for each subject.

PATHOLOGICAL PERIODONTAL POCKETS (STUDIES I- IV)

Pocket depth (mm) was recorded by using a graded periodontal probe (LM-instruments OY, Finland) and measured to the nearest mm. The occurrence of pathological periodontal pocket was classified when the subject exhibited one or more sites with a pocket depth of ≥4mm.

CALCULUS (STUDIES I AND II)

Supragingival calculus was recorded on all teeth as present or absent when clearly visible. Subgingival calculus was recorded as present or absent on proximal surfaces of first molar and premolars on the radiographs taken as well as clinically after probing the gingival sulcus.

INCIPIENT ALVEOLAR BONE LOSS (STUDIES I AND II)

In order to determine marginal alveolar bone loss, 2 bitewing radiographs were taken using standardized technique. The distance between cemento-enamel junction (CEJ) and alveolar bone crest (AC) on the radiographs was measured on the mesial and distal surfaces of premolars and first molar by using a Peak scale loupe (Carton Optic Tokyo Japan; 7-fold magnification). Incipient marginal alveolar bone loss was classified as positive when the distance from CEJ to AC on the radiographs was ≥2 mm [138].

CREVICULAR FLUID SAMPLES (STUDY I)

GCF was collected at two sites, 16 and 41 from each subject using a paper strip (Periopaper, Proflow, Inc Amityville, N.Y., USA). The strip was inserted into the gingival crevice and left for 30 seconds. The strip was then analyzed using Periotron 8000 sensor and the volume was calculated by interpolation from a standard curve and expressed as µL GCF. The periopaper was placed in 120 µL assay buffer containing 0.9% Na Cl, 0.01 M EDTA, 0.3% bovine-globulin, 0.005 % Triton-X-100, 0.05% sodium azide, 0.0255 M NaH_2PO_4, 0.0245 M Na_2HPO_4, PH 6.8 and kept frozen at -70^{o}C [23].

The GCF samples were analyzed with respect to the levels of adiponectin, IL-1β, IL-6, IL-8 and PAI-1 by using commercially available kits (Linco Research, Inc., Missouri, USA) in accordance with the manufacturer's instruction. TNF-α levels were measured using Bio-Plex cytokine assay (Bio-Rad laboratories, California, USA).

MICROBIOLOGICAL SAMPLING (STUDY II)

Before sampling, supra gingival plaque was eliminated and the gingival margins were wiped dry with a sterile cotton pellet. Samples of subgingival plaque were taken from one molar in the lower jaw and one incisor site in the upper jaw with a sterile paper point. The paper point (*Precise Dental Internacional S.A de C.V, Mexico. Size 40)* was inserted into the gingival crevice for 5 seconds. The paper points were stored in Eppendorf tubes at -70°C until analyzed with respect to microbiology. We analyzed the samples using the checkerboard DNA-DNA hybridization technique. A total of 37 bacterial species and 3 subspecies were included in the checkerboard panel.

BLOOD PRESSURE (STUDY III)

Systolic and diastolic blood pressure were measured in the sitting position with a mercury sphygmomanometer with a standard pressure cuff of the correct size on the left arm. Blood pressures were measured in duplicates. A third measurement was taken if the two readings differed more than 5 mm Hg and the mean of the two closest readings was used. The same experienced nurse practitioner performed all measurements.

BLOOD SAMPLES (STUDY III)

Blood samples were taken after an overnight fast starting at midnight. Fasting was confirmed by the child and parent before collecting blood. Blood samples for HDL were analyzed immediately. Remaining samples were kept frozen in -80°C until analyzed. High density lipoprotein-cholesterol (HDL-C), TSH and CRP-sensitivity analyses were performed at a certified laboratory (Department of Clinical Chemistry, Karolinska University Hospital). IL-1β, IL-6, IL-8, MCP-1 and Leptin were analyzed using commercially available Luminex kits (Linco Research, Inc., Missouri, USA). TNF-α levels were measured using Bio-Plex cytokine assay (Bio-Rad laboratories, California, USA).

SALIVA (STUDY IV)

Before the saliva collection, the subjects rinsed the mouth with water. Stimulated whole saliva was collected by chewing 1g of paraffin wax for 5 minutes, and during this time the subjects spit the saliva into a test tube. The saliva was then measured and the saliva flow rate was expressed as ml/min. For further analysis, 1 ml of saliva was measured and within 10 minutes frozen and stored at -80°C until further analysis. Total amount of protein in the salivary samples was quantified using a NanoVue Plus spectrophotometer (GE Healthcare, Uppsala, Sweden). The saliva samples were analyzed with respect to the levels of adiponectin, Insulin, Resistin, IL-1β, IL-6, IL-8, TNF-α, IL-4, IL-17 and INF-γ using Luminex technology on a Bioplex Suspension Array System (Bio-Rad Laboratories, Hercules, CA, USA) with commercially available Milliplex® Map kits (EMD Millipore Corporation, Billerica, MA, USA) in accordance with the manufacturer's instruction. Leptin and CRP were analyzed using commercially available ELISA-kits (R&D Systems, Minneapolis, MN, USA).

STATISTICS

STUDIES I-IV

Data analysis was carried out using the statistical software package SPSS, version 16.0-22.0 (SPSS, Chicago, IL, USA). For analyzing the data, frequency tables, cross tables, ANOVA, Chi-square and logistic regression were used. In the logistic regression analysis the Wald test was also performed. The OR and confidence interval (95% CI) were calculated and the level of significance was accepted at P-values less than 0.05.

STUDY I

Bivariate analyses association was carried out between the dependent variable "pathological periodontal pocket (PD≥ 4mm)" and the independent variables by applying logistic regression binary model. In the multiple logistic regression analysis with pathological periodontal pocket as dependent variable, the independent variable BMI-sds was adjusted for potential confounders.

STUDY II

Bivariate analyses of associations were carried out between the dependent variable obesity and the potential independent variables. Pearson's correlation test was used to determine intra correlations between potential confounders. In the multivariate logistic regression with obesity as dependent variable the variable "sum of bacterial cells" was adjusted for the strongest variable of the intracorrelating potential confounders. In case of the microbiological variables, the Bonferroni test estimated, a P-value of < 0.01 was required to declare significance to avoid chance of mass significance. In addition, the sensitivity and specificity for bacterial sum as a discriminator of obesity were also determined.

STUDY III

Bivariate analyses were carried out between the dependent variable diastolic blood pressure or systolic blood pressure and the independent variables by applying a linear regression model. In the multiple linear regression analysis with diastolic blood pressure as dependent variable, the independent variable PD ≤ 4mm was adjusted for potential confounders (BMI-sds, age, gender, mother's country of birth, BOP >25%, IL-6, IL-8, Leptin, MCP-1, TSH and total cholesterol).

Confidence Interval 95% was calculated. Level of significance was accepted at P-values less than 0.05. In case of biochemical variables, the Bonferroni test adjusted for intracorrelations between the biomedical variables and a P-value of <0.01 was required to declare significance to avoid chance of mass significance.

STUDY IV

Bivariate and multivariate logistic regression models were used to check for potential confounders. Bivariate analyses of associations were carried out between the dependent variable "flow rate of stimulated whole saliva less than the median value (<1.5 ml/min)" and independent variables by logistic regression binary model. In the multivariate logistic regression, with "flow rate of stimulated whole saliva (< 1.5 ml/min)" as dependent variable, the independent variables BMI-sds, IL-1β and IL-8 were adjusted for potential confounders. The OR and 95% CI was calculated and the level of significance was accepted at P-values less than 0.05.

RESULTS AND DISCUSSION

This chapter gives a brief overview of the results in the studies included in the thesis and discusses them in relation to current literature. This thesis investigates oral health in adolescents with obesity with focus on periodontal inflammation, subgingival microflora and salivary flow rate. **Study I** focuses on periodontal risk indicators and their association to obesity. **Study II** was designed to test whether microbiota in the oral biofilm is linked with obesity. **Study III** takes the subject further and investigates if periodontal diseases in terms of pathological periodontal pockets may be associated with raised blood pressure and other risk markers for CVD. **Study IV** was designed and conducted to investigate the association between obesity and reduced stimulated whole salivary flow. The first three studies have been published and the fourth study is in manuscript form. All four studies can be found in their entirety in the appendix.

SOCIODEMOGRAPHIC AND MEDICAL CONDITION OF THE SUBJECTS

There was no difference between the obese and normal weight subjects in regards to parents' country of birth or level of education **(Studies I, II and IV).** There are several other socio-economic variables that we did not take into consideration, which could differ between the groups and affect the results.

Obese subjects were significantly more likely to have a chronic disease diagnosis and more likely to take one or more prescribed medication **(Studies I, II and IV).** This might also have an impact on the results, as chronic illnesses such as diabetes type 2, CVD, chronic kidney diseases and celiac disease are associated with negatively impacted oral health [139, 140]. As chronic disease and metabolic factors are also highly associated with obesity, it is hard to evaluate the effect of each components on the inter-relationship between oral health, obesity and chronic disease [141].

ORAL HYGIENE AND GINGIVAL INFLAMMATION

The obese subjects demonstrated significantly lower frequency of tooth brushing ($P<0.05$) **(Studies I, II and IV),** use of dental floss ($P<0.05$) **(Study I)** and significant higher frequency of VPI >25% ($P<0.05$) **(Studies I, II and IV).** Adolescents with obesity exhibited higher prevalence of BOP >25% **(**$P<0.05$) **(Studies I, II and IV).** Our results concerning both oral hygiene habits and gingival inflammation have been concurred by several later studies investigating oral health in obese children worldwide [105, 142-145].

PATHOLOGICAL PERIODONTAL POCKETS (STUDY I)

Obese subjects demonstrated higher frequency of PD≥ 4mm (P< 0.001) than their normal weight counterparts. We further tested the association between BMI-sds and PD≥ 4mm using a multiple logistic regression model. The variable BMI-sds was significantly (P=0.030) associated with the occurrence of PD≥ 4mm even after adjusting for the variables BOP (>25%), and subgingival calculus. Altogether the multivariate analysis demonstrates that obese adolescents exhibit an enhanced relative risk for exhibiting pathological periodontal pockets that is not caused by worse oral hygiene alone.

There are very few published studies on pathological periodontal pockets and obesity in a population under 18 years old. Reeves et al. found an association between periodontal pockets >3mm and obesity in a population 17 to 21 years of age but not in 13-16 year olds [102]. Fadel et al. did not demonstrate an association between obesity and periodontal pockets >5mm [144]. As to date, there are no longitudinal studies on obesity and periodontal disease involving children and adolescents.

ADIPOKINES IN GCF (STUDY I)

IL-1β and IL-8 in GCF were significantly higher in the obese subjects compared with normal weight controls (**Figure 3**).

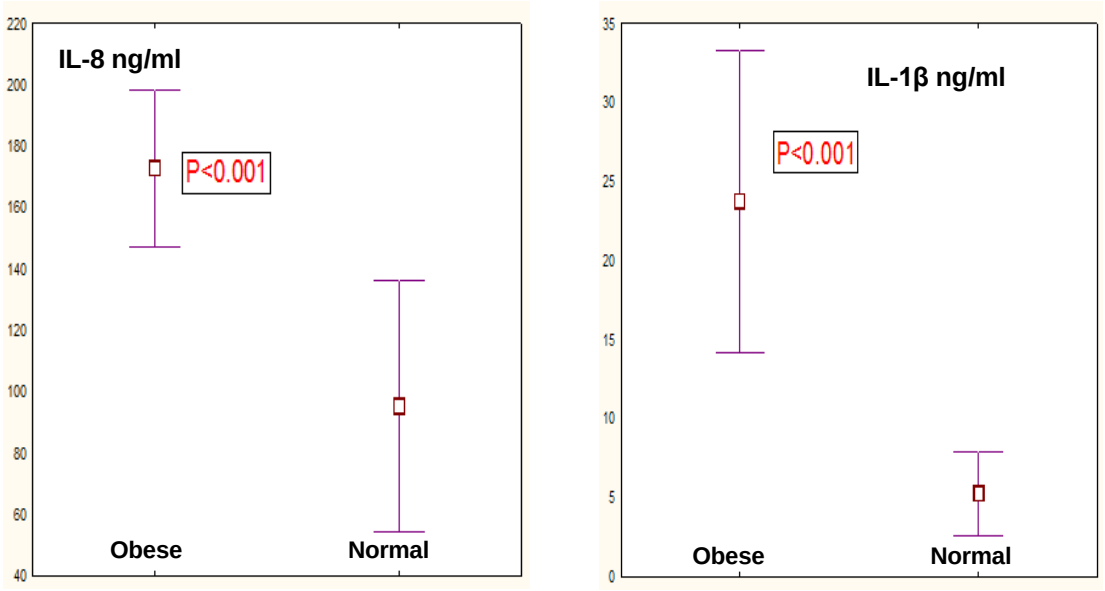


Figure 3. Levels of Il-8 and Il-1β in GCF.

Levels of Adiponectin, PAI-1 and TNF-α in GCF did not differ significantly between the groups.

A possible explanation of the enhanced relative risk of periodontal disease in obesity seems to lie with adipose derived adipokines present in periodontal disease [146]. The enhanced level of cytokines in GCF reported in obese subjects may to some extent be produced by adipose tissue and we hypothesize that obesity either directly or indirectly contributes to an enhanced pro-inflammatory milieu in the periodontal

tissue that triggers the inflammatory response to periodontal pathogens residing in the biofilm. Adipokines bind receptors on target cells and initiate intercellular signaling cascades resulting in phenotypic changes to the cell through altered gene expression and regulation. These adipocytokines activate monocytes which increas the production of inflammatory cytokines and thereby plays an important role in initiation of periodontal disease [146].

However, according to the bivariate logistic regression analysis in the present study, there was no significant difference in cytokine concentration in GCF between subjects with or without pathological periodontal pockets.

The enhanced level of the pro-inflammatory cytokines IL-1β and IL-8 demonstrated in GCF from the obese adolescents are in line with the findings in adults demonstrating an association between obesity and IL-1β as well as PGE2 levels in GCF[147]. Previously, our research group reported that the level of TNF-α in GCF was correlated with BMI of the most severe obese subjects [107]. The current study, however, did not demonstrate higher GCF level of TNF-α in obese subjects compared to controls, which probably is due to the fact that the subjects were younger and BMI in the present obese subjects was lower, indicating a shorter duration and/or less severe obesity.

MICROBIOTA IN THE ORAL SUBGINGIVAL BIOFILM (STUDY II)

The sum of bacterial cells was significantly higher among subjects in the obesity group ($P < 0.001$) as compared to the controls. On average, approximately three-fold higher amounts of bacterial cells were found in the samples from the obesity subjects compared with normal weight controls. Out of six bacterial phyla determined, five were found at higher counts in the obese subjects and counts of all families under these phyla were significantly higher ($P < 0.001$) in the obese subjects (**figure 4**). Of the Proteobacteria phylum, the bacteria *Campylobacter rectus* and *Neisseria mucosa* were present in six-fold higher amounts among obese subjects compared with the normal weight group**.** There was no difference between the groups concerning bacteria in the Spirochaetes phylum.

From the total of 40 different bacterial species determined in the bacterial samples, 32 species were present in significantly higher amounts ($P < 0.01$ level) in the obese subjects compared to the normal weight controls.

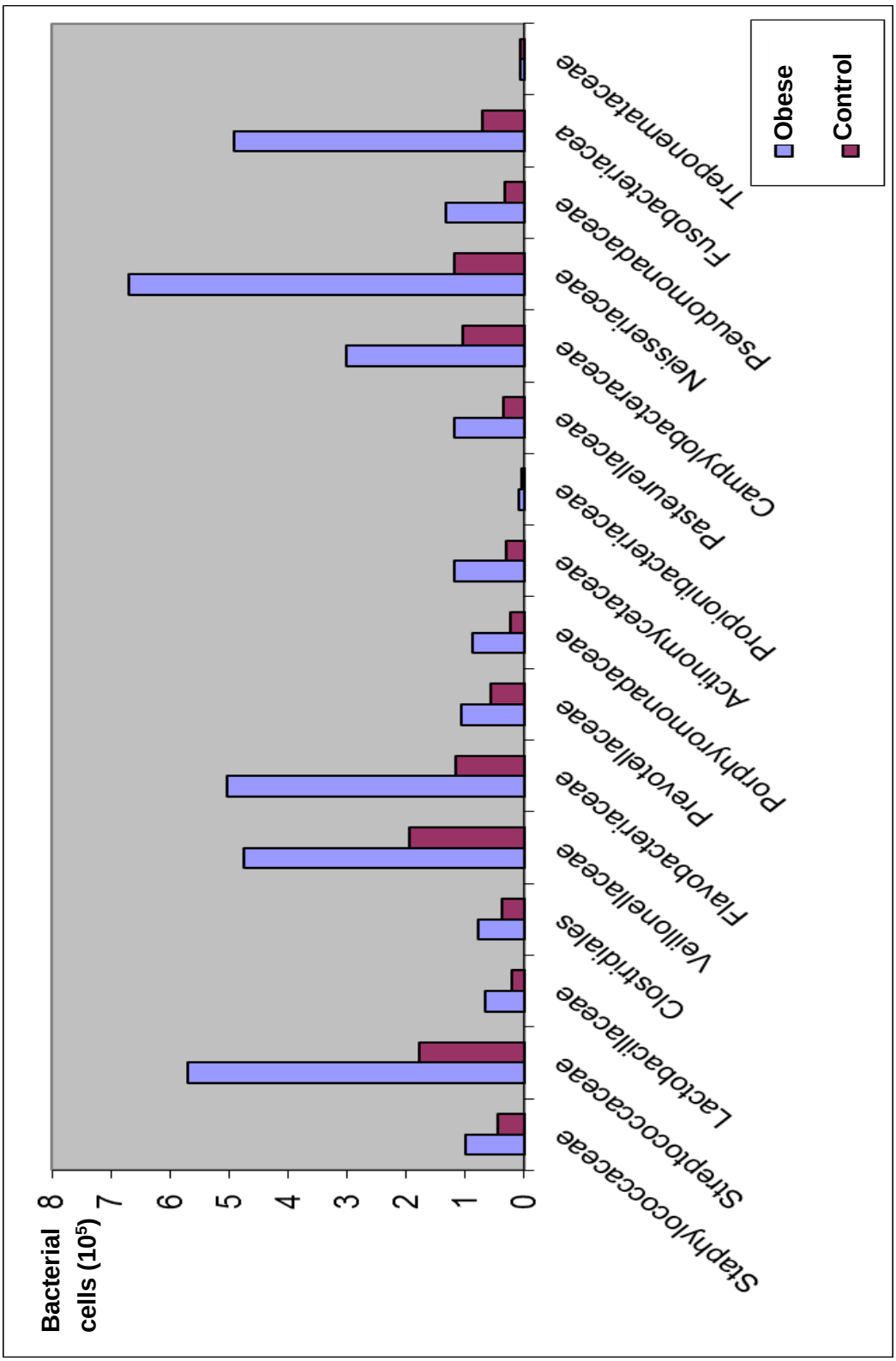


Figure 4. The sum of assessed bacterial cells (x 10^5) from obese and normal weight subjects. Presented by family.

After adjusting for VPI%, BOP%, chronic disease, tooth brushing habits or salivary flow rate (ml/min), there was a significant association between bacterial cell count and the dependent variable obesity (OR= 1.05, P= 0.006). Although the OR of "sum of bacterial cells" seems to be low (1.05), one has to consider that an increase from $1x10^5$ to $1x10^6$ bacteria enhances the risk for obesity by 50%, indicating potential clinical relevance.

This was the first study demonstrating an association between obesity and an alteration of the subgingival microbiota in adolescents, although increased counts of *T. forsythia* colonizing the periodontal pockets have previously been demonstrated in obese adults [108]. We demonstrated that traditional periodontal pathogens such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* and *Parvimonas micra* [148, 149], were present at approximately three-fold increase in the dental biofilm of obese adolescents compared with the normal weight controls. We found approximately a three-fold higher level of the phylum Firmicutes and approximately a four-fold higher level of the phylum Bacteroidetes in the subgingival biofilm of obese adolescents. There was no significant difference of the relative proportion of Firmicutes and Bacteroidetes in the dental biofilm between obesity and controls. In the gut, however, obese subjects had relatively more Firmicutes but less Bacteroidetes compared with lean controls [30]. Results from animal studies support the hypothesis that gut bacteria influences development of obesity, especially the Firmicutes phylum that in mice promotes absorption of monosaccharides in the gut that results in enhanced lipogenesis [150]. Approximately one gram of oral bacteria corresponding to approximately 10^{11} cells are swallowed daily with the 500 to 1500 ml of saliva produced [148]. The high amount of oral microbiota ingested may affect energy harvesting in the gut and thereby be involved in the development of obesity. However, it is unknown in what amounts and proportions oral bacteria ingested will survive the defense barriers of the gastrointestinal tract.

Oral microbiota plays a major role in the nitrate to nitrite conversion which in its turn is important in regulating blood pressure [151, 152]. Interestingly, it was reported that the number of nitrate reducing bacteria was greatly reduced in the oral cavity of rats treated with daily application of antiseptic mouthwash indicating a possible role of oral microbiota in regulating blood pressure and thereby in general health [153]. In addition, a link between an increased loading of periodontal pathogens in subgingival plaque and hypertension has been demonstrated [154].

Whether the alteration of the oral microbiota promotes the development of obesity in adolescents or is a consequence of the obese condition or, alternatively, is induced by life-style associated conditions is an essential issue for further research.

PATHOLOGICAL PERIODONTAL POCKETS, DIASTOLIC BLOOD PRESSURE AND INFLAMMATORY MARKERS IN BLOOD SERUM (STUDY III)

Adolescents with PD≥ 4mm (n=14) had significantly higher means of diastolic blood pressure (P= 0.008) than adolescents without PD≥ 4mm (n= 61). There was no significant difference between adolescents with and without pathological periodontal pockets (PD≥ 4mm) in regards to age, gender, BMI-sds, tooth brushing habits, sociodemographic factors, medical history or VPI> 25%. Generally, the average diastolic blood pressure for 14.5 year old normal weight adolescents is 63 mmHg, and the cut off point for prehypertension has been suggested to be at 78 mmHg[155]. Based on this consideration, approximately 12% of the obese subjects in our study exhibited prehypertension on the day the measurements were taken. The average diastolic blood pressure for obese adolescents is reported to vary from 62 mmHg to 70 mmHg[156, 157] with a frequency of prehypertension of approximately 20% [158, 159]. Our finding that there is an association between pathological periodontal pockets and diastolic blood pressure is compatible with the finding in a similar age group that overweight or obese adolescents had significantly higher blood pressure and more gingival inflammation than their normal weight counterparts [145].

Obese adolescents with PD≥ 4mm had significantly higher levels of IL-6 (P< 0.001), Leptin (P= 0.018), MCP-1 (P= 0.049) and TSH (P= 0.004) in blood serum than adolescents without PD≥ 4mm (n= 61). Presence of calculus, IL-1β, IL-8, TNF-α, Cholesterol, Low density lipoprotein (LDL)/HDL quotient, adult Hemoglobin 1c (Hba1c) or CRP sensitivity did not differ significantly between the two groups.

TSH has not previously been connected to periodontal disease but is reported to be enhanced in serum in obese subjects[160]. In this study, the subjects without PD≥ 4mm had TSH levels corresponding to average levels in healthy individuals, whilst the subjects with PD≥ 4mm had relatively higher levels. Interestingly, it has been reported that increases in TSH levels are associated with increases in both systolic and diastolic blood pressure as well as other cardiovascular risk factors [161-163]. The higher levels of IL-6, TSH and Leptin in serum in obese adolescents with PD≥ 4mm further supports the notion that periodontal disease is part of a systemic inflammation that might, in the long run, lead to atherosclerosis and CVD [164, 165].

In addition, a recent study demonstrated that adolescents diagnosed with three or more metabolic syndrome parameters are more likely to display gingival inflammation than healthy individuals [166]. The systemic inflammatory response that accompanies periodontal disease has been proposed as a possible link between periodontal disease, atherosclerosis and its cardiovascular ill-effects [167, 168].

There is a well-established association between oral and cardiovascular co-morbidity in adults [169] and diastolic blood pressure at 18 years of age has been suggested as a stronger predictor for cardiovascular mortality than systolic blood pressure [170]. However, more longitudinal studies are needed before pathological

periodontal pockets in children with obesity can be suggested as an early marker for future cardiovascular events.

SALIVARY FLOW AND INFLAMMATORY BIOMARKERS (STUDY IV)

Obese subjects demonstrated significantly lower flow rate of stimulated whole saliva ($p< 0.001$). The mean value of stimulated whole saliva flow rate was 2.0 ml/min of the normal weight controls compared with 1.3 ml/min of obese subjects.

A bivariate logistic regression analysis was performed with stimulated whole saliva (<1.5ml/min) as dependent variable. Obesity, in terms of BMI-sds as continuous variable, was significantly associated with salivary flow rate (<1.5ml/min) ($p< 0.001$).

The significant association between salivary flow rate <1.5ml/min and BMI-sds remained even when adjusting for age, gender, chronic disease, drugs affecting salivary flow, PD< 4mm, IL-1β, IL-8 and Insulin.

Salivary flow rate <1.5ml/min was significantly associated with the biomarkers IL-1β ($p= 0.026$), IL-8 ($p= 0.013$) and Insulin ($p= 0.043$). Using multivariate logistic regression models we further tested the association between salivary flow rate (<1.5ml/min) and the significant independent variables IL-1β and IL-8. In the multivariate logistic regression model, the levels of the biomarkers IL-1β and IL-8 were significantly associated with salivary flow rate <1.5ml/min even after adjusting for age, gender, chronic disease, drugs affecting salivary flow, PD> 4mm and protein content in saliva. In agreement with previous findings we here demonstrate that obese adolescent's exhibit reduced salivary flow rate compared to normal weight adolescents [80, 171] which also has been reported among young adults [80, 81], but not in children under 12 years [83, 84]. There are several reasons why younger children do not exhibit an association between reduced salivary flow and obesity. There is the possibility that sample taking is more difficult in a very young population. In addition, one has to consider that the duration and exposition time to obesity is shorter, which might have an influence on the results.

The finding that obese subjects present reduced salivary flow rate is well compatible with a recent study reporting that in a group of morbidly obese patients, the stimulated salivary flow significantly increased after weight loss following gastric bypass surgery [172].

Obese adolescents also demonstrated higher levels of salivary Insulin ($p< 0.001$), Leptin ($p= 0.005$), CRP ($p= 0.002$) and INF-γ ($p= 0.011$). The quota between T-helper cells (Th)-1 and Th-2 related cytokines (INF-γ/ IL-4) was higher in the obese group (0.73) compared to normal weight adolescents (0.44) ($p=0.020$).

Although the salivary levels of Leptin, Insulin and CRP were significantly higher in obese adolescents than in their normal weight counterparts, these inflammatory biomarkers were, according to the results, not associated with reduced salivary flow rate.

The mean CRP level in saliva was approximately five times higher in obese adolescents than in their normal weight counterparts. Salivary CRP concentration has been found to correlate well with serum concentration [173]. Human studies have associated high levels of CRP with metabolic syndrome and type 2 diabetes [83, 174].

Leptin level in saliva was 11 times higher in the obese subjects compared to normal weight subjects. Leptin is produced by adipocytes and binds to leptin receptor in hypothalamus to suppress food intake and increase energy consumption. Reduced sensitivity to this molecule might trigger the onset of obesity [95]. More recently, Leptin has also been demonstrated to influence the immune systems balance [175], and it is interesting to note that leptin concentrations in saliva are significantly lower in subjects with chronic periodontal disease, whilst serum levels are significantly higher and relate closely to current disease activity [176].

Concentration of Insulin in saliva is known to correlate well with serum levels of Insulin [177] and the results in the present study are well in line with previous findings describing increased Insulin levels in saliva from obese individuals [83].

Presumably, the higher levels of CRP, Leptin and Insulin in saliva are reflecting a systemic metabolic change in obesity but does not seem to be directly associated with reduced saliva flow rate. However, as the increased levels of CRP, Leptin and insulin does reflect a systemic inflammation, they might indirectly affect salivary flow. Systemic inflammation and autoimmune disease, such as Sjögren's syndrome and rheumatoid arthritis, often involve salivary glands with reduced salivary flow as a consequence [178].

LIMITATIONS

All studies in this thesis are of a cross-sectional design, giving us results at one moment in time. Longitudinal studies are needed before any permanent conclusions can be drawn. In addition, experimental studies would be necessary to evaluate cause and effect mechanisms behind cross-sectional and longitudinal statistical associations.

We did not register the duration of obesity of the subjects in any study, which in addition to the severity of obesity is of importance when looking upon the association between obesity and oral health issues during adolescence.

One weakness of these studies is that the amount of preventive care given to our patients is not possible to analyze. One cannot rule out that prevention care differs between the groups, which may affect the strength of the link between obesity and oral co-morbities.

Another weakness in these studies is the possible influence of socioeconomic factors. Although we took into consideration parents' education and country of birth there are other socioeconomic variables that could affect the subjects' oral and general health.

A weakness to take into consideration as well is that in **Study III,** one-time blood pressure measurement in a resting position was used, which is an acceptable screening method [179]. However, to diagnose a child or adolescent with hypertension, a 24 hour evaluation is recommended. [155, 180].

In **Study III**, as well, no normal weight participants were included and therefore, the association between periodontal disease and raised diastolic blood pressure might be specific for the obese condition. Future research is needed and should include a normal weight control group in order to investigate if there is a general association between blood pressure and periodontal disease.

We used the median value of flow rate of stimulated whole saliva (1.5ml/min) as the cut off value in the multiple logistic regression analysis in **Study IV** since there is lack of valid reference in adolescents regarding reduced flow rate of whole saliva.

Saliva samples were mostly taken between one and three o'clock in the afternoon but not strictly at the same time, which may to some extent influence the secretion rate due to circadian rhythm of salivary flow rate [81]. Clinical studies using standardized saliva collection procedure that includes point of time are needed to confirm the current and previous observation of reduced salivary flow rate in subjects with obesity.

MAIN FINDINGS

- There is an association between obesity and pathological periodontal risk indicators in adolescents.
- Oral bacterial cells counts in the subgingival biofilm are associated with obesity in adolescence.
- Periodontal disease in terms of pathological periodontal pockets is associated with diastolic blood pressure in obese adolescents.
- Obesity in adolescents is associated with reduced flow rate of stimulated whole saliva.
- Low salivary flow rate in adolescents is associated with enhanced levels of inflammatory markers IL-1β and IL-8 in saliva.

CLINICAL IMPLICATIONS

Obesity in adolescents is associated with inadequate oral hygiene habits, declined oral health in terms of VPI% and BOP% as well as PD≥ 4mm, indicating more periodontal inflammation than in their normal weight counterparts.

The Public Dental Service may want to consider the knowledge that periodontal disease is more frequent and stimulated whole saliva flow rate is reduced in adolescents with obesity in developing a prevention plan for this group of patients.

In conclusion, these results call for collaboration between pediatric dentists and pediatric medical physicians in developing a prevention program for obesity and its associated disorders in children and adolescents.

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This journey, whilst working and writing on this thesis, has taken me places I never expected and lead me to people I will never forget. It started with a hopeful e-mail to my would-be supervisor asking if perhaps there were any ongoing projects, preferably in a health-nutrition-weight related area in the pediatric dental department. Lucky me! There was such a project! And this is where it took me.

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