

From the Departments of Cariology and Endodontology,
Institute of Odontology
Karolinska Institutet, Stockholm, Sweden

and

Centre for Clinical Research
Uppsala University, Västerås, Sweden

Studies on the prevalence of
reduced salivary flow rate
in relation to general health
and dental caries,
and effect of iron supplementation

Håkan Flink



**Karolinska
Institutet**



LANDSTINGET
VÄSTMANLAND



UPPSALA
UNIVERSITET

Stockholm 2007

OPPONENT

Professor Downen Birkhed, Göteborg University, The Sahlgrenska Academy
Department of Cariology, Institute of Odontology
Göteborg, Sweden

EXAMINING COMMITTEE

Professor Lars Gahnberg
Folktandvården Västra Götalandsregionen
Göteborg, Sweden

Professor Jukka H Meurman, University of Helsinki
Department of Oral Infectious Diseases
Institute of Dentistry, Faculty of Medicine
Helsinki, Finland

Professor Thomas Modéer, Karolinska Institutet
Department of Pedodontics, Institute of Odontology
Huddinge, Sweden

SUPERVISORS

Professor Folke Lagerlöf, Karolinska Institutet
Department of Cariology and Endodontology, Institute of Odontology
Huddinge, Sweden

Professor Åke Tegelberg, Uppsala University
Centre for Clinical Research
Västerås, Sweden

Associate Professor Anette Oliveby, Karolinska Institutet
Department of Cariology and Endodontology, Institute of Odontology
Huddinge, Sweden

Associate Professor Maud Bergdahl, University of Tromsø
Institute of Odontology, Faculty of Medicine
Tromsø, Norway

Cover: A drop from a dripping water tap has about the same volume as the unstimulated whole saliva produced during one minute or less by an individual with average salivary flow rate. When salivary flow rates are very low, it takes three minutes or more to produce the drop.

All published papers are reproduced with permission from the respective publisher

Published by Karolinska Institutet.
© Håkan Flink, 2007
ISBN 978-91-7357-429-7
ISSN 0348-6672

Printed by



REPROPRINT AB
Stockholm 2007

www.reproprint.se
Gårdsvägen 4, 169 70 Solna

To my family
Eva, Emil, Mirna and Pilar

Håkan Flink
Centre for Clinical Research
Central Hospital
721 89 Västerås

hakan.flink@ltv.se

ABSTRACT

Background: Reduced salivary flow is a condition that affects oral health. Its prevalence is unknown in young and middle-aged adults and there is no known treatment that permanently increases the salivary flow rate. Reduced salivary flow is related to dental caries, the most common oral disease. Reduced salivary flow is often found in individuals with insufficient food intake and thereby insufficient nutrition to the salivary glands. One nutrition related factor that has been proposed to effect salivary flow rate is iron deficiency. **Aims:** The aims of the thesis were to investigate i) the prevalence of reduced salivary flow rate in different age groups of adults, ii) the relationship between reduced salivary flow rate, general health and dental caries, iii) the influence of time of measurement on reduced salivary flow rate, and iv) if reduced salivary flow rates could be increased by iron supplementation. **Material and methods:** In Study I saliva was collected from 1427 individuals aged 20-69 years. A questionnaire was answered regarding subjective oral dryness, general diseases, use of drugs, BMI (Body Mass Index) and use of tobacco. In Study II saliva was collected from 48 patients with active caries and 48 caries-inactive patients. A blood sample was analysed for serum ferritin. In Study III the unstimulated salivary flow rate was tested at 7:30 and 11:30 a.m. in 108 individuals, age 15-46 years. The participants were allocated to one of three groups (very low <0.1 mL/min, low 0.1-0.2 mL/min and normal >0.2 mL/min) based on the the unstimulated salivary flow rate at 7:30 a.m. Different aspects of the perception of oral dryness were rated using Visual Analogue Scales. In Study IV a double-blind, randomized controlled trial was carried out on 50 individuals with a low unstimulated whole salivary flow rate and low serum ferritin. Half the individuals received 60 mg of iron orally twice a day for 3 months, while the other half received placebo. **Results:** In Study I it was found that the prevalence of very low (<0.1 mL/min) and low (0.10-0.19 mL/min) unstimulated salivary flow rate were similar for different age groups up to 50 years, ranging between 10.9-17.8% and 17.3-22.7%, respectively. Multiple logistic regression revealed that above age 50, female gender, 'having fewer than 20 teeth', and taking xerogenic drugs significantly increased the risk of very low unstimulated salivary flow rate. In Study II 32 individuals (67%) in the caries active group had low unstimulated salivary flow rate compared with 13 individuals (27%) in the caries inactive group. There was no difference in serum ferritin levels between the two groups. Study III showed for all groups a statistically significant increase in unstimulated salivary flow rate at 11:30 a.m. compared with 7:30 a.m., all of similar magnitude (0.08-0.09 mL/min). In the group with very low salivary flow rate, 70% at 11:30 a.m. exceeded the 0.1 mL/min limit. There were significant difference in perception of oral dryness between the normal group and both the low and the very low groups. In Study IV no statistically significant difference was found between the groups after treatment for the unstimulated flow rate and in the subjective assessments of oral dryness. **Conclusions:** The prevalence of reduced salivary flow rates is consistent and prevalent in younger and middle-aged adults (<50 years). Very low salivary flow rates are related to high Body Mass Index (BMI) and diagnosed diseases in younger adults, but to medication in older adults. Reduced salivary flow rate in young adult women is related to caries. The time of measurement of salivary flow rates influences diagnosis of hyposalivation. Iron supplementation does not enhance salivary flow.

Keywords: Unstimulated whole saliva, stimulated whole saliva, resting saliva, xerostomia, dry mouth, hyposalivation, caries, remaining teeth, body mass index, iron deficiency, serum ferritin, circadian rhythm.

LIST OF PUBLICATIONS

- I. Flink H, Bergdahl M, Rosenblad A, Tegelberg Å, Lagerlöf F.
Prevalence of reduced salivary flow rates in relation to general health, body mass index and remaining teeth in different age groups of adults.
Submitted.
- II. Flink H, Tegelberg Å, Sörensen S.
Hyposalivation and ironstores among individuals with and without dental caries.
Acta Odontologica Scandinavica. 2000; 58:265-71.
- III. Flink H, Tegelberg Å, Lagerlöf F.
Influence of the time of measurement of unstimulated human whole saliva on the diagnosis of hyposalivation.
Archives of Oral Biology. 2005; 50: 553-9.
- IV. Flink H, Tegelberg Å, Thörn M, Lagerlöf F.
Effect of oral iron supplementation on unstimulated salivary flow rate: a randomized, double-blind, placebo-controlled trial.
Journal of Oral Pathology & Medicine. 2006; 35: 540-7.

CONTENTS

Preface	1
Introduction	3
Saliva origin and composition.....	3
Functions of saliva in relation to oral health.....	3
Measurement of saliva flow rates (Sialometry)	4
Reduced salivary flow rate.....	6
Malnutrition and salivary gland hypofunction.....	7
Dental caries.....	8
Reduced salivary flow rates and caries	9
Caries and iron deficiency.....	9
Aims	10
Materials and methods	11
Study designs	11
Cross-sectional study (<i>Study I</i>).....	11
Case-control study (<i>Study II</i>).....	11
Methodological study (<i>Study III</i>).....	11
Randomized controlled trial (<i>Study IV</i>).....	11
Ethical considerations	11
Subjects	11
Collection of saliva	13
Unstimulated whole saliva	13
Stimulated whole saliva.....	13
Caries-related variables.....	13
Number of teeth.....	13
Manifest dental caries.....	14
Blood samples.....	14
Serum ferritin.....	14
Capsular reactive protein (CRP).....	14
Questionnaires.....	15
Related to general health	15
Subjective evaluation of the salivary and tear gland function.....	15
Statistics	15
Results	17
Prevalence of reduced salivary flow rates (<i>Study I</i>).....	17
Caries active compared to caries inactive individuals (<i>Study II</i>)	19
Influence of time of measurement on diagnosis of hyposalivation (<i>Study III</i>)	21
Effect of iron supplementation on salivary flow rates (<i>Study IV</i>).....	23
Discussion	24
Future directions.....	35
Conclusions	36
Epilogue and implications	37
Svensk sammanfattning (Swedish summary)	38
Acknowledgements	40
References	43

PREFACE

She is a 45 year-old nurse' assistant at her yearly dental checkup in my dental office. She seems to be quite nervous: only four times has she been free from decay which required filling. She considers herself healthy. As a mother of two sporting teenagers she is conscientious about good eating habits, even if they could be better. She is aware of her oral hygiene and she brushes her teeth more than twice a day. She regards this as "more than others", and she compares herself with her husband who eats more sweets, never bothers much about brushing, but never gets cavities. Once when the children were young and the family budget was tighter, she wanted to save money and postponed her annual recall for one year. It cost her a summer holiday ruined by toothache, one root filling, extraction of one tooth, and a lot of money, an experience she does not want to have again. Therefore, annual checkups are very important to her. When I measured her salivary flow rate it was found to be low. Her first question was "What can I do to get more saliva?"

While working as a general practicing dentist for more than twenty years at the same public dental clinic, I have had the privilege of getting to know my patients well, but have also experienced challenge and sometimes frustration when trying to help patients with problems like those described above. These experiences made me realize that there is a need for further studies on reduced salivary flow rate and its relationship to reduced defence against dental caries. Writing this thesis has allowed me to do so.

INTRODUCTION

Saliva is a very important body fluid often taken for granted. It is critical to the maintenance of oral health, yet it receives little attention until its quantity or quality is diminished (Humphrey and Williamson 2001). There has been much recent research on the topic of salivary dysfunction related to disease (von Bultzingslöwen, et al. 2007) or as a side effect of certain medications (Scully 2003). Saliva has also become useful as a non-invasive alternative for blood in medical diagnosis and research (Aps and Martens 2005). Consequently, it is necessary for the dentist to have good knowledge concerning the normal salivary flow and its function.

SALIVA ORIGIN AND COMPOSITION

Normally the daily production of saliva ranges between 0.5 and 0.6 L/24h (Dawes 2004). It is composed of more than 99% water and less than 1% solids, mostly proteins and electrolytes (Whelton 2004). Saliva is secreted by the major salivary glands (parotid, submandibular and sublingual glands) and by hundreds of minor salivary glands located in the palate, lip, cheek and tongue (Nauntofte, et al. 2003)(Figure 1). Saliva is predominantly produced in the acinar cells of these glands divided into serous and mucous cells (Whelton 2004). Saliva from the sublingual, labial and palatal glands is rich in high molecular weight mucins, in contrast to the parotid glands, which secrete a more watery or serous type of saliva. The submandibular saliva has a more seromucous character (Nauntofte, et al. 2003). Unstimulated whole saliva consists of a mixture of the secretions of individual salivary glands, the most dominating being the submandibular glands (Kerr 1961). After stimulation by e.g. chewing or acids will the salivary flow will increase, mainly from the parotid glands (Kerr 1961). Saliva excreted by the various salivary glands differs in biochemical composition and whole saliva is therefore influenced by the net flow rate from the different types of salivary glands and by the various types of stimulation (Söderling 1989).

FUNCTIONS OF SALIVA IN RELATION TO ORAL HEALTH

The multiple functions of saliva relate to its quantity and quality. The salivary fluids solubilise food, facilitate taste and bolus formation, dilute and clear food remnants, lubricate the oral soft tissues, and facilitate mastication, swallowing and speech (Nauntofte, et al. 2003). The functions of its components include protection of the teeth by buffering acids, by maintaining the saliva sufficiently saturated with respect to hydroxyapatite to prevent dissolution of the dental hard tissues, and by formation of a protective barrier, the enamel pellicle (Hay 1969). There are also several antimicrobial defence mechanisms by different salivary proteins (Amerongen and Veerman 2002).

As reduced salivary flow nearly always results in an altered salivary composition normal salivary flow is important for oral health (for reviews see Humphrey and Williamson 2001, Amerongen and Veerman 2002, Van Nieuw Amerongen et al. 2004, Tabak 2006). Hyposalivation, defined as an objectively measured abnormal reduction in salivary flow, has been associated with the symptom xerostomia, which has been defined as the subjective perception of oral dryness (Nederfors 2000, Orellana, et al. 2006). The terminology regarding reduced salivary flow has been confusing as hyposalivation and xerostomia have sometimes been used as synonyms.

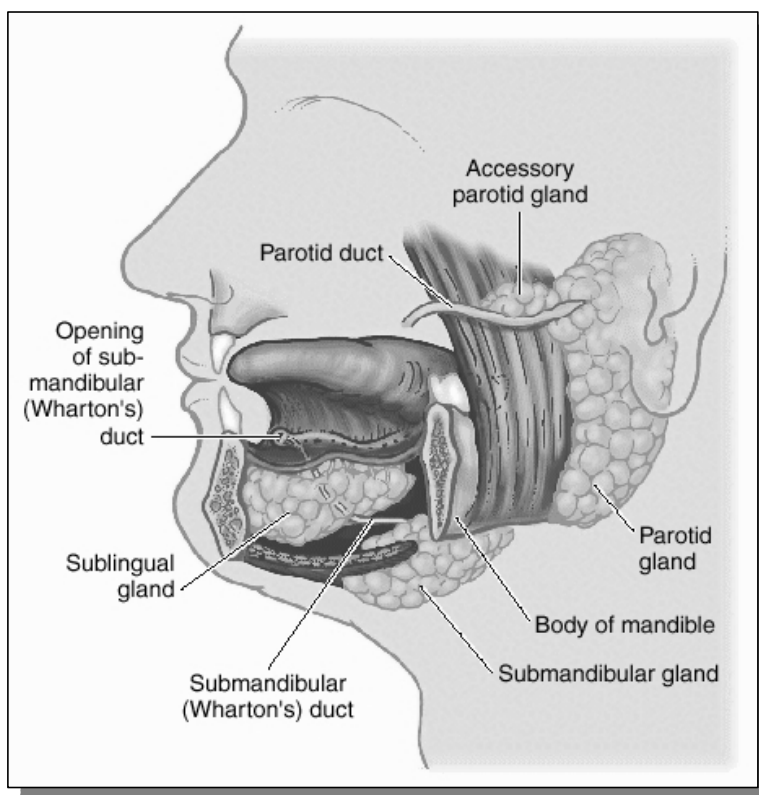


Figure 1. Salivary glands.
Dorland's Medical Dictionary for Health Consumers. © 2007

In an attempt to clarify the nomenclature the term 'Salivary Gland Hypofunction (SGH)' has been proposed as an all-embracing term for subjective symptoms and objective signs of dry mouth (Nederfors 2000). Salivary gland hypofunction is subdivided into three concepts: xerostomia, hyposalivation, and dysfunctionally altered saliva composition, that usually occur together. However, hyposalivation may exist separately from xerostomia.

Other symptoms of hyposalivation are thirst, difficulties in speaking and eating dry food (Sreebny, et al. 1992). If severe, hyposalivation could affect quality of life (Baker, et al. 2006, Baker, et al. 2007, Larsson, et al. 2004, McMillan, et al. 2004) and lead to dental diseases, such as dental caries (Leone and Oppenheim 2001) and to inflammatory conditions in the oral cavity (Sreebny, et al. 1992).

MEASUREMENT OF SALIVA FLOW RATES (SIALOMETRY)

Various sialometric methods have been described for unstimulated and stimulated saliva from separate glands or for whole saliva (Manthorpe and Axéll 1990, Navazesh and Christensen 1982, Ship, et al. 1991). The lack of standardized methodology to measure saliva has been stressed (Leone and Oppenheim 2001)

The unstimulated whole saliva flow rate has been proposed as more sensitive for detecting reduced salivary flow, since it may be reduced even when the stimulated whole saliva is unaffected (Becks and Wainwright 1939, Sreebny 1989, Wang, et al. 1998). The standardization of its measurement has included restriction in eating, drinking, tooth brushing, and use of tobacco for 1-2 hours prior saliva collection (Birkhed and Heintze 1989, Navazesh and Christensen 1982). Instructions on how to perform the collection include having the subject sitting bent forward in a relaxed location, the tongue placed on the lingual surfaces of the upper incisors, the mouth kept open, and passively letting the saliva drip into a cup held to the lower lip (Dawes 1974).

The length of the collection time has varied from 2 to 15 min in different studies. Dawes (1974) proposed that the minimum time needed to collect a sufficient volume to accurately estimate flow rate is probably 5 min. Collection times between 5 and 10 min seem to be the most common in clinical studies (Fure and Zickert 1990, Heintze, et al. 1983, Kavanagh, et al. 1998, Nederfors and Dahlöf 1992, Percival, et al. 1994, Rantonen and Meurman 1998, Risheim, et al. 1992). However, in diagnosis of Sjögren's syndrome 15 min is used (Vitali, et al. 2002).

Since a circadian rhythm has been described for the unstimulated whole saliva flow rate (Dawes 1972) attention has also been focused on the time of day when the testing ought to be performed. Lower flow rates are found early in the morning compared with late in the afternoon (Figure 2)(Dawes 1972). For individuals with a normal unstimulated whole saliva flow rate, the increase during 3-4 morning hours has been estimated to be 25-50% or around 0.1 mL/min (Nederfors and Dahlöf 1992, Rantonen and Meurman 1998)(Figure 2). However, the influence of the circadian rhythm on the saliva flow in individuals with hyposalivation is, to the best of my knowledge, unknown, despite possibly having a decisive impact on the diagnosis of Sjögren's syndrome, on caries risk assessment, and in clinical trials aiming to increase the salivary flow rate.

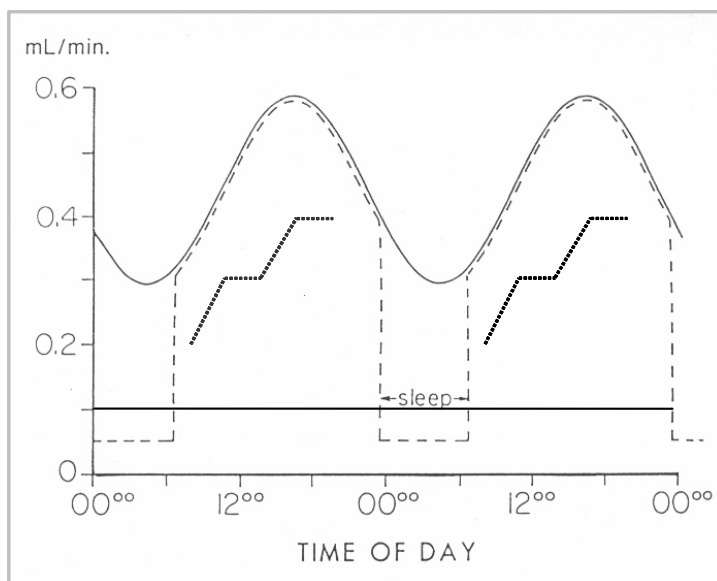


Figure 2. The circadian rhythm in unstimulated salivary flow rate and the idealized effect of sleep (dashed line) Modified from Dawes 1972 with data from Rantonen and Meurman 1998 (dotted lines) and including the limit for very low flow rates 0.1 mL/min.

It is generally accepted in the literature that the limit for a very low unstimulated whole saliva flow rate is ≤ 0.1 mL/min (Ericsson and Hardwick 1978, Sreebny and Valdinì 1988). This limit is also used for the diagnosis of Sjögren's syndrome (Pedersen and Nauntofte 2001). Values between 0.1 and 0.2 mL/min have been suggested as low values, while those higher than 0.2 mL/min should be regarded as normal (Sreebny and Valdinì 1988). It has been shown that subjective symptoms of oral dryness, xerostomia, are often present below a flow rate of about 0.1-0.2 mL/min (Wang, et al. 1998, Wolff and Kleinberg 1998). Furthermore, a limit in the range 0.12-0.16 mL/min has been proposed as an indicator of increased caries risk. This is based on the findings of i) a prolonged glucose clearance below an unstimulated flow rate of 0.14 mL/min (Hase and Birkhed 1988), ii) an increased dental mineral loss below 0.16 mL/min (Bardow, et al. 2001), and iii) the possibility to separate individuals with salivary gland hypofunction from those with normal gland function (Navazesh, et al. 1992).

For stimulated whole saliva, flow rates less than 1.0 mL/min have been regarded as low and flow rates less than 0.7 mL/min as very low (Ericsson and Hardwick 1978, Nauntofte, et al. 2003). The relationship between unstimulated and stimulated whole salivary flow rates has been reported to be significantly correlated (Heintze, et al. 1983, Sreebny and Valdinì 1988).

REDUCED SALIVARY FLOW RATE

It has been suggested that reduced salivary flow is caused by many factors, e.g. systemic diseases (von Bultzingslöwen, et al. 2007), drugs (Scully 2003), or dehydration (Walsh, et al. 2004). No treatment is known to permanently normalize the salivary flow rate (Givens 2006), apart from substitution of drugs inducing hyposalivation or alter the time of the day when medications are taken (Atkinson, et al. 2005). Systematic therapies are available but have substantial side effects (Grisius 2001). Therefore, the aim of the treatment is mainly limited to reducing symptoms (Fox 2004, Sreebny, et al. 1992).

In adults, 20-69 years of age, the prevalence of reduced salivary flow rates (defined as an unstimulated whole saliva flow rate of ≤ 0.1 mL/min) has been reported to be 15% among men and 22% among women (Bergdahl 2000). This corresponds well with the observation that hyposalivation is more common among women than men. Heintze et al. (1983) found in a population aged 15-74 years that only 3% of men had an unstimulated salivary flow rate below 0.1 mL/min. The corresponding value for women was 10%. It has also been shown that the prevalence of hyposalivation is high among subjects being treated for medical diseases and conditions, such as rheumatoid arthritis, diabetes mellitus, high blood pressure (Sreebny 1989) and HIV (Navazesh, et al. 2000). The prevalence of hyposalivation increases in old age, mainly because of an increase in the use of prescribed drugs that affect saliva secretion (Ship, et al. 2002). Even if hyposalivation is common among older individuals, it has also been found in younger adults (Sreebny and Valdinì 1988).

Few studies on salivary flow rate are based on large general populations including individuals younger than 50 years of age (Becks and Wainwright 1943, Bergdahl 2000, Billings, et al. 1996, Heintze, et al. 1983, Yeh, et al. 1998). To the best of my knowledge, there is no population-based study that has shown the prevalence of reduced salivary flow rates in young and middle-aged adults.

The variation of the salivary flow rate in a population has been described by the standard deviation, and the mean as a measure of central tendency. However, since the distribution of salivary flow rates is not symmetrical but right-skewed, the use of median in combination with percentiles has been suggested as more appropriate (Sreebny 2000, von Bultzingslöwen, et al. 2007). Table 1 summarizes the results from the four population based studies identified on unstimulated saliva flow rate. It can be seen from the table that the studies vary concerning the choice of measures of central tendency and variability. Furthermore, the choice of cohorts varies quite a lot and the studies are therefore difficult to compare. An important issue when comparing different studies is that the sample is selected randomly from a well defined population. This has not been declared for any in the studies of Table 1.

Table 1. Median and mean values of unstimulated whole saliva flow rates (mL/min) from four different population based studies. Presented for different age groups 15-80+ years of age and separated by gender.

Gender	Heintze et al. 1983				Percival et al. 1994				Billings et al. 1996				Yeh et al. 1998		
	Age	n	Median		Age	n	Mean		Age	n	Mean	SD	Age	n	Mean
Men	15-29	84	0.34		20-39	17	0.70		<30	55	0.27	0.26			
	30-44	104	0.44						30-49	59	0.26	0.21	35-44	56	0.55
	45-59	58	0.33		40-59	10	0.50						45-54	87	0.47
	60-74	40	0.30		60-79	16	0.49		50-69	61	0.21	0.15	55-64	96	0.48
					80+	12	0.30						65-69	83	0.42
									70+	51	0.20	0.23	70-74	90	0.40
													75+	32	0.43
All		286	0.36			55	0.50			226				444	
Women	15-29	95	0.25		20-39	12	0.55		<30	99	0.24	0.19			
	30-44	133	0.31						30-49	166	0.21	0.15	35-44	60	0.36
	45-59	70	0.22		40-59	20	0.36						45-54	111	0.34
	60-74	45	0.20		60-79	12	0.39		50-69	110	0.18	0.22	55-64	126	0.27
					80+	17	0.18						65-69	115	0.28
									70+	109	0.13	0.12	70-74	107	0.26
													75+	43	0.20
All		343	0.26			61	0.33			484				562	
Total		629				116				710				1006	

MALNUTRITION AND SALIVARY GLAND HYPOFUNCTION

Hyposalivation has been associated with insufficient intake of food and thereby insufficient nutrition to the salivary glands, for example, in children with malnutrition (Johansson, et al. 1992), inadequate intake in older adults (Rhodus and Brown 1990) or individuals with eating disorders (Öhrn, et al. 1999). In individuals with iron deficiency, the oral signs are similar to those with hyposalivation. These signs include glossitis, angular cheilitis, stomatitis, dysphagia and candida infections (Fergusson 1975, Fletcher, et al. 1975, Sreebny, et al. 1992). In the 1940s it was reported that the treatment of iron-deficient patients often resulted in the elimination of these signs (Faber 1943). A more recent study has suggested that treatment with iron increases salivation in iron-deficient patients and alleviates glossal pain (Osaki, et al. 1999).

The effects of malnutrition on the salivary gland function have recently been reviewed by Lingström and Moynihan (2003), who stated that due to the diversity of the saliva functions, there is an overwhelming risk that factors compromising salivary gland function may also severely affect oral and general health. These authors also pointed out that apart from general malnutrition, deficiency of specific nutrients, such as protein, minerals and vitamins, have been related to impaired gland function and salivary gland morphology. Animal studies have shown that iron deficiency reduced the salivary secretion rate and peroxidase antibacterial activity (Johansson and Fagermäs 1994).

DENTAL CARIES

Despite the large decrease of caries prevalence during the last 50 years (Marthaler 2004), caries remains the most common oral disease world-wide (Petersen, et al. 2005). Annually approximately 20-30% of the adult population develops new caries lesions that need treatment (Bader, et al. 2005, Zickert, et al. 2000). Dental caries is an infectious disease, which damages the structure of teeth. Caries lesions are consequences of caries and may be classified according to their activity (Fejerskov, et al. 2003). A lesion considered progressive would be described as an active lesion. This is a very important concept that influences the management of the disease, although the clinical distinction between active and inactive lesions is often difficult. In a recent review of aspects of saliva as indicators of risk for dental caries (Leone and Oppenheim 2001) it was shown that comparisons between studies were difficult due to the lack of a uniform terminology and definition of caries activity. For example, some studies defined their “high-caries” group as having individuals with ≥ 5 carious lesions whereas others defined caries activity with as little as one carious lesion. For many studies it was inferred from the data that a cavitated lesion was the primary outcome variable in caries assessments.

A patient may be allocated to one of the following caries activity and caries risk categories based on history and examination (Kidd and Nyvad 2003).

- *Caries inactive/caries controlled*: no (or maximally one) active lesion and no history of recent restorations.
- *Caries active but all relevant risk factors can potentially be changed* (e.g. plaque control, fluoride, diet): presence of active lesions and a yearly increment ≥ 2 new/progressing/filled lesions in the preceding 2-3 years.
- *Caries active but some risk factors cannot be changed* (e.g. some dry mouths, some medications) *or risk factors cannot be identified*: presence of active lesions and a yearly increment ≥ 2 new/progressing/filled lesions in the preceding 2-3 years.

A three groups caries risk assessment classification has been used by different insurance systems to guide the practical management of the patients (Table 2) (Bader, et al. 2005, Zickert, et al. 2000).

Table 2. The caries score for different risk groups used in three different insurance systems, the risk groups' classification also incorporated other risk factors not listed here.

Risk groups	Zickert et al. 2000	Bader et al.2005 Plan A	Bader et al.2005 Plan B
Low	No new caries lesion	No caries in the last 3 years	No active caries
Medium	1-2 new caries lesions	1-2 caries in the last 3 years	No or non-progressive incipient caries detected Evidence of 1-5 lesions
High	≥ 3 new caries lesions	3 or more lesions in the last 3 years	Rapidly progressing caries or evidence of 6 or more lesions

REDUCED SALIVARY FLOW RATES AND CARIES

Increased caries activity together with a severely impaired salivary secretion are often found after radiation therapy in the head and neck region (Vissink, et al. 2003), after taking drugs with a side-effect of hyposalivation (Atkinson and Fox 1992), or in individuals with Sjögren's syndrome (Ravald and List 1998). In milder forms of reduced salivary flow its relationship with caries is less clear (Mandel 1974, Sreebny 1983, Sweeney 1979). The most recent systematic review of saliva as a factor in dental caries concluded that normal salivary output, as quantified by the flow rate, is an extremely important intrinsic host factor providing protection against caries (Leone and Oppenheim 2001). Based on twenty-one studies, chronically low unstimulated salivary flow rate was found to be the strongest indicator of an increased risk of caries. However, thirty-four studies did not show this relationship, which was attributed to confounding experimental factors. In particular, whenever differences in disease severity were very small between groups an effect due to salivary flow was difficult to establish.

CARIES AND IRON DEFICIENCY

Iron deficiency is characterized by decreased marrow iron stores mirrored by low serum ferritin values (Jacobs 1985). Small iron stores are not unusual in growing children (Siimes, et al. 1974). During childhood there are two major periods of growth, one pre pubertal (6-7 and 7-8 years of age for girls and boys, respectively) and at puberty (Björk 1972, Björk 1975). These periods correspond with increased caries activity (Massler 1969). After the last period of growth there is a steady rise to adult iron levels in boys but not for girls, a difference that remains throughout childbearing years (Burman 1974). Adolescent girls and women develop dental caries more often than boys and men, even though females have better oral hygiene (Haugejorden 1996, Stephen and Purdell-Lewis 1992). Because of menstruations and childbearing, women have a higher prevalence of iron deficiency than men (Hallberg and Rossander-Hulthén 1989). Approximately half of the iron stores are lost after one pregnancy (Kaneshige 1981). Therefore there is an increased risk of iron deficiency in mothers having additional pregnancies after short time intervals as serum ferritin levels recover slowly (Jacobs, et al. 1972). The saying 'a tooth per child' has been debated repeatedly over the last century (Lukacs and Largaespada 2006), and some support have been presented in a study on twins (Christensen, et al. 1998). Maternal serum ferritin levels are correlated to concentrations of ferritin in blood from the umbilical cord and mothers with iron deficiency will have a higher risk of having children with iron deficiency (Fenton, et al. 1977, Kaneshige 1981). Recently the birth order, being the second or later child, was found to be related to higher risk of caries (Nicolau, et al. 2005). It has been shown in animal studies that treatment with iron supplements significantly reduced dental caries activity (Emilson and Krasse 1972, Miguel, et al. 1997).

AIMS

The general aims of this thesis were to investigate:

- The prevalence of reduced salivary flow rate in adults of different age groups
- The relationship between reduced salivary flow rate, general health and dental caries
- The influence of time of measurement on reduced salivary flow rate
- Whether reduced salivary flow rates could be increased by iron supplementation

The specific aims were:

- To determine the prevalence of reduced salivary flow rate in different age groups of adults aged 20–69 years (*Study I*).
- To analyse the relationship between reduced salivary flow rate and subjective oral dryness, presence of general diseases, regular use of prescribed drugs, BMI, number of remaining teeth and use of tobacco (*Study I*).
- To evaluate the frequencies of low unstimulated whole saliva flow rate levels and low serum ferritin levels among individuals with and without dental caries activity, and to analyse the relationship between UWS and S-f levels (*Study II*).
- To investigate whether individuals with hyposalivation will have an increase in unstimulated whole saliva flow rate measured at two different time-points in the morning (*Study III*).
- To compare the perception of oral dryness between individuals with hyposalivation and individuals with normal saliva secretion (*Study III*).
- To investigate whether oral iron supplementation increases salivary flow rate in individuals with hyposalivation and low serum ferritin levels (*Study IV*).

MATERIALS AND METHODS

STUDY DESIGNS

Cross-sectional study (*Study I*)

A random and by age and gender stratified sample of adults based on a population of dental patients in northern Sweden undertook tests of unstimulated and stimulated whole salivary flow rates and answered a questionnaire.

Case-control study (*Study II*)

Caries active individuals were matched by age and gender to caries inactive individuals (controls). All participants were examined clinically, and unstimulated whole salivary flow rate and serum ferritin values were determined.

Methodological study (*Study III*)

Unstimulated whole salivary flow rates were determined at two different time points, separated by four hours and a questionnaire was answered on both occasions.

Randomized controlled trial (*Study IV*)

Unstimulated whole salivary flow rate and the serum ferritin value were measured before and after three months treatment with iron or a placebo, and questionnaires were answered on both occasions. The participants were randomly assigned to receive either 60 mg of Fe^{2+} in the form of ferrous fumarate (Erco-Fer, Orion Pharma, Sollentuna, Sweden) or a placebo twice a day for 3 months - a total of 180 tablets. The study design followed the CONSORT recommendations for random controlled trials.

ETHICAL CONSIDERATIONS

Study I was approved by the Ethics committee of the University of Umeå, and *Study II*, *III* and *IV* were approved by the Ethics committee of the University of Uppsala. Regarding *Study IV* the design and protocol of the study was also approved by the Swedish Medical Products Agency (Läkemedelsverket). All studies were conducted in accordance with the Helsinki Declaration and all participants gave their informed consent. The statistical analyses were performed with unidentifiable data.

SUBJECTS

In *Study I* a total of 1000 men and 1000 women, aged 20-69 yrs, were randomly selected from a population consisting of 48,500 patients attending 14 different dental clinics in two counties of northern Sweden (Västernorrland and Västerbotten) with approximately half a million inhabitants together. The selection was stratified into 10 groups, each spanning 5 years and containing 100 individuals each for men and women. Of the invited individuals 1427 (70%) volunteered to participate in the study. Of the 573 non-participants, all but 69 were reached by telephone and agreed to have a short interview. The following reasons for non-attendance were given: unwillingness to participate (45%), moved from the area (33%), lack of time (10%), too ill to attend (9%), or unable to read or understand the language in the communication (3%).

In *Study II*, *III* and *IV* the participants were consecutively included patients, 15-46 years of age, at the Public Dental Clinic in Sala, Sweden. The clinic comprises eight dentists who examine and treat approximately 4000 patients in these age groups following an individual recall system. The municipality of Sala is situated in the county of Västmanland in the central part of Sweden and has 22,000 inhabitants. The numbers of individuals recruited to the different studies are seen in Figure 3.

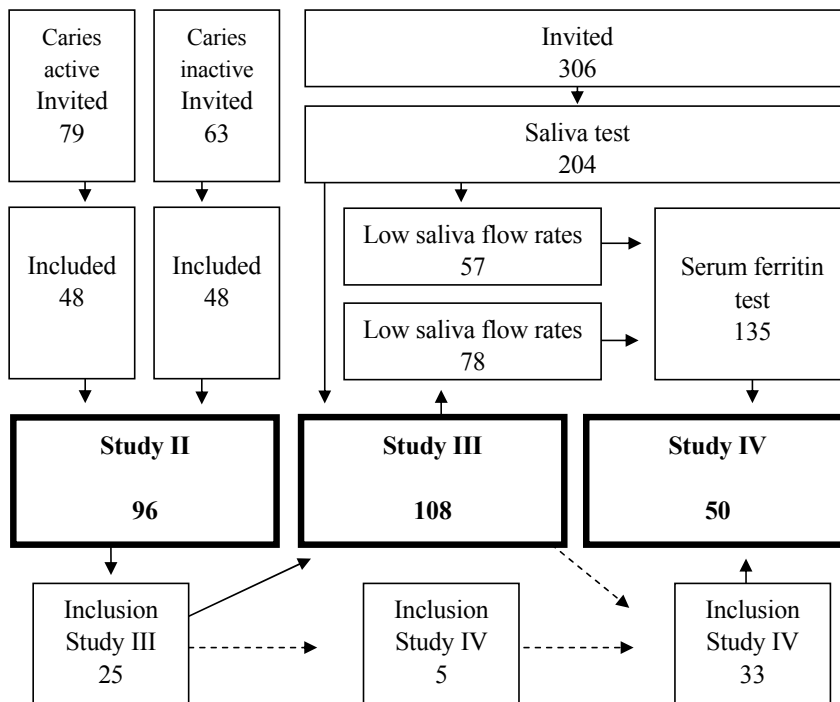


Figure 3. The participants included in Study II-IV. Dotted lines = number of individuals included from previous study.

In *Study II* 48 caries active individuals were matched with regard to sex and age to 48 caries inactive individuals aged 15-40 years, 30 women and 18 men. The caries active individuals, mean age 28.4 (SD 7.0) years, had developed manifest caries in two or more teeth since the last examination or manifest caries in one tooth since the last examination combined with a history of recurrent caries disease (for more than three years). Seventy-nine caries active individuals were invited, 27 declined participation and four were excluded. The caries inactive individuals, mean age 29.3 (SD 7.9) years, had been free from manifest caries for more than three years. Sixty-three caries inactive were invited, 13 declined participation and two were excluded. None of the participants was pregnant and all considered themselves healthy.

In *Study III* 108 individuals, aged 15-46 years, 86 women and 22 men participated. The subjects were allocated to three groups based on the results of a saliva test at 7:30 a.m.: 37 with very low secretion rate, 41 with low and 30 with normal. The mean age in these groups was 33 (SD 9), 33 (SD 9) and 32 (SD 8) years, respectively. Subjects taking

medication with antihypertensives, diuretics, antidepressants and antihistamines, or other drugs associated with dry mouth were excluded.

A total of 306 patients who had had a previous or planned saliva test for the assessment of caries risk were invited to participate in *Study IV*. Of these patients, 204 agreed to perform a saliva test out of which 135 had low salivary flow rates and were tested for serum ferritin. Finally, *Study IV* included 50 individuals, aged 15–46 years, 46 women and four men. The participants were randomly allocated to a treatment (all women) or a placebo (21 women and 4 men) group, mean age 34.7 (SD 8.2) and 34.0 (SD 10.5) years, respectively. None of the participants was pregnant or taking medications with xerogenic drugs.

COLLECTION OF SALIVA

Unstimulated whole saliva

The saliva collections were undertaken between 9 am and 11 am (*Study I*) and between 7.00 and 9.30 a.m (*Study II*). In *Study III* and *IV* all tests were conducted at fixed time points. The subjects were requested to refrain from eating, drinking, tooth brushing and tobacco use for at least one (*Study I* and *II*) or two hours (*Study III* and *IV*) prior to the saliva collection. Participants were relaxed and sat bent forward in an ordinary chair during the saliva collection. In *Study I* no test was conducted during acute illness and in *Study II* all participants were requested to postpone the date of testing with at least three weeks if they now had or had recently had any kind of general infection or inflammation. In *Study I* the staff of the involved clinics received practical training in measurement of salivary flow rate, while in *Study II, III* and *IV* all saliva collections were supervised by specially trained assistants with lengthy experience of the procedure.

Unstimulated whole saliva was collected by the passively draining method for 10 (*Study I*) or 15 minutes (*Study II, III* and *IV*) (Nauntofte, et al. 2003). In *Study I* collection time was reduced or extended when the flow rate was extreme.

Salivary flow rate was determined by volume using a glass centrifuge tube graded in 0.1 mL increments up to 10 mL (KEBO, Spånga, Sweden) (*Study I*) or a 3-mL syringe marked in increments of 0.1 mL (*Study II*). In *Study III* and *IV* salivary flow rate was determined by weight, using a scale with an accuracy of 0.01 g (Sartorius, BP310P, Sartorius AG, Göttingen, Germany), presuming that 1 g of saliva is equivalent to 1 mL.

Stimulated whole saliva

In *Study I* directly after the collection of unstimulated saliva, the participants chewed on 1 g of paraffin wax until soft. After swallowing, the masticatory stimulated saliva produced for 3 min was collected. Collection time was reduced or extended when the flow rate was extreme.

CARIES-RELATED VARIABLES

Number of teeth

The number of remaining teeth was recorded by intraoral inspection (*Study I*).

Manifest dental caries

Caries prevalence, decayed, missing and filled teeth (DMF-T) and decayed, missing and filled surfaces (DMF-S) were recorded for all patients. The caries incidence in the caries active group was determined by registering the number of teeth with new manifest dental caries since the last examination (D-T) (*Study II*). The patients' regular dentist did the registration by clinical and radiographic examination (bite-wings).

In the caries active group, the number of caries active years was determined as time passed since the patients had been free from manifest caries for a period of more than 3 years. This was possible to trace in the records of 43 of the final 48 patients. The other five patients (not fully traceable) were traced back as far as possible; all of them reported that they had experienced recurrent manifest caries for many years prior to the first registration in the records. In the caries inactive group, the number of caries inactive years was counted from the time the patient had had manifest caries. This was done by consulting the records of 44 of the patients and by relying on estimations made by four of the patients (members of the dental staff) in the final study group.

BLOOD SAMPLES

Venous blood samples were drawn immediately after the collection of the saliva test (*Study II* and *IV*).

Serum ferritin

The blood samples were immediately frozen and stored (*Study II*). After all the patients in both groups had undergone saliva collection, the blood samples were analysed for serum ferritin levels using an immunoanalyser (IMx Immunoanalyzer, Abbott Laboratories, Chicago, IL, USA) using the MEIA (microparticle enzyme immunoanalyser) technology. All samples were analysed on the same day using the same reagent batch to minimise analytical errors.

In *Study IV* serum ferritin was measured with an analyser for heterogeneous immunoassays (Elecsys 1010, Roche Diagnostics, Mannheim, Germany). For the purpose of inclusion of subjects in the study, samples were analysed immediately. For comparison between the iron and placebo groups the blood samples were stored frozen and analysed after the intervention period on the same day using the same reagent batch.

Capsular reactive protein (CRP)

Capsular reactive protein (CRP) was measured in order to detect inflammation or infections, which might elevate serum ferritin levels. Blood samples were analysed for CRP at the time of the saliva test (*Study II*) using immunoturbidometry (Cobas Mira with the reagents Unimate 3 CRP, Roche, Basel, Switzerland). A CRP level >5 mg/L would exclude the subject from the study. In *Study IV* CRP was analysed after the intervention period on samples that had been frozen using immunoturbidometry (Synchron CX5 Delta, Beckman Coulter, Fullerton, CA, USA).

QUESTIONNAIRES

Related to general health

After the saliva sampling (*Study I*) the subjects received a questionnaire that included questions about diagnosed diseases, regularly prescribed drugs or over-the counter medication, use of tobacco, and self assessed weight and height. The question Does your mouth usually feel dry? was used as an indicator of subjective oral dryness. Based on the answers in the questionnaire, each participant was then interviewed by the specially trained dental personnel regarding current diseases and ongoing medication. Systemic diseases were classified according to ICD-10 (International Statistical Classification of Diseases and Related Health Problems). Drugs were classified according to the WHO guidelines for ATC (Anatomical-Therapeutical- Chemical).

Subjective evaluation of the salivary and tear gland function

All the participants (*Study III*) assessed six variables related to the function of the salivary glands using Visual Analogue Scales (VAS) before each saliva collection. At baseline and after treatment (*Study IV*), all the participants assessed eleven variables related to the function of the salivary glands and tear glands using visual analogue scales (VAS).

STATISTICS

Parametric (normally distributed) data were analysed using t-test in *Study II* and *IV*. Paired t-test were used in *Study II* and when comparing differences between baseline and after treatment within groups in *Study IV*, while unpaired t-test were used to analyse differences between groups.

Mann-Whitney U test were used to analyse non-parametric (non-normally distributed) data in *Study I* and *IV*, such as differences in salivary flow rate between genders (*Study I*) and the questionnaire data (*Study IV*).

In *Study I, II* and *IV* Chi-square test was used for categorical variables.

Spearman rank correlation test was used in *Study I* to analyse correlations between the unstimulated and stimulated whole salivary flow rates, while Pearson's correlation test was used in *Study II* and *IV* to analyse correlations between the salivary flow rate and the serum ferritin levels.

In *Study III* intra-individual differences in salivary flow rate and VAS assessment were tested using Wilcoxon's signed-rank test and differences between groups were tested using the Kruskal-Wallis test. The agreement between the salivary flow rates at different time points was assessed using the kappa test.

In *Study I* associations between reduced salivary flow rates and risk factors were analysed by multiple logistic regression with stepwise backward elimination. Low and very low flow rates for unstimulated and stimulated saliva were used as dependent variables. Independent variables were gender, presence of diagnosed general disease, regular use of drugs, tobacco use and variables created by dichotomisation after analyses of prevalence; age >50 years, risk drugs, >2 drugs, <20 remaining teeth,

<27 remaining teeth and BMI >25. An omnibus test of model coefficients was used to evaluate how well the model performed. Cox & Snell R^2 and Nagelkerke R^2 were used to estimate the variance of the model.

All statistical tests were two-sided and P-values of less than 5% were considered significant. The statistical analyses were carried out using SPSS statistical software (SPSS, Chicago, IL, USA).

RESULTS

PREVALENCE OF REDUCED SALIVARY FLOW RATES (STUDY I)

Prevalence of low and very low unstimulated whole salivary flow rates

The prevalence of very low (<0.1 mL/min) and low (0.10-0.19 mL/min) unstimulated whole salivary flow rates in the different age groups are seen in Figure 4. The prevalence of very low flow rates was consistent in all age groups of women up to 50-69 years, where the prevalence was significantly higher compared to the lower age groups (Figure 4). For men there was a similar pattern up to 60-69 years. The prevalence of low unstimulated whole salivary flow rate showed no age-related differences.

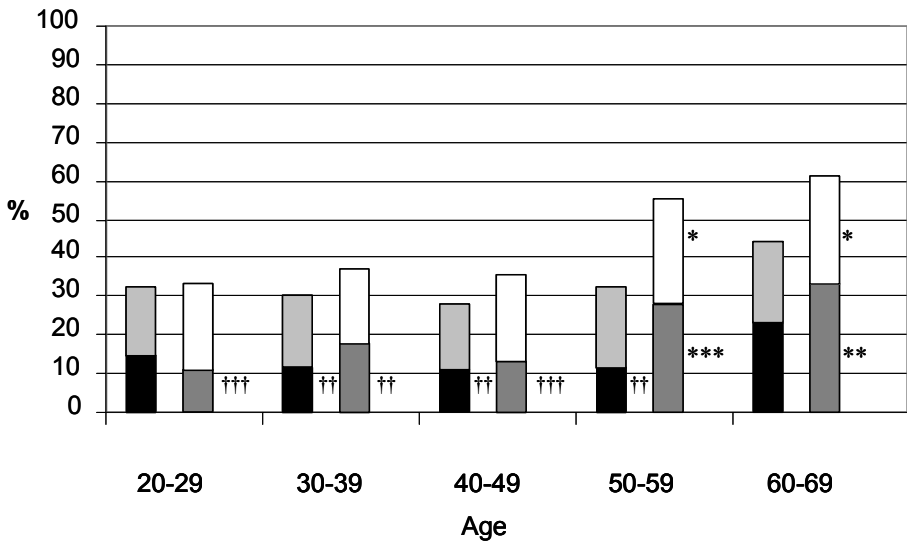


Figure 4. The prevalence of very low (<0.1 mL/min) and low (0.1–0.19 mL/min) unstimulated whole salivary flow rates in different age-groups from 20–69 years of age, divided by sex.

(■ Men <0.1 mL/min, □ Men 0.1–0.19 mL/min, ■ Women <0.1 mL/min, □ Women 0.1–0.19 mL/min)

* = P<0.05, ** = P<0.01 and *** = P<0.001 difference between sex.

† = P<0.05, †† = P<0.01 and ††† = P<0.001 compared to 60-69 years of age.

Prevalence of low and very lows stimulated whole salivary flow rates

The proportions of individuals having very low (<0.7 mL/min) or low (0.7-1.0 mL/min) salivary flow rates of stimulated whole saliva were markedly lower than for those having low or very low unstimulated whole salivary flow rates, ranging 0-5.5% and 0.8-8.2%, respectively.

Relationship between reduced salivary flow rate and general health and remaining teeth

The multiple regression models for very low and low unstimulated whole salivary flow rate included three mutual variables. These variables were ‘age >50 years’ (OR 1.78, $P=0.001$ and OR 1.45, $P=0.018$, for respectively very low and low unstimulated whole salivary flow rate) and ‘gender women’ (OR 1.94, $P<0.001$ and OR 1.70, $P<0.001$, respectively) and ‘remaining teeth <20’ (OR 1.84, $P=0.003$ and OR 1.57, $P=0.029$, respectively). For very low flow rates the variable denoted ‘risk drugs’, i.e. any drug from the ATC categories: cardiovascular system (C), musculo-skeletal system (M), nervous system (N) or respiratory system (R) was also included in the model (OR 1.67, $P=0.005$). For very low stimulated whole salivary flow rate a relationship was found for ‘remaining teeth <20’ (OR 2.36, $P=0.027$) and for ‘using more than two drugs’ (OR 5.52, $P<0.001$). For low stimulated whole salivary flow rate only ‘gender women’ (OR 2.82, $P=0.001$) was significant.

In subset analyses of those younger than 50 years very low flow rate of unstimulated and stimulated saliva was related to ‘BMI>25’ (OR 1.56, $P=0.047$) and ‘having a diagnosed disease’ (OR 3.90, $P=0.009$), respectively. While for low flow rates a relationship was found for the interaction ‘gender women’ \times ‘remaining teeth<27’ (OR 2.78, $P<0.001$) in unstimulated saliva and for ‘gender women’ (OR 3.03, $P=0.011$) in stimulated saliva.

Xerostomia and salivary flow rates

When the question “Does your mouth usually feel dry?” was used as an indicator of subjective oral dryness (xerostomia) there were statistically significant differences between the genders in all age groups except the youngest. When the participants had salivary flow rates below any of the four limits used for low and very low flow rate there were statistically significant differences in reported xerostomia compared to those having normal flow rates (Table 3). The experience of xerostomia was significantly higher for women than for men in all groups of unstimulated whole salivary flow rates (Table 3).

Table 3. Xerostomia (Does your mouth usually feel dry? n=317) in relation to salivary flow rate groups according to sex

Salivary flow rate	Sex	Total	No, n (%)	Yes, n (%)	P higher flow rates	P sex
Unstimulated						
≥0.2 mL/min	M	435	386 (89)	49 (11)		0.012
	F	410	339 (83)	71 (17)		
0.10-0.19 mL/min	M	129	105 (81)	24 (19)	0.029 ^a	0.003
	F	182	120 (66)	62 (34)	<0.001 ^a	
<0.1 mL/min	M	99	71 (72)	28 (28)	0.084 ^a (<0.001 ^b)	<0.001
	F	162	79 (49)	83 (51)	0.001 ^a (<0.001 ^b)	
Stimulated						
≥1.0 mL/min	M	625	539 (86)	86 (14)		<0.001
	F	671	496 (74)	175 (26)		
0.70–0.99 mL/min	M	18	10 (56)	8 (44)	<0.001 ^a	0.796
	F	50	26 (52)	24 (48)	0.001 ^a	
<0.7 mL/min	M	15	9 (60)	6 (40)	0.797 ^a (0.004 ^b)	0.170
	F	24	9 (38)	15 (63)	0.242 ^a (<0.001 ^b)	

^a Difference from the group having unstimulated or stimulated whole saliva flow rates larger than this group

^b Difference compared to normal flow rates

CARIES ACTIVE COMPARED TO CARIES INACTIVE INDIVIDUALS (STUDY II)

Dental caries

The mean number of teeth with new manifest dental caries (D-T) in the active caries group was 3.2 (SD 2.3), and there was no significant difference between females and males. In the inactive caries group, the mean number of months free from caries was 129.8 (SD 68.3). The corresponding mean number of months with dental caries activity in the active caries group was 155.1 (SD 112.1). There was no significant difference between the genders for these two variables.

Unstimulated whole saliva

The distributions of unstimulated whole salivary flow rate in the active and inactive caries groups are shown in Figure 5. Both distributions are skewed to the right, but less for the caries active group. Frequencies of very low and low flow rate of unstimulated whole saliva were compared with frequencies of normal levels in the two groups (Table 4). In the active caries group, 32 individuals (67%) had very low or low unstimulated whole salivary flow rate compared with 13 individuals (27%) in the inactive caries group. This difference was statistically significant ($P<0.001$).

The mean for the caries active and the caries inactive groups differed significantly: 0.20 (SD 0.13) and 0.33 (SD 0.24), respectively (P=0.002).

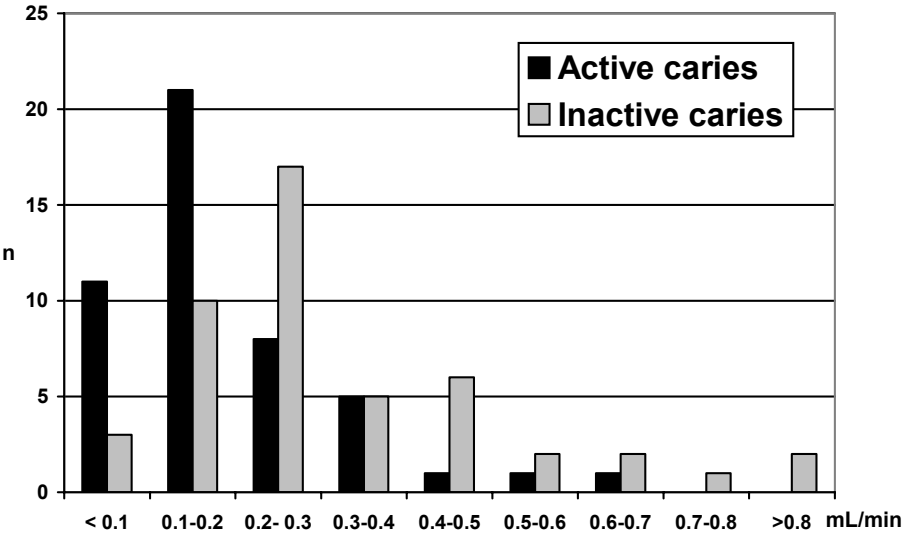


Figure 5. Distribution of unstimulated whole saliva flow rates in the two study groups, the active caries group and the inactive caries group.

Table 4. Frequencies of very low, low, normal levels and means of unstimulated whole saliva flow rates in the active caries and inactive caries groups divided by sex.

	Very low n (%)	Low n (%)	Normal n (%)	Mean (SD)
Active caries-women	4 (13)	17 (57)	9 (30)***	0.19 (0.11)**
Inactive caries-women	1 (3)	6 (20)	23 (77)	0.35 (0.21)
Active caries-men	7 (39)	4 (22)	7 (39)	0.21 (0.16)
Inactive caries -men	2 (11)	4 (22)	12 (67)	0.32 (0.28)

* = P<0.05, ** = P<0.01 and *** = P<0.001 difference between active- and inactive caries groups.

Serum ferritin

The mean (SD) serum ferritin values were lower in the caries active group compared to the caries inactive group: 43.6 (32.9) and 50.4 (44.7) µg/L, respectively. However, the differences between the two groups were not statistically significant. Neither was there any statistically significant difference in the numbers of individuals with very low or low serum ferritin levels between the two groups: 25 (52%) and 24 (50 %), respectively. In both groups, only the women had very low serum ferritin levels. No correlation was found between serum ferritin and the unstimulated whole saliva flow rate.

INFLUENCE OF TIME OF MEASUREMENT ON DIAGNOSIS OF HYPOSALIVATION (STUDY III)

Unstimulated whole saliva flow rates

All three groups (very low, low and normal) displayed a statistically significant increase in unstimulated whole saliva flow rate when comparing the tests at 7:30 and 11:30 a.m. (Figure 6). There were no significant differences in the size of the increase in unstimulated whole saliva flow rate between the three groups.

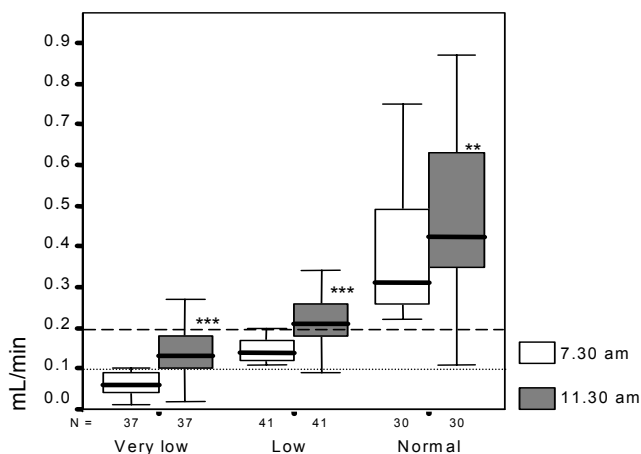


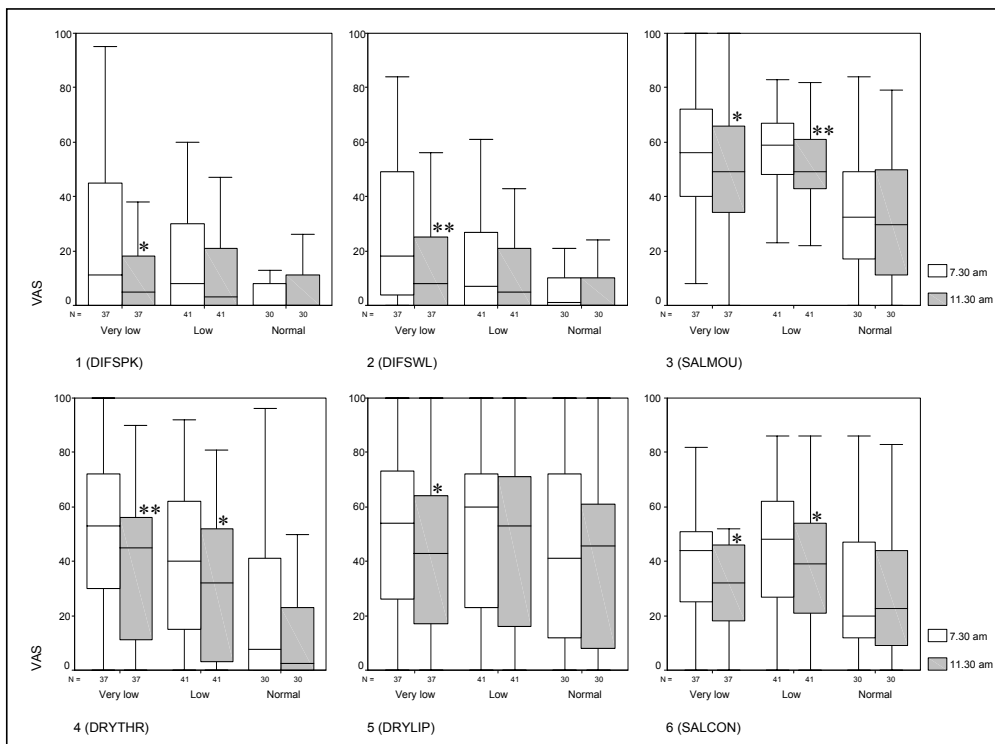
Figure 6 The unstimulated whole saliva flow rate at 7:30 and 11:30 a.m. for the three different groups: very low (<0.1 mL/min), low (0.11-0.2 mL/min) and normal (>0.2 mL/min) flow rates. The box plots show the median and interquartile range. Horizontal lines indicate limits for very low (.....) and low (---) unstimulated whole saliva flow rates. N denotes the number of subjects.

P<0.01; *P<0.001.

There was a disagreement between the unstimulated whole saliva flow rates at 7:30 and 11:30 a.m. ($\kappa=0.28$). The frequency of normal unstimulated whole saliva flow rates was twice as high at 11:30 a.m. compared with 7:30 a.m. and the frequency of individuals with a very low unstimulated whole saliva flow rates, 70%, shifted to the low or normal groups.

Subjective evaluation of salivary gland function

The median VAS scores for salivary function at 7:30 a.m. were significantly higher when compared with the scores at 11:30 a.m. for all six VAS items in the very low group, for three VAS items in the low group and for none of the items in the normal group (Figure 7).



1. (DIFSPK) Rate the difficulty you experience in speaking due to dryness (Not difficult at all–Very difficult)
2. (DIFSWL) Rate the difficulty you experience in swallowing due to dryness (Not difficult at all–Very difficult)
3. (SALMOU) Rate how much saliva there is in your mouth (A lot–None)
4. (DRYTHR) Rate the dryness of your throat (Not dry at all–Very dry)
5. (DRYLIP) Rate the dryness of your lips (Not dry at all–Very dry)
6. (SALCON) Rate the consistency of your saliva (Watery–Very viscous)

Figure 7. The perceived function of the salivary glands registered by six VAS items at 7.30 and 11.30 a.m. in the three groups; very low (<0.1 mL/min), low (0.11–0.2 mL/min) and normal (>0.2 mL/min) flow rates. Box plots show the median and interquartile range.

* = $P < 0.05$, ** = $P < 0.01$.

EFFECT OF IRON SUPPLEMENTATION ON SALIVARY FLOW RATES

(STUDY IV)

For the primary outcome variable, difference in unstimulated whole salivary flow rate between baseline and end-point, there was no statistically significant difference between the groups. In the placebo group, there was a significant increase in unstimulated whole salivary flow rates ($P=0.008$), but this did not apply to the iron group. For serum ferritin a statistically significant difference ($P<0.001$) was found between the two groups when comparing the change from baseline. A significant increase in serum ferritin was found in the iron group ($P<0.001$) but not in the placebo group. No statistically significant correlation was found between serum ferritin and unstimulated whole salivary flow rate. There were no differences found in the values of subjective assessment of salivary and tear function.

DISCUSSION

Limits for reduced salivary flow rate

The limits for low and very low salivary flow rates used in this thesis are based on the recommendations by Ericsson and Hardwick (1978), Sreebny and Valdin (1988) and Wolff and Kleinberg (1998). The validity of these limits seems not to have been confirmed and is open for discussion. It has been recommended that reference values be established by following well-defined nomenclature and procedures (Solberg 1987). Further studies on salivary flow rate should therefore be aimed at establishing valid reference values stratified for gender and ages.

Related to these limits are the frequently used definition of hyposalivation (Nauntofte, et al. 2003) as being <0.1 mL/min of unstimulated or <0.7 mL/min of stimulated whole saliva. This definition might seem misleading since the results of *Study I* gives the impression that these two limits are two different magnitudes of reduced salivary flow and not fully compatible. It might be argued that the definition of hyposalivation as abnormal reduction in salivary flow only should relate to flow rates <0.1 mL/min of unstimulated whole saliva and that the limit <0.7 mL/min of stimulated whole saliva should just be regarded as a more severe form of hyposalivation than the former.

Prevalence of reduced salivary flow rate

The population based prevalence study (*Study I*) is to the best of my knowledge the only study present on prevalence of reduced salivary flow rate in different age groups including young adults. External validity might be limited to the relatively homogenous Swedish population. However, there are a few studies reporting median or mean values in different age groups (Billings, et al. 1996, Heintze, et al. 1983, Percival, et al. 1994, Yeh, et al. 1998). The results from these studies show differences in flow rate between the genders, and a tendency for decreasing flow rates with age (Figure 8-9). It can be seen from the figures that the medians and the mean show a high variability between the studies, which may be explained by differences in the methodology (Table 5). The time of saliva collection influences the salivary flow rate as shown in *Study III*, but was similar for all studies bar one for which no information is given. The length of collection differed, but the influence of this factor on salivary flow rates is unknown. The number of individuals in the different age groups might have some impact, but the most important factor is probably the selection of the study population. In this regard the selection process used in *Study I* was randomised and stratified on age and gender, which is appropriate for epidemiological studies (Petrie, et al. 2002).

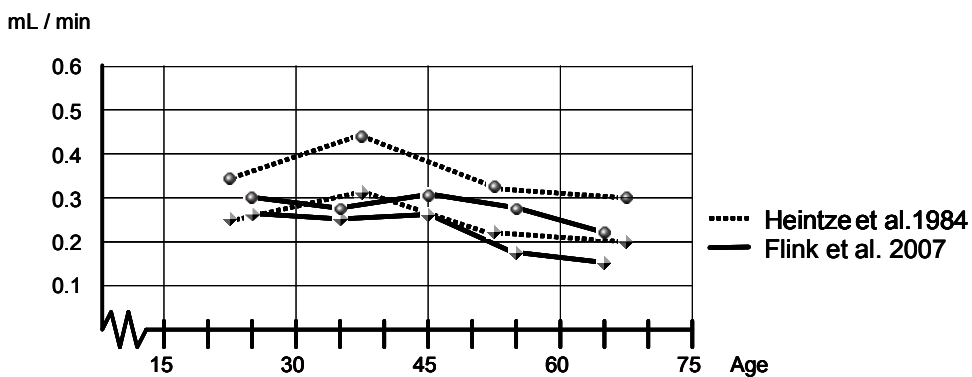


Figure 8. Median flow rate values of unstimulated whole saliva flow rates from population based studies by Heintze et al. 1984 and Flink et al. 2007 (*Study I*) (● = males and ▲ = females).

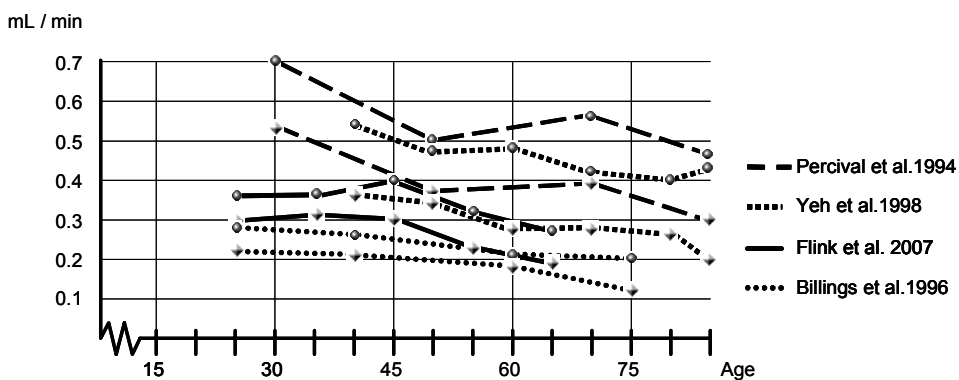


Figure 9. Mean flow rate values of unstimulated whole saliva flow rates from population based studies by Billings et al. 1996, Flink et al. 2007, Yeh et al. 1998, and Percival et al. 1994 (*Study I*) (● = males and ▲ = females).

Table 5. Different sampling parameters for unstimulated whole saliva; time of collection, length of collection, restrictions before collection, mean age, number of participants and study selections in five population based studies of salivary flow rates (ND = No Data).

Study	Time of collection	Length of collection	Restrictions before collection	Mean age (Range)	n	Selection
Heintze et al. 1984	9.00-11.00	5 min (+5 min)	Avoid oral intake, smoke -1hour	About 40 (15-79)	629	Mostly caries active
Percival et al. 1994	9.00-11.00	10 min	ND	ND (20-80+)	116	Healthy non medicated
Billings et al. 1996	ND	2 min	ND	ND (19-88)	710	Convenience
Yeh et al. 1998	8.00-10.00	5 min	Not eat, drink or OH after 24.00	60.6 (ND)	1006	Demographic stratification
Flink et al. 2007	9.00-11.00	10 min (± 5 min)	Restrained eat, drink, tooth-brush, smoke -1hour	46.7 (20-69)	1427	Randomised and stratified by age and gender

Relationship between reduced salivary flow rate and general health

In *Study I* a relationship between very low salivary flow rate and high BMI, and the presence of diagnosed disease was found in individuals younger than 50. This implies that a reduced salivary flow rate might be an early sign of chronic disease. A relationship between very low salivary flow rate and medication was also found. In Figure 8 the highest mean values are seen in the only study that includes only healthy individuals (Percival et al. 1994), which agrees with the findings in *Study I*.

The World Health Organization (WHO) recently published an expert review of the evidence of the effects of diet and nutrition on chronic diseases, among these obesity, and dental diseases (WHO Report 2003). By including dental diseases within the broader field of chronic diseases, the WHO has acknowledged the significance of dental conditions. Future action at clinical and community levels therefore needs to adopt a holistic approach rather than a narrow disease focus (Watt 2003). In the WHO report, a life-course perspective has been adopted, which highlights the impact of diet and nutrition at key life stages and identifies opportunities for intervention. The five key life stages include i) foetal development and maternal environment; ii) infancy; iii) childhood and adolescence; iv) adulthood; and v) old age (Watt 2003). These life stages are closely linked to biological and social factors influencing oral health. The life course concept may be important for understanding how exposure to risk factors may act differently in different critical periods (Ben-Shlomo and Kuh 2002). In its purest form, this model advocates that exposure in a critical period result in permanent and irreversible damage or disease.

The effect of risk reduction and health promotion during growth is shown schematically in Figure 9 (Halfon, et al. 2000), and may also apply for saliva secretion.

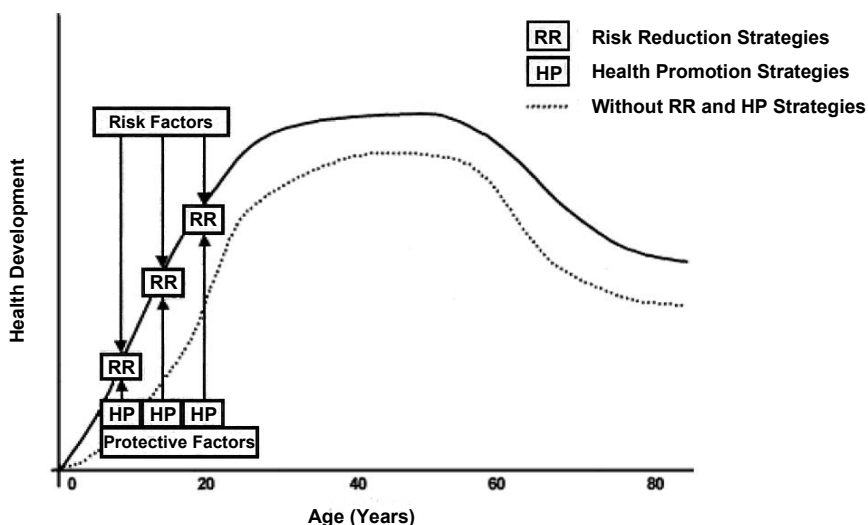


Figure 9. How risk reduction and health promotion strategies influence health development. This figure illustrates how risk reduction strategies can mitigate the influence of risk factors on the developmental trajectory, and how health promotion strategies can simultaneously support and optimize the developmental trajectory. In the absence of effective risk reduction and health promotion, the developmental trajectory will be suboptimal (dotted lines) from Halfon et al. (2000).

The consistent prevalence of reduced unstimulated whole saliva flow rates between 20–50 years (*Study I*) suggests that reduced salivary flow may develop before adulthood, which is in line with the life course perspective. However, the limitation in the cross sectional study design has to be considered. Very little is known about how salivary flow rates fluctuate in individuals over time, since there are few longitudinal studies. Repeated measurements of unstimulated whole salivary flow rate over periods shorter than two weeks have shown high reproducibility with correlation coefficients between $r=0.75–0.91$ (Mulligan, et al. 1995, Navazesh, et al. 1992, Navazesh and Christensen 1982). To the best of my knowledge, there is no data on periods longer than two weeks, other than on individuals with Sjögren’s syndrome (Haga, et al. 1999, Jorkjend, et al. 2004). It has been suggested that longitudinal studies are necessary in order to investigate the life-course concept (Nicolau, et al. 2007). Well-designed longitudinal studies have been proposed to increase our understanding of the presence of xerostomia, changes in flow rate and the relationship to diseases and drug use over time in aging people (Billings, et al. 1996).

The higher prevalence of low and very low unstimulated whole salivary flow rates found in women aged 50–69 years compared to men (*Study I*) is similar to the findings in a cohort of older Australians (Thomson, et al. 1999). This emphasises a clear gender difference in the older age groups that deserves the attention of the dental profession. The causes for the gender difference in dental caries have recently been discussed from an anthropological perspective (Lukacs and Largaespada 2006). Referring to clinical

studies, these authors suggested as main causes to the gender difference in caries prevalence, salivary factors, hormonal fluctuations in puberty, menstruation, and pregnancy. Interest in these gender-specific burdens has also been focused on the aetiology of other diseases in relation to nutrition (Bartley, et al. 2005). However, to identify the onset and circumstances behind this higher prevalence, once again, repeated measurements of salivary flow rates are of great interest.

Relationship between reduced salivary flow rate and dental caries

Definitions of caries activity and caries inactivity have been expressed in many different ways (Leone and Oppenheim 2001). Caries activity has been determined clinically by estimating the progress of the caries lesions (Fejerskov, et al. 2003, Kidd, et al. 2003), which requires repeated measuring from at least two time points. In a US-based insurance system for dental care the definition for low caries risk and caries inactivity was set to three years without new caries lesions (Bader, et al. 2005). Besides this information very little is found in the dental literature about recommendations of the appropriate time span to use when classifying a patient as caries inactive in clinical management. Another definition for caries inactivity is “no (or maximally one) active lesion and no history of recent restorations”, where the last part of the sentence reflects an undefined time involvement in the definition (Kidd and Nyvad 2003).

The definitions for caries active (manifest caries in two or more teeth since the last examination or manifest caries in one tooth since the last examination combined with a history of recurrent caries disease for more than 3 years) and caries inactive (free of manifest dental caries for more than 3 years) individuals used in *Study II* include a time aspect that seem appropriate for separating individuals into these two categories. Validation of the group assignments showed that all participants exceeded the minimum time stipulated for caries activity and caries inactivity.

In *Study II* diagnosis of manifest caries depended on the decisions of the dentists performing the clinical examinations. The variability in decision making between dentists is substantial (Bader and Shugars 1997). Kay and Nuttall (1994) found that the dentists' decision were matched to their views on the relative importance of false-negative or false-positive treatment decision. Mej   et al. (1999) found that the threshold for restorative treatment differed between the metropolitan regions in Sweden, speculating that this could relate to the influence of attitudes of those giving postgraduate courses in each region, or the influence of the undergraduate education programmes. Younger dentists more often than older dentists would postpone restorative treatment of approximal caries until the lesion has reached a relatively advanced stage of progression. Furthermore, dentists in private practice would restore approximal caries at an earlier stage of progression than the dentists in the Public Dental Health Service. Because of this variability in decision making between dentist the need for training and calibration has been emphasised (Anusavice 2003). Calibration exercises were performed repeatedly at the Sala clinic together with recurrent discussions about defining caries activity. The clinic also had a mixture of old experienced dentist and younger less experienced. At the start of the study no calibration was performed since inclusion of patients in *Study II* had to rely on retrospective data. The inclusion was decided by two dentists in concert.

The relationship between caries and reduced salivary flow has been difficult to confirm in cross-sectional studies, except when the salivary flow rate is very low (Leone and Oppenheim 2001, Sreebny 1983). A difficulty in studies of this kind has been the lack of information about actual flow rates during the development of caries cavities (Sreebny 1983). Longitudinal studies are therefore needed to monitor the progress of caries in relationship to risk factors such as hyposalivation (NIH 2001). However, some evidence has been found that indicates an association between hyposalivation and the DMF-index (Percival, et al. 1994, Ravald and List 1998). The importance and need of incorporating the aspect of time in the evolution of caries is best demonstrated by the fact that the most consistent predictor of caries risk is the past caries experience (NIH 2001).

New ways of measuring and analysing rates of recurrent events over time have been proposed (Glynn and Buring 1996). Many diseases and other clinical outcomes may recur in the same patient, e.g. dental caries or tooth loss. Another type of repeated event occurs when a disease can affect paired or multiple organs separately, such as cavities in multiple teeth. One major important advantage with this approach is that they are dealing with repeated events in one individual as dependent and not as independent variables. Multilevel modelling is a regression model that allows repeated measurement at different times in the same individual (Petrie, et al. 2003).

In the case-control design the frequencies of the exposure of interest is compared between a group of diseased subjects (cases) and a group of referent subjects (controls). Case-control studies are efficient and powerful biomedical research tools when properly implemented. However, poorly conducted case-control studies are prone to various form of bias that could be grouped into three general categories: selection, information and confounding bias (Grimes and Schulz 2002). Case-control studies are especially prone to selection bias, meaning that the selection process has made the groups differ in some important aspect aside from the exposure (Lopez, et al. 2007). The selection of the control group is especially important. The optimal control group for a case control study is randomly sampled from the same population that gave rise to the cases during a clearly specified time interval. In *Study II* both cases and controls were regular patients at the same clinic and no one was referred to the clinic. Some of the controls were staff at the clinic and may introduce a so called membership bias (Lopez, et al. 2007). This group may differ in eating and oral hygiene habits. However, the direction of such a bias would decrease the difference between the groups, as the exposure “having reduced salivary flow rate” may exist but the outcome, caries, would not appear due to better habits. Bias may also be present if a large number of eligible subjects are unwilling to participate in the study and the reason for this is related to the exposure (Lopez, et al. 2007). It is difficult to know if this bias is present, but the main reasons for refusal to participate in *Study II* were job interference and anxiety for the needles used in blood sampling. These factors are probably not related to the exposure.

In order to control for confounding (Grimes and Schulz 2002) restriction in the selection was made by excluding individuals who were pregnant or who were taking any medication with known negative effects on saliva secretion (*Study II*), together with the pair wise matching by age and gender (Petrie, et al. 2002). Other factors important for bias are diagnostic criteria (discussed above) and recruitment period

(Lopez, et al. 2007). The recruitment period in *Study II* could induce a bias since there was a time gap between recruitment of cases (mainly during winter) and controls (mainly during summer). Since a circannual rhythm of salivary flow has been described, with lower salivary flow rates during summer and higher during winter time (Kavanagh, et al. 1998, Shannon 1966) an information bias may be induced (incorrect information about the exposure). If this factor has influenced the results in *Study II* the direction would presumably have narrowed the difference.

A limitations for cross sectional and case control studies is that the results could only generate hypotheses and not show a cause-effect relationship (the cause must come before the effect)(Grimes and Schulz 2002). Tooth loss (*Study I*) has been considered an indirect variable for caries, since caries is the most important cause of tooth loss throughout adult life (McCaul, et al. 2001). These authors pointed out that tooth loss due to caries is particularly dominant from 20 to 50 years of age. The relationship found between low unstimulated salivary flow rate and being young woman with less than 27 remaining teeth (*Study I*) is in agreement with the finding in *Study II* that caries active women in similar ages have a higher frequency of reduced salivary flow rate than caries inactive women.

Evidence-based reports support the notion that a very low salivary flow rate is an indicator of a significant risk of developing new caries lesions (Leone and Oppenheim 2001). The finding in *Study I* that the prevalence of very low unstimulated whole salivary flow rates ranged 10.9-17.8% in the age group 20-50 years indicates a need for salivary flow measurement in patients with recurrent caries disease. This is important, since acknowledgement of reduced salivary flow by both patient and dentist identifies a need for more extensive prevention and attempts to control other risk factors in order to avoid caries, as no treatment is known permanently to increase the salivary flow in these patients (Kidd and Nyvad 2003).

A very recent review has given an overview of four current explanations for inequalities in oral health (Sisson 2007). These four are the materialist, the behavioural, the psychosocial and the life course perspectives. The reviewer means that the life course perspective could be seen as an interaction of all three of the other factors over time. Only a few studies have been performed with a life course approach in dental practice (Bedos, et al. 2005, Nicolau, et al. 2003, Poulton, et al. 2002). The results from these studies appear to be useful for understanding oral health disparities (Nicolau, et al. 2007, Sisson 2007). A life course approach to caries disease requires better understanding of the natural history and physiological trajectory of normal biological development of the oral environment. How different periods across the life course influence biological development need to be investigated and critical periods have to be identified in order to define health promotion strategies.

A life course approach to caries disease recognises that reduced defence, in some instances irreversibly reduced, might be present in patients having recurring caries disease. The reduced defence could relate to tooth structure, salivary composition and flow rate (Nicolau, et al. 2005). Nutritional deficiency during childhood has been reported to affect tooth structure (Enwonwu 1973, Seow 1996, Sweeney and Guzman 1966), saliva secretion rate, buffering capacity and immunological systems (Johansson, et al. 1994, Johansson, et al. 1992). Some general health parameters, related to

inadequate nutrition, such as height (Nicolau, et al. 2005) and lower birth weight (Nicolau, et al. 2003) have been associated with high DMFT.

Recently the potential for a life course approach to aid understanding of variations in the health and disease of populations over time, across countries and between social groups has been given more attention (Leon 2000). The life course perspective may help understand the underlying geographical patterns of mortality, particularly East-West differences (Ben-Shlomo and Kuh 2002). The differences in caries prevalence related to geographical pattern have been obvious in several studies involving immigrants (Källestål and Wall 2002, Stecksén-Blicks, et al. 2004) and comparing prevalence of caries between different countries (Bjarnason 1998, Petersen, et al. 2005).

Xerostomia and subjective evaluation of salivary gland function

The relationship between unstimulated and stimulated whole salivary flow rates has been reported to be correlated (Heintze, et al. 1983, Sreebny and Valdinini 1988), which also was found in *Study I*. Reduced flow rates were more common in unstimulated whole saliva than in stimulated whole saliva which is in agreement with other studies (Becks and Wainwright 1939, Wang, et al. 1998). This finding is important with the background that the unstimulated whole salivary flow rate has been proposed as a more sensitive measure in relation to xerostomia than the stimulated whole salivary flow rate (Sreebny and Valdinini 1988, Wang, et al. 1998). Furthermore, mild symptoms of xerostomia have been reported in relation to low unstimulated whole salivary flow rates while more severe symptoms were related to very low unstimulated whole salivary flow rates combined with very low stimulated whole salivary flow rates (Wang, et al. 1998). This is in agreement with the findings in *Study I*, where the highest proportion of individuals experiencing xerostomia was found among those having reduced stimulated whole salivary flow rates compared to other groups (Table 3).

The significant relationship between low and very low flow rates and perception of dry mouth found in *Study I* verifies that xerostomia is associated with low salivary flow rates (Fox, et al. 1987) (Table 3). However, the correlation between xerostomia and reduced salivary flow has been questioned, and may depend on factors other than salivary flow rate (Nagler 2004), especially among older individuals (Nederfors, et al. 1997, Närhi 1994, Thomson, et al. 1999)

The association between xerostomia and low saliva flow rates has been explained by the fact that lower unstimulated whole saliva flow rates are associated with lower mucosal thickness of saliva. At a certain flow rate level there is not enough saliva to wet the various oral surfaces and oral dryness will be perceived (Wolff and Kleinberg 1998, Wolff and Kleinberg 1999). Evaporation during mouth-breathing and water absorption through the mucosa have been proposed as clinically significant for differences in perception of dry mouth, especially if saliva flow rates are low (Dawes 2004). This might be one explanation why not all individuals with low unstimulated flow rate perceive oral dryness. It has also been shown that an experimentally induced reduction of the saliva flow rates of approximately 40-50% results in symptoms of dry mouth independent of the original saliva flow rate (Dawes 1987). However, very little is known about how the salivary flow rate fluctuates in individuals over time.

There are several issues to consider in epidemiological studies of dry mouth, including xerostomia and hyposalivation, that have recently been reviewed (Orellana, et al. 2006, Thomson 2005). The importance of learning more about the ‘natural history’ of xerostomia has been stressed (Thomson 2005). The term ‘natural history’ includes a condition’s progress from pathological onset through a pre-clinical stage to clinical manifestation, and then to persistence, remission, relapse or resolution (Last 2001). The ‘natural history’ is important in order to get a complete understanding of the condition and identifying needs for treatment. This can only be achieved by longitudinal prospective cohort studies (Thomson 2005).

There is limited knowledge about the prevalence of xerostomia in younger individuals (Orellana, et al. 2006, Thomson 2005). Only one previous study has presented data for separate age groups of young adults (Nederfors, et al. 1997). In this study the prevalence was slightly higher for the men but slightly lower for the women compared to *Study I*. However, Nederfors et al. (1997) did not measure the salivary flow rate.

In a majority of studies a single item (question) has been used to verify xerostomia (Thomson 2005), but the definition of this item has been extremely diverse (Orellana, et al. 2006). This diversity leads to the prevalence of xerostomia varying over a wide range (0.9 %-46%). Orellana et al. (2006) noted that if the study with the lowest prevalence values was excluded, the prevalence values were less dispersed (20-46% for women and 13-26% for males).

In *Study I* the question Does your mouth usually feel dry? was used, and also used in other studies (Hochberg, et al. 1998, Nederfors, et al. 1997). The use of the same question might be one reason for the similarities found in prevalence of xerostomia between *Study I* and the study by Nederfors et al (1997).

Some multiple item indexes usable for verifying xerostomia have been developed and tested (Locker 2003, Pai, et al. 2001, Thomson, et al. 1999), but are so far not fully validated (Thomson 2005). Multiple item indexes have been recommended for use with at least one single item measure as a safeguard. These instruments are supposed to have properties which are more sensitive to different levels of severity in the perception of xerostomia and may identify changes over time (Thomson 2005). In *Study III* five items from the multi item index by Pai et al. (2001) were selected, the items that had shown best validity in relation to unstimulated sublingual salivary gland flow rates. These VAS items seemed to work well to identify changes in the perception of oral dryness that were related to salivary flow rates in individuals who had reduced salivary flow rates, but not for those who had normal salivary flow rates.

The gender differences found in *Study I* regarding perception of xerostomia are in agreement with almost all previous studies (Orellana, et al. 2006). Medication is one of the major risk factors known to cause xerostomia and hyposalivation. Many epidemiological studies have used a simple count of number of chronic diseases or drugs used in attempts to control for general health, a method that is imprecise (Thomson 2005). However, in *Study I* this was one of the approaches that were used in the multiple logistic regression. Surprisingly, ‘diagnosed diseases’ was significantly related to very low stimulated whole salivary flow rate among those less than 50 years of age. The number of medications taken has been significantly associated with xerostomia (Locker 1993, Nederfors, et al. 1997). This was another factor investigated in *Study I*, and it was significantly related to very low stimulated whole salivary flow rate among all individuals. Medications have also been classified and listed as

xerogenic or non xerogenic. There are major problems with this approach as the evidence for the xerogenic effect is not from clinical or epidemiological studies but mostly from case reports and clinicians' observations. Another more informative approach is recommended and this is to examine by therapeutic category of medication (Thomson 2005). This was done in *Study I* by creating a variable including the four categories having the highest prevalence of reduced unstimulated whole salivary flow rates, denoted 'Risk drugs'. This variable was significantly related to very low unstimulated whole salivary flow rates among all individuals.

The limitation of the cross sectional study design is that it does only provide data on the current exposure. To further investigate xerostomia and hyposalivation, and the relationship to a particular class of medicines, prospective cohort study design are the only possible solution (Thomson 2005).

Xerostomia is not a serious condition, even though it may cause a lot of subjective suffering to the patient and affect quality of life for both older (Locker 2003) and younger (Thomson, et al. 2006) individuals.

Influence of time of measurement on diagnosis of reduced salivary flow

The circadian rhythm for unstimulated whole saliva in individuals with normal salivary flow rates has previously been described in the introduction. The results from *Study III* showed a pattern that might relate to circadian rhythm for salivary flow rates also among those who have reduced salivary flow rates (Figure 10). In the very low group the mean values would pass the very low limit at approximately 9 a.m. if the increase was supposed to be linear. However, it is difficult to determine whether the increase in salivary flow rate found between the two time points is related to circadian rhythm or other factors. The increase found in all groups might be important when diagnosing reduced salivary flow in the clinical management of Sjögrens syndrome and caries risk assessment. Furthermore, it is important to reduce systematic error in research studies especially in clinical trials aiming to increase salivary flow rates. It is therefore suggested that the collection of saliva should be performed at a fixed time-point or during a limited time interval as early as possible in the morning.

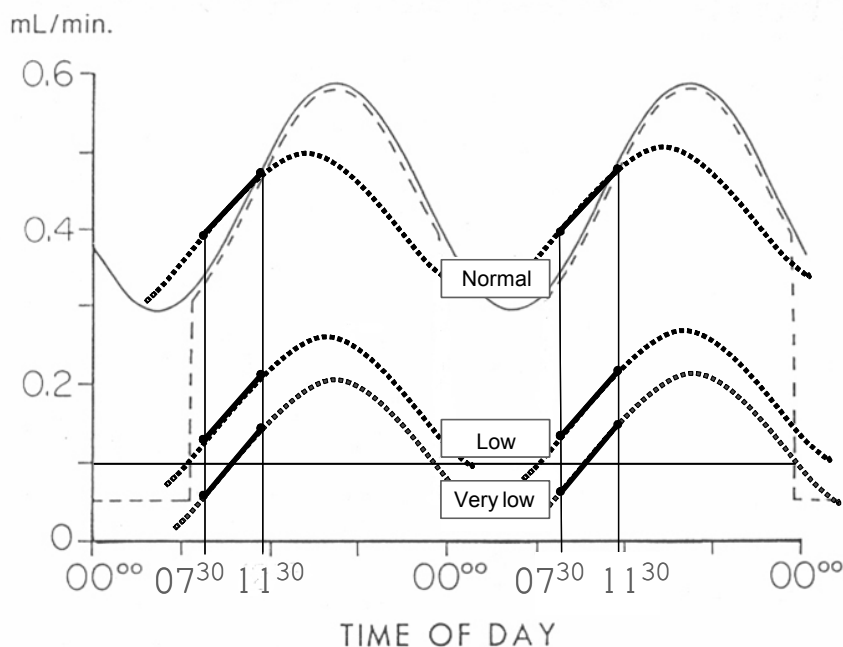


Figure 10. The circadian rhythm in unstimulated salivary flow rate (from Dawes), modified with data Study III, very low, low and normal flow rates measured at 7.30 and 11.30 and extrapolated to a cosines pattern similar to the circadian rhythm (dotted lines). The limit for very low flow rates 0.1 mL/min were passed for the very low group (if linear) at approximately 9.00 a.m.

Effect of iron supplementation on salivary flow rates

Therapeutic trials regarding treatment effects of salivary gland hypofunction have been systematically reviewed (Brennan, et al. 2002, Grisius 2001). It was found that among the evaluated clinical trials the experimental quality has varied considerably. A system to assess the quality of clinical trials (Hadorn, et al. 1996) was used on randomised controlled trials (RCT) for the management of xerostomia. Out of 52 evaluated studies only 4 could be classified in the highest evidence group (level A) (Brennan, et al. 2002). When assessing the quality of *Study IV* according to Hardon et al. (1996) it will in my opinion qualify for level A evidence.

The small increase in unstimulated whole salivary flow rate in both the placebo and iron groups after 3 months treatment found in *Study IV* may be explained by the statistical phenomenon regression to the mean (Barnett, et al. 2005), which could also explain the similar increase reported by Osaki et al. (1999). This effect could probably have been reduced by repeated baseline saliva tests (Barnett, et al. 2005).

The results from *Study IV* showed no effect from iron supplementation for three months on unstimulated salivary flow rate in individuals with hyposalivation and low serum ferritin values. It seems very likely that the result from *Study IV* is a fact but it may be appropriate to address some alternative possibilities that may relate to the length of treatment and other deficiencies together with iron deficiency. Reference values for serum ferritin ranges widely: a value $<15 \mu\text{g/L}$ confirms the diagnosis of iron deficiency while a value $>100 \mu\text{g/L}$ rules out iron deficiency (Guyatt, et al. 1992). The likelihood of iron deficiency does not start to drop until the serum ferritin values are $>40 \mu\text{g/L}$. Even if the serum ferritin levels were elevated in the iron group in *Study IV*, it is not certain that the three month treatment period was sufficient.

In treatment of iron deficiency anaemia, iron supplements are frequently reported as failing to restore haemoglobin concentrations to normal (Allen 2002). In some of these reports, it is possible that other micronutrient deficiencies are limiting the haemoglobin response to iron supplements. In women most attention has been focused on a few micronutrients, for example iron and folate, while multiple micronutrient deficiencies occur simultaneously when diets are poor (Allen 2005). A superior effect has been shown for supplementations with multiple micronutrients compared to iron supplementation for children in developing countries (Smuts, et al. 2005). The multiple micronutrients treatment resulted in a significantly greater weight gain, was more effective for controlling anaemia and iron deficiency, besides improving zinc, retinol, tocopherol, and riboflavin status in children.

FUTURE DIRECTIONS

The limitation of the cross-sectional study design have been recognised for caries risk assessment studies in general, emphasising a need for longitudinal studies (NIH 2001). Therefore, future longitudinal studies are needed to learn more about reduced salivary flow i) in adolescents and young adults, ii) in relationship to dental caries, iii) in aging people and its relationship to gender, diseases and drug use. It is important that these studies include broad information about other potential predictors and confounders in regression by multilevel modelling.

CONCLUSIONS

These conclusions can be drawn from the results presented in this thesis:

- Prevalence of reduced salivary flow rates was consistent and prevalent in younger and middle-aged adults (<50 years).
- Very low salivary flow rates were related to high Body Mass Index (BMI) and diagnosed diseases in younger adults, but to medication in older adults.
- Reduced salivary flow rates in young adult women were related to caries.
- Time of measurement of the salivary flow rate influences diagnosis of hyposalivation.
- Iron supplementation did not enhance salivary flow.

EPILOGUE AND IMPLICATIONS

She is a 45 year-old nurse' assistant at her yearly dental checkup in my dental office. She seems to be quite nervous: only four times has she been free from decay which required filling

.....Therefore, annual checkups are very important to her. When I measured her salivary flow rate it was found to be low. Her first question was "What can I do to get more saliva?"

Has this thesis made any contribution that may help us in the clinical management of patients like the one mentioned above?

The results show that she is not a rare individual having reduced salivary flow and being less than 50 years old. For women this seems to be a special health problem in relation to caries. Furthermore, the finding that reduced salivary flow is also related to general health in different degrees depending on the age, indicate a need to have a broader perspective on reduced salivary flow that might an early sign of chronic disease. The hypothesis, trying to enhance salivary secretion by iron supplementation, was tested but did not work out.

Until further research has identified causes and treatments for reduced salivary flow the dentist will have to acknowledge reduced salivary flow to be a factor that needs to be evaluated in patients with recurring caries. This is important for both patient and dentist as it identifies a need for a more extensive prevention and attempts to control other risk factors in order to avoid caries.

SVENSK SAMMANFATTNING (SWEDISH SUMMARY)

Bakgrund

Munhälsan påverkas negativt av nedsatt salivsekretion. Förekomsten av nedsatt salivsekretion hos unga vuxna individer är okänd och det finns ingen känd behandling som ökar salivsekretionen. Nedsatt salivsekretion ger en ökad risk för karies, som fortfarande är den vanligaste och mest utbredda sjukdomen i munhålan. Nedsatt salivsekretion har noterats i samband med undernäring. En typ av nutritionsstörning som är relaterad till undernäring och vilken föreslagits påverka salivsekretionen är järnbrist. Ett ökat intresse har på senare år fokuserat på gemensamma orsaksfaktorer när det gäller kost och nutrition för flera kroniska sjukdomar, däribland fetma och karies.

Målsättning

Målsättningen med avhandlingen är att i) beskriva förekomsten av nedsatt salivsekretion i olika åldersgrupper, ii) undersöka relationen mellan karies, sjukdomsförekomst, medicinering, övervikt och nedsatt salivsekretion iii) undersöka hur tidpunkten för salivprovtagningen påverkar nedsatt salivsekretion samt iv) pröva om järntillskott kan öka salivsekretionen.

Material och metod

I *Studie I* som är en epidemiologisk tvärsnittsstudie valdes 1000 män och 1000 kvinnor slumpvis från 48,500 patienter vid 14 tandvårdskliniker i Västernorrland och Västerbotten i åldrarna 20-69 år (lika många män och kvinnor i fem olika åldersgrupper). Av dessa undersöktes 1,427 med avseende på vilosalivflöde, tuggstimulerat salivflöde och antalet kvarvarande tänder. Deltagarna fyllde även i ett frågeformulär kring upplevd muntorrhet, medicinering, sjukdomsdiagnoser, tobaksvanor samt längd och vikt. I *Studierna II, III och IV* var deltagarna konsekutivt utvalda patienter, i åldrarna 15-46 år, vid Folktandvården i Sala.

I *Studie II*, en fall-kontrollstudie, matchades 48 kariesaktiva individer med avseende på ålder och kön mot 48 kariesinaktiva individer i åldrarna 15-40 år, 30 kvinnor och 18 män.

Hos dessa mättes vilosalivflöde, serum ferritin (järndepåer) och tidigare karieserfarenhet.

I *Studie III*, en metodstudie, undersöktes tidpunkten för salivprovtagningens inverkan på salivflödet hos 108 individer i åldrarna 15-46 år, 68 kvinnor och 22 män. Salivflödet mättes två gånger under samma dag, kl. 7.30 och 11.30. Försökspersonerna delades in i tre grupper beroende av resultatet från det första salivprovet; 37 individer med mycket låga, 41 med låga och 30 med normala salivflöden. Vid båda tillfällena uppskattade personerna den subjektiva upplevelsen av salivfunktionen med hjälp av VAS-skalar. I *Studie IV*, en randomiserad och placebokontrollerad behandlingsstudie, deltog 50 individer med nedsatt salivsekretion och små järndepåer i åldrarna 15-45 år. Patienterna slumpades till behandling med järntabletter (2×60 mg/dag i 3 månader) eller placebo i motsvarande dosering. Vilosalivflöde och serumferritin mättes före och efter behandling. Patienterna uppskattade även saliv och tårkörtelfunktionen och efter behandling med hjälp av VAS-skalar.

Resultat

I *Studie I* var förekomsten av mycket låga (<0.1 mL/min) och låga (0.10-0.19 mL/min) vilosalivflöden likartade upp till 50 års ålder, mellan 10,9-17,8% respektive 17,3-22,7%.

Multiple logistisk regressionsanalys visade att "ålder över 50 år", "kön-kvinna", "färre än 20 kvarvarande tänder" samt "intag av mediciner med salivsekretionshämmande inverkan" signifikant ökade risken för mycket låga vilosalivflöden. I *Studie II* hade 32 individer (67%) i den kariesaktiva gruppen låga vilosalivflöden jämfört med 13 individer (27%) i kariesinaktiva gruppen. Det var ingen skillnad i serumferritin-nivåer mellan grupperna. I *Studie III* fanns det en statistiskt signifikant ökning av vilosalivflödet kl. 11.30 jämfört med 7.30 hos samtliga tre grupper av likartad storlek (0.08-0.09 mL/min). I gruppen med mycket låga salivflöden hade 70% passerat gränsen för mycket lågt (0.1 mL/min) kl. 11.30. Det var en signifikant skillnad i upplevd muntorrhet mellan den normala gruppen och båda grupperna med låga och mycket låga salivflöden. I *Studie IV* fanns ingen skillnad mellan grupperna efter behandling avseende vilosalivflöde eller upplevd muntorrhet.

Slutsatser

Förekomsten av nedsatt salivsekretion är relativt vanligt även hos yngre och medelålders vuxna (<50 år). Mycket låga salivflöden har samband med övervikt (hög BMI) och diagnostiserad sjukdom hos yngre vuxna, men till medicinering hos äldre. Nedsatt salivsekretion hos yngre kvinnor har samband med karies. Tidpunkten för salivprovet påverkar diagnostiken av nedsatt salivsekretion. Oralt tillfört järn ökar inte salivflödet.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to everyone who has supported and helped me with this thesis. I would especially like to thank:

Professor **Åke Tegelberg**, my co-supervisor, tutor and excellent mentor for many years, who introduced me to scientific research. He has, during a very long journey, guided me to the information I needed and towards the right contacts. Any kind of obstacle, financial or practical, has been swept from my path by his skilful supervision. Always incredibly fast in returning an encouraging answer to a “desperately” mailed question, or giving his opinion on a manuscript. His efforts have given me the opportunity to focus on my research without bothering about bureaucratic matters, I am deeply grateful.

Professor **Folke Lagerlöf**, my supervisor and tutor, for very generously sharing a lot of his time. It has been a very pleasant and relaxed period filled with humour and discussions. In a stimulating and very inspiring way he has shared his vast knowledge of cariology and research in general. At conferences and meetings he has also introduced me to several of his many friends in the caries research field. His long experience as an editor has afforded me a great deal of important and invaluable information on the art of writing and submitting scientific papers. He definitely made this thesis possible, without him there would have been no thesis. I owe him a lot.

Dr **Per Byström**, former head of the Public Dental Clinic in Sala, my dentist since I was a child, my colleague and clinical tutor, for his support and enthusiasm for my research and clinical work. Furthermore, I am very grateful for stimulating discussions about epidemiological data and clinical management to improve the health and efficient treatment of our patients.

Associate Professor **Anette Oliveby**, my co-supervisor, for giving valuable advice on saliva collection and the management of salivary research and for encouraging discussions about my research and clinical implementations.

Associate Professor **Maud Bergdahl**, my co-supervisor, for gladly sharing her large and impressive dataset (*Study I*) with me, and for very interesting and stimulating discussion on our findings as well as constructive criticism as co-author of the manuscript.

Professor **Jerzy Leppert**, Head of the Centre for Clinical Research Västerås, for facilitating my time as a PhD student in a very inspiring environment. Always encouraging my work.

Director **Jan-Erik Andersson**, Head of Public Dental Health in Västmanland, for having the insight to start the collaboration with the Centre for Clinical Research Västerås, which has given me the opportunity to combine clinical work and research.

All the staff at the Public Dental Clinic in Sala, for their professionalism, friendship and for a lot of fun. I am especially grateful for my ever trusty nurses **Ann Ljunggren-Larsson** and **Gun-Britt Andersson**, for coordinating and supervising all the saliva tests and making the daily work easy. Also the dedicated group of dental hygienist, **Malin Karlson**, **Barbro Asp**, **Ulrika Pehrsson**, **Gunnel Boman**, **Margareta Jansson** and **Veronica Sundstedt** for their interest in my research and the clinical management of our patients. Doctor **Anna Hägglund** and **Maud Söderström**, Head and principal nurse at the Public Dental Clinic in Sala, for supporting my research and not complaining when I reduced my time at the clinic.

Doctor **Mari Thörn**, specialist in internal medicine, for participating in the study design, and taking care of the referred patients with extreme serum ferritin values in *Study IV*.

Professor Emeritus **Leif Hallberg**, Göteborg University, for his valuable advice on iron deficiency.

Professor **Gunnar Birgegård**, Uppsala University, for providing very valuable information and advice on serum ferritin.

Associate Professor **Kristina Sundquist**, Karolinska Institutet, Centre for Family and Community Medicine, for constructive criticism and good ideas regarding the manuscript in *Study IV*.

At the Institute of Odontology, Karolinska Institutet, Huddinge, I would especially thank Professor **Anders Gustafsson**, the Director of Postgraduate Studies, and Department administrator **Kerstin Smedberg** for very kind and helpful guidance during my time as a PhD student in the department.

The entire staff and associates at the Centre for Clinical Research Västerås, for their help and support. Especially the administrators **Maria Delluva Karlsson**, **Gun Nyberg**, **Katarina Ringström**, **Tony Wiklund**, and **Marja-Lena Ojutkangas**.

Professor **Göran Nilsson**, my room mate, for good company and interesting reflections on research in general, chronic disease and ethical matters.

Doctor **Kenth Nilsson**, my next door neighbor, always available for questions and discussion, always in a very supportive and positive mood.

Doctor **Andreas Rosenblad**, Statistician, for explaining statistical interpretations and never complaining when I ask him dumb questions, and for constructive criticism as co-author of the manuscript in *study I*.

Associate Professor **Stefan Sörensen** and Research Assistant **Petra Whalén** for their advice and support, giving me my first basic introduction to statistics.

For good discussions within the group of PhD students, at the Centre for Clinical Research Västerås, sharing the same kind of problems despite the disparities in research fields **Per Andersson, Ann-Christin Johansson, Eva Thors-Adolfsson, Tuula Wallsten, Anders Westman, Eva Nohlert, Marie-Louise Södersved Källestedt, and Cecilia Åslund.**

Monica Forssberg and her staff at the Hospital laboratory in Sala, for taking care of all the blood sampling, and **Rosanne Forberg**, head of the clinical laboratory at the Central Hospital in Västerås, for assisting with laboratory management.

Eva Eriksson at the local pharmacy in Sala, for handling all the required paperwork in connection with the clinical trial *Study IV*.

My first very good contact back at the academy with Professor Emeritus **Bo Krasse**, who gave very valuable advice and pointed me in the right direction.

Associate Professor **Ann-Marie Wiktorsson**, my former teacher in Cariology, for encouraging me to continue.

The County Council of Västmanland, Colgate Research Fund, Swedish Patent Revenue Fund and Swedish Dental Society for financial support

My wife **Eva Flink**, for her endless love and support, and our children **Emil, Mirna** and **Pilar**, for joy and happiness – I love you all.

All the patients from whom I learned so much, and not only in relation to hyposalivation and caries.

REFERENCES

- Allen, L. H.** Iron supplements: scientific issues concerning efficacy and implications for research and programs. *J Nutr* 2002;132:813S-9S.
- Allen, L. H.** Multiple micronutrients in pregnancy and lactation: an overview. *Am J Clin Nutr* 2005;81:1206S-12S.
- Amerongen, A. V. and Veerman, E. C. I.** Saliva--the defender of the oral cavity. *Oral Dis* 2002;8:12-22.
- Anusavice, K.** The maze of treatment decisions. In: O. Fejerskov and E. A. M. Kidd, editors. *Dental caries: the disease and its clinical management*. Oxford: Blackwell Munksgaard; 2003. p. 251-65.
- Aps, J. K. M. and Martens, L. C.** Review: The physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* 2005;150:119-31.
- Atkinson, J. C. and Fox, P. C.** Salivary gland dysfunction. *Clin Geriatr Med* 1992;8:499-511.
- Atkinson, J. C., Grisius, M. and Massey, W.** Salivary hypofunction and xerostomia: diagnosis and treatment. *Dent Clin North Am* 2005;49:309-26.
- Bader, J. D., Perrin, N. A., Maupome, G., Rindal, B. and Rush, W. A.** Validation of a simple approach to caries risk assessment. *J Public Health Dent* 2005;65:76-81.
- Bader, J. D. and Shugars, D. A.** What do we know about how dentists make caries-related treatment decisions? *Community Dent Oral Epidemiol* 1997;25:97-103.
- Baker, S. R., Pankhurst, C. L. and Robinson, P. G.** Utility of two oral health-related quality-of-life measures in patients with xerostomia. *Community Dent Oral Epidemiol* 2006;34:351-62.
- Baker, S. R., Pankhurst, C. L. and Robinson, P. G.** Testing relationships between clinical and non-clinical variables in xerostomia: a structural equation model of oral health-related quality of life. *Qual Life Res* 2007;16:297-308.
- Bardow, A., Nyvad, B. and Nauntofte, B.** Relationships between medication intake, complaints of dry mouth, salivary flow rate and composition, and the rate of tooth demineralization in situ. *Arch Oral Biol* 2001;46:413-23.
- Barnett, A. G., van der Pols, J. C. and Dobson, A. J.** Regression to the mean: what it is and how to deal with it. *Int J Epidemiol* 2005;34:215-20.
- Bartley, K. A., Underwood, B. A. and Deckelbaum, R. J.** A life cycle micronutrient perspective for women's health. *Am J Clin Nutr* 2005;81:1188S-1193S.
- Becks, H. and Wainwright, W.** Human Saliva. IX. The effect of activation of salivary flow. *J Dent Res* 1939;18: 447-56.
- Becks, H. and Wainwright, W.** Human saliva: XIII. Rate of flow of resting saliva of healthy individuals. *J Dent Res* 1943:391-6.
- Bedos, C., Brodeur, J. M., Arpin, S. and Nicolau, B.** Dental caries experience: a two-generation study. *J Dent Res* 2005;84:931-6.
- Ben-Shlomo, Y. and Kuh, D.** A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. *Int J Epidemiol* 2002;31:285-93.
- Bergdahl, M.** Salivary flow and oral complaints in adult dental patients. *Community Dent Oral Epidemiol* 2000;28:59-66.
- Billings, R. J., Proskin, H. M. and Moss, M. E.** Xerostomia and associated factors in a community-dwelling adult population. *Community Dent Oral Epidemiol* 1996;24:312-6.

- Birkhed, D. and Heintze, U.** Salivary secretion rate, buffer capacity, and pH. In: J. Tenovou, editor. *Human Saliva: Clinical chemistry and microbiology*. Boca Raton: CRC Press; 1989. p. 25-73.
- Bjarnason, S.** High caries levels: problems still to be tackled. *Acta Odontol Scand* 1998;56:176-8.
- Björk, A.** Timing of interceptive orthodontic measures based on stages of maturation. *Trans Eur Orthod Soc* 1972:61-74.
- Björk, A.** Kaebernes relation til det øvrige kranium (Danish). In: A. Lundström, editor. *Nordisk lärobok i ortodonti*. 4th ed. Stockholm: Tandläkarförbunds Förlagsförening; 1975. p. 102-10.
- Brennan, M. T., Shariff, G., Lockhart, P. B. and Fox, P. C.** Treatment of xerostomia: a systematic review of therapeutic trials. *Dent Clin North Am* 2002;46:847-56.
- Burman, D.** Iron metabolism in infancy and childhood. In: A. Jacobs and M. Worwood, editors. *Iron in biochemistry and medicine*. London and New York: Academic Press; 1974. p. 544-62.
- Christensen, K., Gaist, D., Jeune, B. and Vaupel, J. W.** A tooth per child? *Lancet* 1998;352:204.
- Dawes, C.** Circadian rhythms in human salivary flow rate and composition. *J Physiol* 1972;220:529-45.
- Dawes, C.** Rhythms in salivary flow rate and composition. *Int J Chronobiol* 1974;2:253-79.
- Dawes, C.** Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J Dent Res* 1987;66 Spec No:648-53.
- Dawes, C.** Factors influencing salivary flow rate and composition. In: W. M. Edgar, C. Dawes and D. M. O'Mullane, editors. *Saliva and oral health*. 3rd ed. London: British Dental Association; 2004. p. 32-49.
- Dawes, C.** How much saliva is enough for avoidance of xerostomia? *Caries Res* 2004;38:236-40.
- Emilson, C. G. and Krasse, B.** The effect of iron salts on experimental dental caries in the hamster. *Arch Oral Biol* 1972;17:1439-43.
- Enwonwu, C. O.** Influence of socio-economic conditions on dental development in Nigerian children. *Arch Oral Biol* 1973;18:95-107.
- Ericsson, Y. and Hardwick, L.** Individual diagnosis, prognosis and counselling for caries prevention. *Caries Res* 1978;12 Suppl 1:94-102.
- Faber, M.** Xerostomi: En ofte overset lidelse (Danish). *Tandlægebladet* 1943;47:177-90.
- Fejerskov, O., Nyvad, B. and Kidd, E. A.** Clinical and histological manifestations of dental caries. In: O. Fejerskov and E. A. M. Kidd, editors. *Dental caries: the disease and its clinical management*. Oxford: Blackwell Munksgaard; 2003. p. 71-97.
- Fenton, V., Cavill, I. and Fisher, J.** Iron stores in pregnancy. *Br J Haematol* 1977;37:145-9.
- Fergusson, M.** Oral mucous membrane markers of internal disease: Part II. In: A. Dolby, editor. *Oral mucosa in health and disease*. Oxford: Blackwell; 1975.
- Fletcher, J., Mather, J., Lewis, M. J. and Whiting, G.** Mouth lesions in iron-deficient anemia: relationship to *Candida albicans* in saliva and to impairment of lymphocyte transformation. *J Infect Dis* 1975;131:44-50.
- Fox, P. C.** Salivary enhancement therapies. *Caries Res* 2004;38:241-6.

- Fox, P. C., Busch, K. A. and Baum, B. J.** Subjective reports of xerostomia and objective measures of salivary gland performance. *J Am Dent Assoc* 1987;115:581-4.
- Fure, S. and Zickert, I.** Root surface caries and associated factors. *Scand J Dent Res* 1990;98:391-400.
- Givens, E., Jr.** Update on xerostomia: current treatment modalities and future trends. *Gen Dent* 2006;54:99-101.
- Glynn, R. J. and Buring, J. E.** Ways of measuring rates of recurrent events. *BMJ* 1996;312:364-7.
- Grimes, D. A. and Schulz, K. F.** Bias and causal associations in observational research. *Lancet* 2002;359:248-52.
- Grisius, M. M.** Salivary gland dysfunction: a review of systemic therapies. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001;92:156-62.
- Guyatt, G. H., Oxman, A. D., Ali, M., Willan, A., McIlroy, W. and Patterson, C.** Laboratory diagnosis of iron-deficiency anemia: an overview. *J Gen Intern Med* 1992;7:145-53.
- Hadorn, D. C., Baker, D., Hodges, J. S. and Hicks, N.** Rating the quality of evidence for clinical practice guidelines. *J Clin Epidemiol* 1996;49:749-54.
- Haga, H. J., Hultén, B., Bolstad, A. I., Ulvestad, E. and Jonsson, R.** Reliability and sensitivity of diagnostic tests for primary Sjögren's syndrome. *J Rheumatol* 1999;26:604-8.
- Halfon, N., Inkelas, M. and Hochstein, M.** The health development organization: an organizational approach to achieving child health development. *Milbank Q* 2000;78:447-97, 341.
- Hallberg, L. and Rossander-Hulthén, L.** Prevalence of iron deficiency in European countries and attempts to analyze possible causes of differences. *Bibl Nutr Dieta* 1989;94:105.
- Hase, J. C. and Birkhed, D.** Salivary glucose clearance, dry mouth and pH changes in dental plaque in man. *Arch Oral Biol* 1988;33:875-80.
- Haugejorden, O.** Using the DMF gender difference to assess the "major" role of fluoride toothpastes in the caries decline in industrialized countries: a meta-analysis. *Community Dent Oral Epidemiol* 1996;24:369-75.
- Hay, D. I.** Some observations on human saliva proteins and their role in the formation of the acquired enamel pellicle. *J Dent Res* 1969;48:806-10.
- Heintze, U., Birkhed, D. and Björn, H.** Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. *Swed Dent J* 1983;7:227-38.
- Hochberg, M. C., Tielsch, J., Munoz, B., Bandeen-Roche, K., West, S. K. and Schein, O. D.** Prevalence of symptoms of dry mouth and their relationship to saliva production in community dwelling elderly: the SEE project. *Salisbury Eye Evaluation. J Rheumatol* 1998;25:486-91.
- Humphrey, S. P. and Williamson, R. T.** A review of saliva: normal composition, flow, and function. *J Prosthet Dent* 2001;85:162-9.
- Jacobs, A.** Ferritin: an interim review. *Curr Top Hematol* 1985;5:25-62.
- Jacobs, A., Miller, F., Worwood, M., Beamish, M. R. and Wardrop, C. A.** Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br Med J* 1972;4:206-8.
- Johansson, I. and Fagernäs, C.** Effect of iron-deficiency anaemia on saliva secretion rate and composition in the rat. *Arch Oral Biol* 1994;39:51-6.
- Johansson, I., Lenander-Lumikari, M. and Saellström, A. K.** Saliva composition in Indian children with chronic protein-energy malnutrition. *J Dent Res* 1994;73:11-9.

- Johansson, I., Saellström, A. K., Rajan, B. P. and Parameswaran, A.** Salivary flow and dental caries in Indian children suffering from chronic malnutrition. *Caries Res* 1992;26:38-43.
- Jorkjend, L., Johansson, A., Johansson, A. K. and Bergenholtz, A.** Resting and stimulated whole salivary flow rates in Sjogren's syndrome patients over time: a diagnostic aid for subsidized dental care? *Acta Odontol Scand* 2004;62:264-8.
- Kaneshige, E.** Serum ferritin as an assessment of iron stores and other hematologic parameters during pregnancy. *Obstet Gynecol* 1981;57:238-42.
- Kavanagh, D. A., O'Mullane, D. M. and Smeeton, N.** Variation of salivary flow rate in adolescents. *Arch Oral Biol* 1998;43:347-52.
- Kay, E. J. and Nuttall, N. M.** Relationship between dentists' treatment attitudes and restorative decisions made on the basis of simulated bitewing radiographs. *Community Dent Oral Epidemiol* 1994;22:71-4.
- Kerr, A. C.** The physiological regulation of salivary secretion in man. A study of the response of human salivary glands to reflex stimulation. Oxford, London, New York, Paris: Pergamon Press; 1961.
- Kidd, E. A., Mejäre, I. and Nyvad, B.** Clinical and radiographic diagnosis. In: O. Fejerskov and E. A. M. Kidd, editors. *Dental caries: the disease and its clinical management*. Oxford: Blackwell Munksgaard; 2003. p. 110-28.
- Kidd, E. A. and Nyvad, B.** Caries control for the individual patient. In: O. Fejerskov and E. A. M. Kidd, editors. *Dental caries: the disease and its clinical management*. Oxford: Blackwell Munksgaard; 2003. p. 303-12.
- Källestål, C. and Wall, S.** Socio-economic effect on caries. Incidence data among Swedish 12-14-year-olds. *Community Dent Oral Epidemiol* 2002;30:108-14.
- Larsson, P., List, T., Lundström, I., Marcusson, A. and Ohrbach, R.** Reliability and validity of a Swedish version of the Oral Health Impact Profile (OHIP-S). *Acta Odontol Scand* 2004;62:147-52.
- Last, J.** A dictionary of epidemiology. 4th ed. New York: Oxford University Press; 2001.
- Leon, D.** Common threads: underlying components of inequalities in mortality between and within countries. In: D. Leon and G. Walt, editors. *Poverty, inequality and health: An international perspective*. Oxford: Oxford University Press; 2000. p. 58-87.
- Leone, C. W. and Oppenheim, F. G.** Evidence Report - Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. In: *Diagnosis and Management of Dental Caries Throughout Life*; 2001: National Institutes of Health; 2001.
- Leone, C. W. and Oppenheim, F. G.** Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. *J Dent Educ* 2001;65:1054-62.
- Lingström, P. and Moynihan, P.** Nutrition, saliva, and oral health. *Nutrition* 2003;19:567-9.
- Locker, D.** Subjective reports of oral dryness in an older adult population. *Community Dent Oral Epidemiol* 1993;21:165-8.
- Locker, D.** Dental status, xerostomia and the oral health-related quality of life of an elderly institutionalized population. *Spec Care Dentist* 2003;23:86-93.
- Lopez, R., Scheutz, F., Errboe, M. and Baelum, V.** Selection bias in case-control studies on periodontitis: a systematic review. *Eur J Oral Sci* 2007;115:339-43.
- Lukacs, J. R. and Largaespada, L. L.** Explaining sex differences in dental caries prevalence: saliva, hormones, and "life-history" etiologies. *Am J Hum Biol* 2006;18:540-55.
- Mandel, I. D.** Relation of saliva and plaque to caries. *J Dent Res* 1974;53:246-66.

- Manthorpe, R. and Axéll, T.** Xerostomia. *Clin Exp Rheumatol* 1990;8 Suppl 5:7-12.
- Marthaler, T. M.** Changes in dental caries 1953-2003. *Caries Res* 2004;38:173-81.
- Massler, M.** Teen-age cariology. *Dent Clin North Am* 1969;13:405-23.
- McCaul, L. K., Jenkins, W. M. and Kay, E. J.** The reasons for extraction of permanent teeth in Scotland: a 15-year follow-up study. *Br Dent J* 2001;190:658-62.
- McMillan, A. S., Leung, K. C., Leung, W. K., Wong, M. C., Lau, C. S. and Mok, T. M.** Impact of Sjögren's syndrome on oral health-related quality of life in southern Chinese. *J Oral Rehabil* 2004;31:653-9.
- Mejàre, I., Sundberg, H., Espelid, I. and Tveit, B.** Caries assessment and restorative treatment thresholds reported by Swedish dentists. *Acta Odontol Scand* 1999;57:149-54.
- Miguel, J. C., Bowen, W. H. and Pearson, S. K.** Effects of iron salts in sucrose on dental caries and plaque in rats. *Arch Oral Biol* 1997;42:377-83.
- Mulligan, R., Navazesh, M. and Wood, G. J.** A pilot study comparing three salivary collection methods in an adult population with salivary gland hypofunction. *Spec Care Dentist* 1995;15:154-7.
- Nagler, R. M.** Salivary glands and the aging process: mechanistic aspects, health-status and medicinal-efficacy monitoring. *Biogerontology* 2004;5:223-33.
- National Institutes of Health Consensus Development Conference statement. Diagnosis and management of dental caries throughout life, March 26-28, 2001. *J Am Dent Assoc* 2001;132:1153-61.
- Nauntofte, B., Tenovuo, J. and Lagerlöf, F.** Secretion and composition of saliva. In: O. Fejerskov and E. A. M. Kidd, editors. *Dental caries: The disease and its clinical management*. Oxford: Blackwell Munksgaard; 2003. p. 7-27.
- Navazesh, M., Christensen, C. and Brightman, V.** Clinical criteria for the diagnosis of salivary gland hypofunction. *J Dent Res* 1992;71:1363-9.
- Navazesh, M. and Christensen, C. M.** A comparison of whole mouth resting and stimulated salivary measurement procedures. *J Dent Res* 1982;61:1158-62.
- Navazesh, M., Mulligan, R., Komaroff, E., Redford, M., Greenspan, D. and Phelan, J.** The prevalence of xerostomia and salivary gland hypofunction in a cohort of HIV-positive and at-risk women. *J Dent Res* 2000;79:1502-7.
- Nederfors, T.** Xerostomia and hyposalivation. *Adv Dent Res* 2000;14:48-56.
- Nederfors, T. and Dahlöf, C.** Effects of the beta-adrenoceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. *Arch Oral Biol* 1992;37:579-84.
- Nederfors, T., Isaksson, R., Mörnstad, H. and Dahlöf, C.** Prevalence of perceived symptoms of dry mouth in an adult Swedish population--relation to age, sex and pharmacotherapy. *Community Dent Oral Epidemiol* 1997;25:211-6.
- Nicolau, B., Marcenes, W., Allison, P. and Sheiham, A.** The life course approach: explaining the association between height and dental caries in Brazilian adolescents. *Community Dent Oral Epidemiol* 2005;33:93-8.
- Nicolau, B., Marcenes, W., Bartley, M. and Sheiham, A.** A life course approach to assessing causes of dental caries experience: the relationship between biological, behavioural, socio-economic and psychological conditions and caries in adolescents. *Caries Res* 2003;37:319-26.
- Nicolau, B., Thomson, W. M., Steele, J. G. and Allison, P. J.** Life-course epidemiology: concepts and theoretical models and its relevance to chronic oral conditions. *Community Dent Oral Epidemiol* 2007;35:241-249.
- Närhi, T. O.** Prevalence of subjective feelings of dry mouth in the elderly. *J Dent Res* 1994;73:20-5.

- Orellana, M. F., Lagravere, M. O., Boychuk, D. G., Major, P. W. and Flores-Mir, C.** Prevalence of xerostomia in population-based samples: a systematic review. *J Public Health Dent* 2006;66:152-8.
- Osaki, T., Ueta, E., Arisawa, K., Kitamura, Y. and Matsugi, N.** The pathophysiology of glossal pain in patients with iron deficiency and anemia. *Am J Med Sci* 1999;318:324-9.
- Pai, S., Ghezzi, E. M. and Ship, J. A.** Development of a Visual Analogue Scale questionnaire for subjective assessment of salivary dysfunction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001;91:311-6.
- Pedersen, A. M. and Nauntofte, B.** Primary Sjögren's syndrome: oral aspects on pathogenesis, diagnostic criteria, clinical features and approaches for therapy. *Expert Opin Pharmacother* 2001;2:1415-36.
- Percival, R. S., Challacombe, S. J. and Marsh, P. D.** Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. *J Dent Res* 1994;73:1416-20.
- Petersen, P. E., Bourgeois, D., Ogawa, H., Estupinan-Day, S. and Ndiaye, C.** The global burden of oral diseases and risks to oral health. *Bull World Health Organ* 2005;83:661-9.
- Petrie, A., Bulman, J. S. and Osborn, J. F.** Further statistics in dentistry. Part 2: Research designs 2. *Br Dent J* 2002;193:435-40.
- Petrie, A., Bulman, J. S. and Osborn, J. F.** Further statistics in dentistry. Part 7: Repeated measures. *Br Dent J* 2003;194:17-21.
- Poulton, R., Caspi, A., Milne, B. J., Thomson, W. M., Taylor, A., et al.** Association between children's experience of socioeconomic disadvantage and adult health: a life-course study. *Lancet* 2002;360:1640-5.
- Rantonen, P. J. and Meurman, J. H.** Viscosity of whole saliva. *Acta Odontol Scand* 1998;56:210-4.
- Ravald, N. and List, T.** Caries and periodontal conditions in patients with primary Sjögren's syndrome. *Swed Dent J* 1998;22:97-103.
- Rhodus, N. L. and Brown, J.** The association of xerostomia and inadequate intake in older adults. *J Am Diet Assoc* 1990;90:1688-92.
- Risheim, H., Arneberg, P. and Birkhed, D.** Oral sugar clearance and root caries prevalence in rheumatic patients with dry mouth symptoms. *Caries Res* 1992;26:439-44.
- Scully, C.** Drug effects on salivary glands: dry mouth. *Oral Dis* 2003;9:165-76.
- Seow, W. K.** A study of the development of the permanent dentition in very low birthweight children. *Pediatr Dent* 1996;18:379-84.
- Shannon, I. L.** Climatological effects on human parotid gland function. *Arch Oral Biol* 1966;11:451-3.
- Ship, J. A., Fox, P. C. and Baum, B. J.** How much saliva is enough? 'Normal' function defined. *J Am Dent Assoc* 1991;122:63-9.
- Ship, J. A., Pillemer, S. R. and Baum, B. J.** Xerostomia and the geriatric patient. *J Am Geriatr Soc* 2002;50:535-43.
- Siimes, M. A., Addiego, J. E., Jr. and Dallman, P. R.** Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. *Blood* 1974;43:581-90.
- Sisson, K. L.** Theoretical explanations for social inequalities in oral health. *Community Dent Oral Epidemiol* 2007;35:81-8.
- Smuts, C. M., Lombard, C. J., Benade, A. J., Dhansay, M. A., Berger, J., et al.** Efficacy of a foodlet-based multiple micronutrient supplement for preventing growth faltering, anemia, and micronutrient deficiency of infants: the four country IRIS trial pooled data analysis. *J Nutr* 2005;135:631S-8S.

- Solberg, H. E.** International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values, and International Committee for Standardization in Haematology (ICSH), Standing Committee on Reference Values. Approved Recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. *J Clin Chem Clin Biochem* 1987;25:337-42.
- Sreebny, L. M.** Salivary flow and dental caries. In: I. D. Mandel, editor. *Cariology today*. Basel: Kager; 1983. p. 56-69.
- Sreebny, L. M.** Salivary flow in health and disease. *Compend Suppl* 1989;13:S461-9.
- Sreebny, L. M.** Saliva in health and disease: an appraisal and update. *Int Dent J* 2000;50:140-61.
- Sreebny, L. M., Bánóczy, J., Baum, B. J., Edgar, W. M., Epstein, J. B., et al.** Saliva: its role in health and disease. Working Group 10 of the Commission on Oral Health, Research and Epidemiology (CORE). *Int Dent J* 1992;42:287-304.
- Sreebny, L. M. and Valdini, A.** Xerostomia. Part I: Relationship to other oral symptoms and salivary gland hypofunction. *Oral Surg Oral Med Oral Pathol* 1988;66:451-8.
- Stecksén-Blicks, C., Sunnegårdh, K. and Borssén, E.** Caries experience and background factors in 4-year-old children: time trends 1967-2002. *Caries Res* 2004;38:149-55.
- Stephen, K. and Purdell-Lewis, D.** Behavioural aspects of oral hygiene. In: G. Embery and G. Rølla, editors. *Clinical and biological aspects of dentifrices.*: Oxford Medical Publications; 1992. p. 13-20.
- Sweeney, E.** Salivary flow and composition in relation to dental caries - methods and problems in studying this relationship in humans and animals. In: I. Kleinberg, S. Ellison and I. D. Mandel, editors. *Saliva and dental caries*; 1979; New York: Information Retrieval Inc., New York; 1979.
- Sweeney, E. A. and Guzman, M.** Oral conditions in children from three highland villages in Guatemala. *Arch Oral Biol* 1966;11:687-98.
- Söderling, E.** Practical aspects of salivary analyses. In: J. Tenovou, editor. *Human Saliva: Clinical chemistry and microbiology*. Boca Raton: CRC Press; 1989. p. 1-24.
- Tabak, L. A.** In defense of the oral cavity: the protective role of the salivary secretions. *Pediatr Dent* 2006;28:110-7; discussion 192-8.
- Thomson, W. M.** Issues in the epidemiological investigation of dry mouth. *Gerodontology* 2005;22:65-76.
- Thomson, W. M., Chalmers, J. M., Spencer, A. J. and Ketabi, M.** The occurrence of xerostomia and salivary gland hypofunction in a population-based sample of older South Australians. *Spec Care Dentist* 1999;19:20-3.
- Thomson, W. M., Chalmers, J. M., Spencer, A. J. and Williams, S. M.** The Xerostomia Inventory: a multi-item approach to measuring dry mouth. *Community Dent Health* 1999;16:12-7.
- Thomson, W. M., Lawrence, H. P., Broadbent, J. M. and Poulton, R.** The impact of xerostomia on oral-health-related quality of life among younger adults. *Health Qual Life Outcomes* 2006;4:86.
- Walsh, N. P., Laing, S. J., Oliver, S. J., Montague, J. C., Walters, R. and Bilzon, J. L.** Saliva parameters as potential indices of hydration status during acute dehydration. *Med Sci Sports Exerc* 2004;36:1535-42.
- Van Nieuw Amerongen, A., Bolscher, J. G. M. and Veerman, E. C. I.** Salivary proteins: protective and diagnostic value in cariology? *Caries Res* 2004;38:247-53.

- Wang, S. L., Zhao, Z. T., Li, J., Zhu, X. Z., Dong, H. and Zhang, Y. G.** Investigation of the clinical value of total saliva flow rates. *Arch Oral Biol* 1998;43:39-43.
- Watt, R. G.** New WHO diet and nutrition review: implications for dental disease prevention. *Nutrition* 2003;19:1028-9.
- Whelton, H.** Introduction: the anatomy and physiology of salivary glands. In: M. Edgar, C. Dawes and D. O'Mullane, editors. *Saliva and oral health*. 3rd ed. London: British Dental Association; 2004. p. 1-13.
- WHO Report: Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech Rep Ser* 2003;916:i-viii, 1-149.
- Vissink, A., Burlage, F. R., Spijkervet, F. K., Jansma, J. and Coppes, R. P.** Prevention and treatment of the consequences of head and neck radiotherapy. *Crit Rev Oral Biol Med* 2003;14:213-25.
- Vitali, C., Bombardieri, S., Jonsson, R., Moutsopoulos, H. M., Alexander, E. L., et al.** Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554-8.
- Wolff, M. and Kleinberg, I.** Oral mucosal wetness in hypo- and normosalivators. *Arch Oral Biol* 1998;43:455-62.
- Wolff, M. S. and Kleinberg, I.** The effect of ammonium glycopyrrolate (Robinul)-induced xerostomia on oral mucosal wetness and flow of gingival crevicular fluid in humans. *Arch Oral Biol* 1999;44:97-102.
- von Bultzingslöwen, I., Sollecito, T. P., Fox, P. C., Daniels, T., Jonsson, R., et al.** Salivary dysfunction associated with systemic diseases: systematic review and clinical management recommendations. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;103 Suppl:S57 e1-15.
- Yeh, C. K., Johnson, D. A. and Dodds, M. W.** Impact of aging on human salivary gland function: a community-based study. *Aging (Milano)* 1998;10:421-8.
- Zickert, I., Jonson, A., Klock, B. and Krasse, B.** Disease activity and need for dental care in a capitation plan based on risk assessment. *Br Dent J* 2000;189:480-6.
- Öhrn, R., Enzell, K. and Angmar-Månsson, B.** Oral status of 81 subjects with eating disorders. *Eur J Oral Sci* 1999;107:157-63.