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Karolinska Institutet, Stockholm, Sweden

# **UNSTIMULATED HUMAN WHOLE SALIVA FLOW RATE IN RELATION TO HYPOSALIVATION AND DENTAL CARIES**

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To Eva, Emil, Mirna and Pilar

## **ABSTRACT**

*Introduction:* Salivary secretion is influenced by a large number of factors, including the circadian rhythm, making the detection of reduced salivary flow (hyposalivation) difficult. This is important since severely reduced salivary secretion has been associated with an increased risk for dental caries and may be a sign of a general disease, e.g. Sjögren's syndrome. It has been suggested that iron deficiency is related to hyposalivation.

*Aims:* The aims of this thesis were to investigate i) the unstimulated whole saliva flow rate and serum ferritin among individuals with dental caries, ii) time-dependent changes in the unstimulated whole saliva flow rate in subjects with hyposalivation, and iii) the subjects' perception of salivary gland function.

*Results:* Caries-active women were found to have a significantly lower unstimulated saliva flow rate compared with those with inactive caries, but there were no significant differences in serum ferritin. A significant increase in unstimulated whole saliva flow rate was revealed for individuals with hyposalivation as well as for those with a normal saliva secretion rate, when tested at two time points separated by four hours in the morning. There were significant differences in subjective assessments of oral dryness between individuals with hyposalivation and individuals with normal saliva secretion.

*Conclusions:* It was concluded that a low unstimulated whole saliva flow rate is related to caries activity, thereby strengthening the view that the unstimulated whole saliva flow rate test could be used in the prediction, prevention and treatment of caries. Furthermore, serum ferritin appears not to be related to caries activity, or to the unstimulated whole saliva flow rate. The unstimulated saliva flow rate test should be performed in a narrow time interval early in the morning in order to obtain an accurate diagnosis of hyposalivation. Individuals with salivary gland hypofunction perceive an increase in saliva flow rate more clearly than those with normal unstimulated whole saliva flow rate.

**Keywords:** Dental caries, iron deficiency, serum ferritin, unstimulated whole saliva, resting saliva, xerostomia, dry mouth, circadian rhythm.

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## LIST OF PUBLICATIONS

- I. Flink H, Tegelberg Å, Sörensen S. Hyposalivation and iron stores among individuals with and without active dental caries. *Acta Odontol Scand* 2000;58(6):265-71.
- II. Flink H, Tegelberg Å, Lagerlöf F. Influence of the time of measurement of unstimulated human whole saliva on the diagnosis of hyposalivation. *Arch Oral Biol* 2005, in press.

# 1 INTRODUCTION

More than a century ago, Miller observed that individuals with a diminished flow of saliva developed severe, rapidly spreading carious lesions (Miller, 1904). Since then, numerous investigators have reported similar observations in individuals with impaired salivary secretion, after radiation therapy in the head and neck region, for example (Vissink *et al.*, 2003), after taking medication with a side-effect of hyposalivation (Atkinson and Fox, 1992), or in individuals with Sjögren's syndrome (Ravald and List, 1998). In a recent systematic review of 55 studies of the risk of caries in various populations, the saliva flow rate was found to be a strong indicator of an increased risk in 21 studies (Leone and Oppenheim, 2001). However, in 34 studies, there was no association with caries. The authors of the review emphasized the methodological diversity of these studies, which may have affected the contradictory results. One of these methodological problems was the measurement of the saliva flow rate, sialometry.

Various forms of sialometry have been described for unstimulated and stimulated saliva from separate glands or for whole saliva (Navazesh and Christensen, 1982; Manthorpe and Axéll, 1990; Ship *et al.*, 1991). The measurement of the whole saliva flow rate is convenient for both patients and examiners (Sreebny and Zhu, 1996). In order to detect a reduced salivary flow, the unstimulated whole saliva flow rate has been proposed as the test of choice, as this flow rate may be reduced, even if the stimulated whole saliva is unaffected (Becks and Wainwright 1939; Sreebny, 1989; Wang *et al.*, 1998).

Xerostomia denotes subjective symptoms of dry mouth in contrast to hyposalivation, which denotes an objective sign of reduced saliva flow rate (Fox, 1996; Field *et al.*, 1997; Nederfors *et al.*, 1997). It is generally accepted in the literature that the limit for a very low unstimulated whole saliva flow rate is  $\leq 0.1$  mL/min (Ericsson and Hardwick, 1978; Sreebny and Valdini, 1988). This limit is also used in the criteria 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 Valdini, 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).

A circadian rhythm has been described for the unstimulated whole saliva flow rate (Dawes and Ong, 1973). Lower flow rates are found early in the morning compared with late in the afternoon. 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). However, the influence of the circadian rhythm on the saliva flow in individuals with hyposalivation is not known (Dawes, 1993), but it may have a decisive impact on the diagnosis of Sjögren's syndrome and also on caries risk assessment.

In adults, between 20-69 years of age, the prevalence of hyposalivation (defined as an unstimulated whole saliva flow rate of  $\leq 0.1$  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). The prevalence of hyposalivation increases with age, mainly because of an increase in the use of prescribed drugs that affect saliva secretion (Ship *et al.*, 2002). Even if hyposalivation is more common among older individuals, it has been pointed out that hyposalivation is not uncommon among younger adults (Sreebny and Valdini, 1988). In the 18-34 year age group, 20% have been reported to have a reduced unstimulated whole saliva flow rate. 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 and high blood pressure (Sreebny, 1989) and HIV (Navazesh *et al.*, 2000).

In addition to dental caries, there are several symptoms and signs associated with hyposalivation, such as thirst, difficulty speaking, difficulty eating dry food and oral inflammations (Sreebny *et al.*, 1992). Almost no treatable cause of hyposalivation is currently known, apart from those induced by drugs. The aim of different treatment modalities is therefore mainly limited to reducing symptoms (1988; Sreebny *et al.*, 1992; Fox, 2004).

In the 1940s, it was reported that the treatment of iron-deficient patients usually resulted in the elimination of symptoms, including hyposalivation (Faber, 1943). It was suggested that low serum iron and reduced salivary secretion were expressions of the same basal pathologic process (Bertram, 1967). Furthermore, a more recent study has indicated increased salivation in iron-deficient patients after treatment with iron (Osaki *et al.*, 1999). When comparing studies of hyposalivation and/or xerostomia with studies of iron deficiency, some similarities are found. Glossitis, angular chielitis, stomatitis,



dysphagia and candida infections are observed in patients with xerostomia and/or hyposalivation, as well as in patients with iron deficiency (Fergusson, 1975; Fletcher *et al.*, 1975; Sreebny *et al.*, 1992). Moreover, twice as many women as men suffer from xerostomia or hyposalivation (Sreebny and Valdini, 1988), which corresponds with the fact that iron deficiency is more common among women (Hallberg and Rossander-Hulthén, 1989).

Small iron stores are not unusual in growing children (Siimes *et al.*, 1974). During childhood, there are two major periods of growth (Björk, 1972), which correspond well with increased caries activity (Massler, 1969). Furthermore, after the last period of growth, there is a steady rise to adult iron levels in boys but not in girls. This difference for women remains throughout childbearing age (Burman, 1974). Adolescent girls and women develop dental caries more often than boys and men, even though females have better oral hygiene (Stephen and Purdell-Lewis, 1992). After pregnancy and delivery, iron stores are generally lowered (Kelly *et al.*, 1978; Kaneshige, 1981). This could correspond to the finding that the number of lost teeth in women is associated with the number of children (Christensen *et al.*, 1998).

Treatments with iron supplements have produced significant reduction in dental caries activity in animals (Emilsson and Krasse, 1972; Sintès and Miller, 1983; Miguel *et al.*, 1997). There do not appear to be any studies relating dental caries to iron deficiency in humans.

## 2 AIMS

The aims of this thesis were:

### Study I

- To evaluate the frequency of a low unstimulated whole saliva flow rate and low serum ferritin among individuals with and without dental caries activity
- To evaluate the relationship between unstimulated whole saliva flow rate and serum ferritin levels

### Study II

- To investigate whether individuals with hyposalivation display an increase in unstimulated whole saliva flow rate tested at two different time points in the morning
- To compare the hypothetical increase in unstimulated whole saliva flow rate between individuals with hyposalivation and individuals with normal saliva secretion when the unstimulated whole saliva flow rate was tested at two different time points in the morning
- To compare individual assessments of oral dryness between individuals with hyposalivation and individuals with normal saliva secretion when the unstimulated whole saliva flow rate was tested at two different time points in the morning

The main hypotheses of this thesis were:

- There is a lower unstimulated whole saliva flow rate in caries-active subjects compared with caries-inactive subjects (Study I)
- There is a difference in serum ferritin levels between caries-active and caries-inactive individuals (Study I)
- There is a relationship between unstimulated whole saliva flow rate and serum ferritin (Study I)
- There is a difference (increase) in unstimulated whole saliva flow rate when collected at two different time-points separated by 4 hours in the morning (Study II)

### **3 MATERIALS AND METHODS**

The local ethics committee at Uppsala University approved the studies (Dnr: 97-363 and 02-109).

#### **3.1 SUBJECTS**

All the participants were regular patients at the Public Dental Clinic in the city of Sala, Sweden.

Study I consisted of 96 individuals, 48 with active dental caries (ADC) and 48 who were dental caries inactive (DCI). The two groups were matched by gender and age, 30 females and 18 males in each group. The mean ages were 28.4 years (SD 7.0) for the ADC group and 29.3 years (SD 7.9) for the DCI group. The inclusion criteria for the ADC group were the development of manifest dental caries in two or more teeth since the last dental examination or the development of manifest dental caries in one tooth since the last examination combined with a history of recurrent caries (for more than 3 years). The inclusion criterion for the patients in the DCI group was that they had to have been free of manifest dental caries for more than 3 years. In the DCI group, the mean number of months free from dental caries was 129.8 (SD 68.3). The corresponding mean number of months with dental caries activity in the ADC group was 155.1 (SD 112.1). The dropouts from Study I were similar in terms of gender and age in both groups. The main reasons for withdrawal from the study were job interference and anxiety about the needles used in blood sampling.

Study II consisted of 108 individuals, 86 females and 22 males. The participants were divided into three groups (very low, low or normal) according to the unstimulated whole saliva flow rate at 7.30 am. The mean age in these groups was 33 years (SD 9), 33 years (SD 9) and 32 years (SD 8) respectively. All three groups had about the same percentages of women (~80%) and men (~20%).

Subjects taking drugs associated with dry mouth were excluded (Studies I and II). In Study I, pregnant women were also excluded.

### **3.2 DENTAL CARIES (STUDY I)**

Manifest dental caries was determined by clinical and radiographic examination (bite-wings). The patient's regular dentist performed the examination. Decayed, missing and filled teeth (DMF-T) and decayed, missing and filled surfaces (DMF-S) were recorded in all patients. The number of teeth with new manifest dental caries since the last examination (D-T) was registered.

### **3.3 SALIVA COLLECTION**

The subject was asked to relax for a couple of minutes before saliva collection. He/she was sitting bent forward in an ordinary chair and was asked to put his/her tongue on the lingual surfaces of the upper incisors and to hold his/her mouth open and remain still, letting the saliva drip into a disposable cup held to the lower lip for 15 minutes. In Study I, the volume was measured with a 3-ml syringe marked in increments of 0.1 mL. In Study II, the unstimulated whole saliva flow rate was determined by gravitation, presuming that 1 g of saliva is equivalent to 1 mL.

In Study I, the saliva collections were performed between 7 and 9.30 in the morning. In Study II, saliva was collected on two different days, with an interval of one or three days between the tests. On the first occasion, saliva was collected at 9.30 am. On the second day, two tests were performed, with an interval of four hours, at 7.30 and 11.30 am. The subjects were instructed not to eat, drink, or use any form of tobacco for one (Study I) or two hours (Study II) before each separate test.

### **3.4 BLOOD SAMPLING AND ANALYSES (STUDY I)**

Two blood samples were collected the same morning as the saliva test. The first blood sample was analyzed directly to determine the level of C-reactive protein (CRP) in order to detect inflammation or infection, which may elevate serum ferritin (S-f) levels (Birgegård *et al.*, 1978). A CRP level of > 5 mg/L excluded the individual from participating in the study. This occurred in two individuals, one from each group.

The second blood sample was immediately frozen and stored. After all the patients in both groups had undergone saliva testing, the second blood samples were analyzed to determine serum ferritin. All the samples were analyzed on the same day using the same reagent batch.

The reference values chosen in the present study were for very low S-f:  $\leq 15$   $\mu\text{g/L}$  (Hallberg and Rossander-Hulthén, 1989), low S-f: 16 - 30  $\mu\text{g/L}$  (females) or 16-

50 µg/L (males), and normal S-f: > 30 µg/L (females) or > 50 µg/L (males) (Jacobs, 1985; Osaki *et al.*, 1999).

### **3.5 SUBJECTIVE EVALUATION OF SALIVATION (STUDY II)**

All the participants assessed six variables related to their perception of the function of the salivary glands using Visual Analog Scales (VAS) before each saliva collection.

The subjects were asked to mark their responses to each item by placing a vertical line on a 100-mm horizontal scale. The six VAS items used were as follows:

1. Difficulty speaking due to oral dryness
2. Difficulty swallowing due to oral dryness
3. Amount of saliva in the mouth
4. Dryness of the throat
5. Dryness of the lips
6. Consistency of saliva

## **4 STATISTICS**

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS for Windows).

### **4.1 STUDY I**

The chi-square ( $\chi^2$ ) test was used for categorical variables. For continuous variables, the differences between the patients and their matched controls were calculated and the difference from zero was tested using the paired *t*-test. The correlation between unstimulated whole saliva flow rate and serum ferritin was tested with Pearson's correlation. The level of statistical significance (p-value) is shown if  $p < 0.05$ ; otherwise it is denoted as not significant (NS).

### **4.2 STUDY II**

Intraindividual differences in unstimulated whole saliva flow rate and VAS assessments at 7.30 and 11.30 am were tested using Wilcoxon's signed-rank test and differences between groups were tested using the Kruskal-Wallis test. The agreement between the unstimulated whole saliva flow rate at 7.30 and 9.30 and between 7.30 and 11.30 am was assessed using a kappa ( $\kappa$ ) test.

## 5 RESULTS

### 5.1 STUDY I

#### 5.1.1 Dental caries

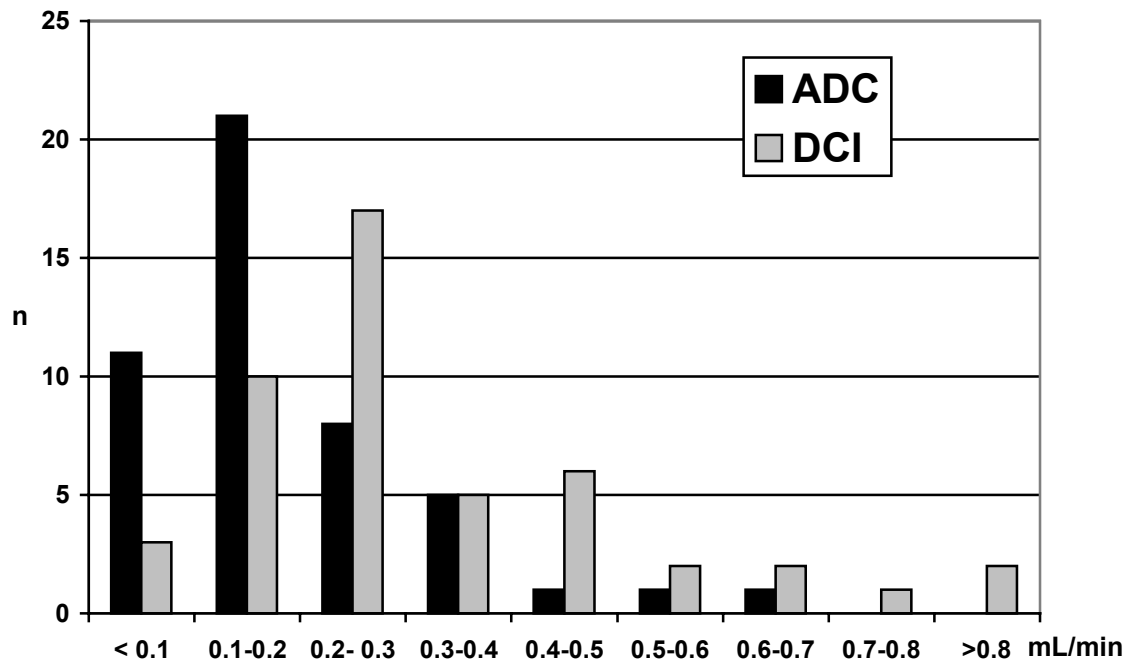
The DMF-T and DMF-S in both groups are shown in Table 1. The mean numbers of teeth with new manifest dental caries (D-T) in the Active Dental Caries group was 3.2 (SD 2.3), with no significant difference between females and males.

**Table 1.** Dental caries experience in the two study groups (n=48 in each group), the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group. Caries experience is expressed by decayed, missing and filled teeth (DMF-T) and decayed, missing and filled surfaces (DMF-S).

	ADC	DCI	
	Mean (SD)	Mean (SD)	p value
DMF-T	12.5 (4.3)	5.3 (4.1)	$p < 0.001$
DMF-S	28.2 (15.1)	6.6 (6.5)	$p < 0.001$

#### 5.1.2 Unstimulated whole saliva flow rate

The distributions of unstimulated whole saliva flow rates were compared between the ADC and DCI groups (Figure 1). The frequency of very low and low levels of unstimulated whole saliva flow rate was compared with the frequency of normal levels in the two groups. In the ADC group, 32 individuals (67%) had a very low or low unstimulated whole saliva flow rate compared with 13 individuals (27%) in the DCI group. This difference was statistically-significant ( $p < 0.001$ ). There was a statistically significant difference for females ( $p < 0.001$ ) but not for males when comparing the frequencies of very low and low levels between the ADC and DCI groups.



**Figure 1.** Distribution of unstimulated whole saliva flow rates in the two study groups, the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group.

The unstimulated whole saliva flow rates differed statistically between the two groups. This difference was true for females but not for males (Table 2).

**Table 2.** Mean values of unstimulated whole saliva (UWS), mL/min, according to gender in the two study groups, the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group.

		<b>ADC</b>	<b>DCI</b>	
	n	Mean (SD)	Mean (SD)	p value
Females	30	0.19 (0.11)	0.35 (0.21)	$p < 0.01$
Males	18	0.21 (0.16)	0.32 (0.28)	NS
Total	48	0.20 (0.13)	0.33 (0.24)	$p < 0.01$

NS = not significant



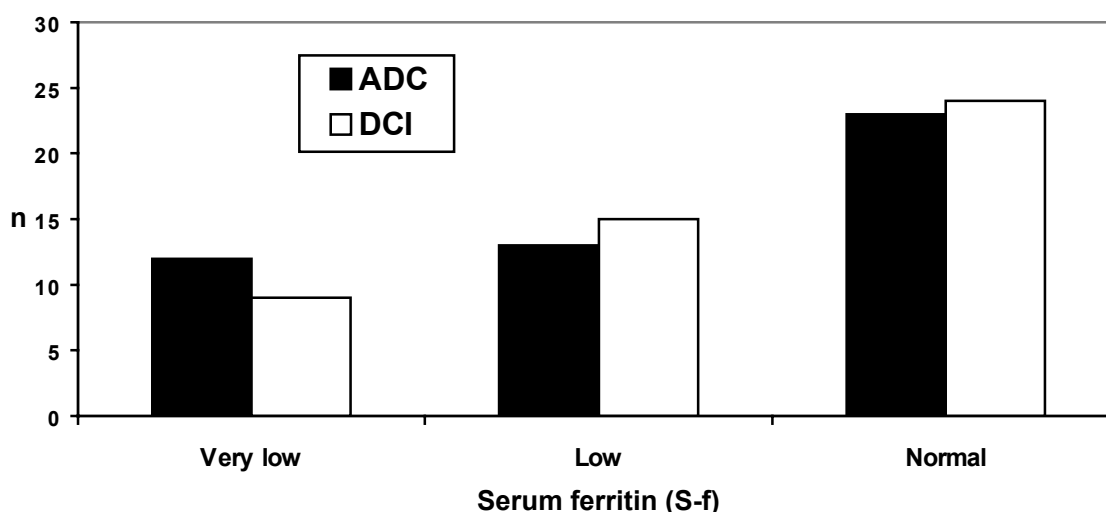
### 5.1.3 Serum ferritin

The mean serum ferritin values were lower in the ADC group, but the differences between the two groups were not statistically significant (Table 3). The frequency of very low ( $\leq 15 \mu\text{g/L}$ ) and low ( $16\text{-}30 \mu\text{g/L}$  for females and  $16\text{-}50 \mu\text{g/L}$  for males) values of serum ferritin was compared with the frequency of normal levels ( $> 30 \mu\text{g/L}$  for females and  $> 50 \mu\text{g/L}$  for males) in the two groups (Figure 2). Nor was there any statistically-significant difference between the two groups in the numbers of individuals with very low, low and normal serum ferritin levels. The subjects with very low serum ferritin levels were all women. No significant correlation was found between serum ferritin and unstimulated whole saliva flow rate.

**Table 3.** Mean values of serum ferritin (S-f),  $\mu\text{g/L}$ , according to gender in the two study groups, the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group.

	n	ADC Mean (SD)	DCI Mean (SD)	p value
Females	30	29.4 (24.9)	30.9 (20.2)	NS
Males	18	67.3 (31.5)	82.6 (55.3)	NS
Total	48	43.6 (32.9)	50.3 (44.7)	NS

NS = not significant

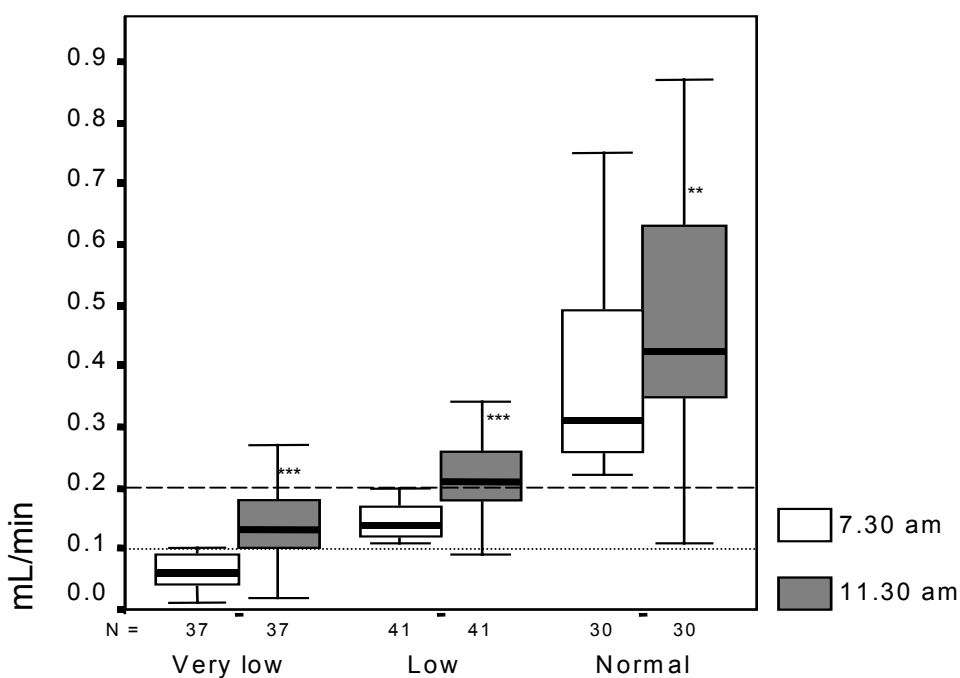


**Figure 2.** Distribution of serum ferritin (S-f) values in the two study groups, the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group. Values are expressed as very low ( $\leq 15 \mu\text{g/L}$ ), low ( $16\text{-}30 \mu\text{g/L}$  for females and  $16\text{-}50 \mu\text{g/L}$  for males) and normal ( $>30 \mu\text{g/L}$  for females and  $>50 \mu\text{g/L}$  for males).

## 5.2 STUDY II

### 5.2.1 Unstimulated whole saliva flow rate

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 am (Figure 3). There were no significant differences in the size of the increase in unstimulated whole saliva flow rate between the three groups. The mean difference and confidence interval at 9.30 and 11.30 am for the three groups are shown in Table 4. The mean unstimulated whole saliva flow rate from the first collection at 9.30 am was 0.12 (SD 0.07), 0.19 (SD 0.08) and 0.35 (SD 0.18) mL/min for the very low, low and normal groups respectively.



**Figure 3.** The unstimulated whole saliva flow rate at 7.30 and 11.30 am for the three different groups: very low ( $\leq 0.1$  mL/min), low (0.1-0.2 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. \*\*\* =  $p < 0.001$ , \*\* =  $p < 0.01$

**Table 4.** The difference ( $\Delta$ ) in unstimulated whole saliva flow rate (UWSFR) between saliva collection at 7.30, 9.30<sup>a</sup> and 11.30 am. The subjects were divided into three groups (very low (< 0.1 mL/min), low (0.11-0.2 mL/min) and normal (>0.2 mL/min) flow rates), based on the saliva collection at 7.30 am.

UWSFR at 7.30	Time	n	$\Delta$ mL (mean(SD))	95% CI	p value
Very low	9.30 <sup>a</sup>	37	0.057 (0.061)	0.038-0.079	$p < 0.001$
	11.30	37	0.094 (0.091)	0.064-0.126	$p < 0.001$
Low	9.30 <sup>a</sup>	40 <sup>b</sup>	0.051 (0.088)	0.022-0.077	$p < 0.01$
	11.30	41	0.085 (0.108)	0.050-0.119	$p < 0.001$
Normal	9.30 <sup>a</sup>	19 <sup>b</sup>	0.052 (0.154)	-0.122-0.027	NS
	11.30	30	0.081 (0.113)	0.045-0.125	$p < 0.01$

<sup>a</sup> = The UWSFR at 9.30 am was not tested on the same day as the test at 7.30 am.

<sup>b</sup> = Twelve participants did not test their UWSFR at 9.30 am.

There was disagreement between the unstimulated whole saliva flow rates at 7.30 and at 11.30 am ( $\kappa = 0.28$ ): the frequency of normal unstimulated whole saliva flow rate was twice as high at 11.30 am compared with 7.30 am and the frequency of the individuals with a very low unstimulated whole saliva flow rate, 70%, shifted to the low or normal groups (Table 5). When comparing the unstimulated whole saliva flow rate at 7.30 and at 9.30 the kappa value was 0.37 and 46% shifted from the very low group to the low or normal groups.

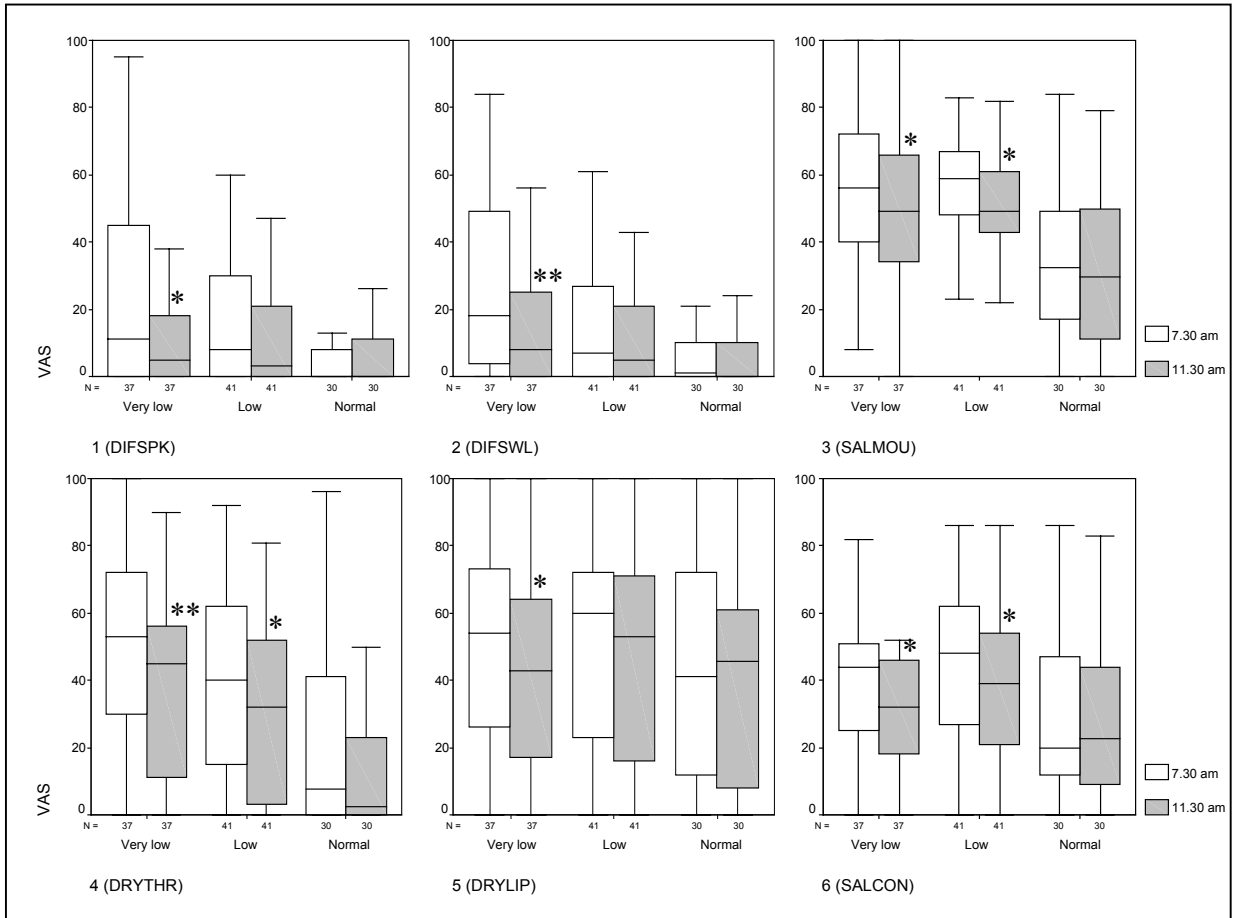
**Table 5.** The number of individuals with very low (< 0.1 mL/min), low (0.11-0.2 mL/min) and normal (>0.2 mL/min) flow rates (UWSFR) at 7.30 and 11.30 am. The agreement between the tests at 7.30 and 11.30 am was tested by means of kappa ( $\kappa$ ).

	UWSFR at 7.30 am	UWSFR at 11.30 am		
		Very low	Low	Normal
Very low	37	11	17	9
Low	41	2	16	23
Normal	30	0	2	28
Total	108	13	35	60

$\kappa = 0.28$

### **5.2.2 Subjective evaluation of salivation**

The median VAS scores for salivary function at 7.30 am were significantly higher compared with the scores at 11.30 am for all six items in the very low group, for three items in the low group and for none of the items in the normal group (Figure 4). At the 7.30 am saliva test, there were significant differences between groups for four of the six items. For items 3 (SALMOU) and 4 (DRYTHR), differences were found between the normal group and both the very low group and the low group ( $p < 0.001$ ). For item 2 (DIFSWL), there was a difference between the normal and the very low group ( $p < 0.01$ ) and, for item 6 (SALCON), a difference was found between the normal group and the low group ( $p < 0.05$ ). At the 11.30 am saliva test, the same differences were still found for items 2 (DIFSWL), 3 (SALMOU) and 4 (DRYTHR).



**Figure 4.** The perceived function of the salivary glands registered by six VAS items at 7.30 and 11.30 am 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. Statistically significant changes \* =  $p < 0.05$ , \*\* =  $p < 0.01$ .

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)

## 6 DISCUSSION

### 6.1 UNSTIMULATED WHOLE SALIVA AND DENTAL CARIES (STUDY I)

In reviews of earlier studies, it has not been possible to demonstrate a clear association between salivary flow and active dental caries, except when the saliva flow rate is very low (Mandel, 1974; Sreebny, 1983; Leone and Oppenheim, 2001). In the present study, there was a difference in unstimulated saliva flow rate between the caries-active group and the caries-inactive group for all participants and also for females. However, the difference between males, when comparing the two groups, was not significant. One explanation of this may be that the number of men included in the study, 18 in each group, was too few and, combined with a large variation, this resulted in insufficient statistical power. Leone and Oppenheim (2001) pointed out that a lack of statistical power is a common problem in studies relating salivary flow to dental caries.

The significant difference in unstimulated whole saliva flow rate found between individuals with active dental caries compared with those with inactive caries supports the idea that unstimulated whole saliva might serve as a factor in caries risk prediction. There may be reasons for recommending a more extensive use of unstimulated whole saliva flow rate in the clinical examination of active dental caries patients, a view also expressed by others (Dawes, 1996; Sreebny, 1996). The unstimulated whole saliva flow rate test is a simple and probably cost-effective complement in the prediction of dental caries and in helping the clinician to choose a prophylactic regimen. Furthermore, the extended use of unstimulated whole saliva flow rate tests would probably also lead to the earlier detection of hyposalivation and thereby in many cases the early detection of underlying diseases.

The long period of caries activity found in the active caries group supports the findings in other studies that one of the best predictors of caries risk is previous caries experience (Verdonschot *et al.*, 1999; Stenlund *et al.*, 2002), and it also indicates that conventional preventive therapies are not effective enough (Hausen *et al.*, 2000). Caries-active individuals continue to be caries active in spite of periods of intensified preventive measures. This corresponds with the insufficient evidence found in a systematic review of caries prevention programs for high-risk groups (Axelsson *et al.*, 2002).

Caries is regarded as a multifactorial disease, but the frequent intake of fermentable carbohydrates has been suggested as the principal cause (Featherstone, 2004). It is tempting to speculate that a cariogenic diet (in the sense of frequent intake of fermentable carbohydrates) is also low in essential nutrients. Insufficient intake of food and thus insufficient nutrition to the salivary glands has been shown to be associated with hyposalivation (Johansson *et al.*, 1992; Öhrn *et al.*, 1999). A cariogenic diet may thus affect the caries process in two ways: directly as substrate for bacterial acid production and indirectly by reducing the saliva flow.

## **6.2 SERUM FERRITIN (STUDY I)**

Serum ferritin levels vary over a wide range. In general, men have higher serum ferritin concentrations than women; the mean values are approximately 100 µg/L for men and 50 µg/L for women (for a review, see Jacobs, 1985). Depleted iron stores have been defined as a serum ferritin concentration of less than 15 µg/L in both genders (Hallberg and Rossander-Hulthén, 1989) and this value was therefore chosen as the limit for “very low” serum ferritin levels. The limit for “low” serum ferritin used in Study I was based on findings in a recent study, in which it was found that the majority of patients with a suspected “latent” iron deficiency had serum ferritin values of less than 30 µg/L for women (Osaki *et al.*, 1999) and less than 50 µg/L for males. Values above these values were registered as “normal”.

The mean serum ferritin values found in Study I were somewhat lower than those found in other studies (Jacobs, 1985). In the present study only women had very low values and the frequency of very low values was only slightly lower compared with other studies of women in Nordic countries (Hallberg and Rossander-Hulthén, 1989).

Osaki *et al.* (1999) demonstrated that iron supplementation increased saliva secretion from 1.6 mL/10 minutes (SD 1.3) to 2.4 mL/10 minutes (SD 1.8) after two months of treatment ( $p < 0.05$ ). However, this finding was contradicted by the absence of a significant correlation between unstimulated whole saliva flow rate and serum ferritin found in Study I. If the results presented by Osaki *et al.* (1999) are reproducible, iron supplementation could be a possible way of treating hyposalivation, especially in women. So far, no controlled clinical study has been performed with the aim of answering this question. As almost no causal treatment of hyposalivation is currently available, studies of the effects of iron supplementation may be of importance.

### 6.3 UNSTIMULATED WHOLE SALIVA FLOW RATE (STUDY I+II)

There is no generally accepted methodology for assessing the unstimulated whole saliva flow rate. However, the reliability and validity of saliva flow rate measurements are of great importance for the diagnosis of some oral and general diseases. Factors that may introduce errors in the measurements therefore need to be controlled. An accurate diagnosis of hyposalivation is especially important in relation to the diagnosis of Sjögren's syndrome, where a limit of 0.1 mL saliva/min is used (Pedersen and Nauntofte, 2001). An erroneous measurement may have an adverse effect on the medical care of the individual. Furthermore, it has been suggested that measurements of saliva flow rate are important when assessing the risk of caries (Dawes, 1996; Nauntofte *et al.*, 2003).

The unstimulated whole saliva flow rates and means in Study I were slightly lower than those reported in other studies (Heintze *et al.*, 1983; Percival *et al.*, 1994). One possible reason for this difference is the time point during the day at which the saliva was collected. This is a factor of importance, as the unstimulated whole saliva flow rate displays a circadian rhythm, with the peak flow rate occurring in the late afternoon and the lowest flow rate in the early morning (Dawes, 1974; Rantonen and Meurman, 1998). In the present study (Study I), the median time for testing was 8 am. When these results are compared with those in studies in which testing was carried out between 9 and 11 am (Heintze *et al.*, 1983), the mean unstimulated whole saliva flow rates found in our study were lower.

The results of Study I were the reason for the aim of Study II, which was to investigate whether individuals with hyposalivation show an increase in their unstimulated whole saliva flow rate when tested at two different, well-defined time points separated by four hours. The results revealed a significant increase in unstimulated whole saliva flow rate for the group with an unstimulated whole saliva flow rate below 0.1 mL/min, as well as for the two groups with an unstimulated whole saliva flow rate above 0.1 mL/min. Our findings are in agreement with those of previous studies of individuals with a normal unstimulated whole saliva flow rate (Nederfors and Dahlöf, 1992; Rantonen and Meurman, 1998) and show that a circadian rhythm may also be found in individuals with hyposalivation.

In a study of individuals with a diagnosis of rheumatoid arthritis or fibromyalgia, differences in unstimulated whole saliva flow rate were found between saliva collected in the morning and at lunchtime (Nederfors *et al.*, 2002). The authors



explained this difference as being caused by the fasting condition before the test, something that also applied in Study II, which revealed similar increases in the unstimulated whole saliva flow rate. It is difficult at this stage to determine whether the increase is related to fasting or circadian rhythm alone or to a combination of the two factors. Nederfors *et al.* (2002) concluded that the fasting before the saliva test was important when using saliva as a diagnostic tool. However, to ensure the lowest unstimulated whole saliva flow rate for an individual when investigating salivary gland hypofunction, early morning and fasting unstimulated whole saliva flow rate tests might have to be recommended until the factors that are involved are better understood.

The large number of subjects displaying hyposalivation at 7.30 am but not at 11.30 am underlines the importance of and need for better definitions of both limit values and diagnostic procedures. From Table 5, it can be seen that about 70% would have been incorrectly diagnosed if the saliva tests had been performed around noon rather than in the morning. This may have an adverse effect on the management of oral diseases in a specific patient. However, it is impossible to determine from this study (limited to only 2-3 saliva tests) which time interval is the best for performing a test of hyposalivation. There is a need for further studies in this field in order to optimize care in diseases such as Sjögren's syndrome or dental caries.

Studies of unstimulated whole saliva flow rate do not always state the time of day at which saliva has been collected. In some studies, the time intervals at which saliva has been sampled have been wide, 2–6 hours (Heintze *et al.*, 1983; Bergdahl, 2000; Bardow *et al.*, 2001), and studies performed before 9 am are rare (Österberg *et al.*, 1984). The influence of the circadian rhythm or fasting on these results is therefore difficult to assess. For future studies, as well as for the clinical use of unstimulated whole saliva flow rate tests, more specific recommendations about the best time interval for performing the test are needed. Our data indicate that the limit for a very low unstimulated whole saliva flow rate is exceeded close to 9 am for the subjects in the very low group, assuming a linear increase. Furthermore, a time interval of two hours will result in a difference in unstimulated whole saliva flow rate of about 0.04-0.05 mL/min between the earliest and the latest test, due to the circadian rhythm (Table 4).

#### 6.4 SUBJECTIVE EVALUATION OF SALIVATION (STUDY II)

The increase in salivary flow between 7.30 am and 11.30 am was clearly perceived by the *very low group*, less clearly perceived by the *low group* and not at all perceived by the *normal group* (Figure 4), in spite of the fact that the increase in unstimulated whole saliva flow rate expressed in mL/min was similar in all three groups. These results could be interpreted as indicating that individuals with hyposalivation perceive an increase in saliva flow rate more clearly than those with a normal unstimulated whole saliva flow rate. One explanation of this may be that a lower unstimulated whole saliva flow rate is associated with a lower thickness of saliva covering the mucosa (Wolff and Kleinberg, 1998; Wolff and Kleinberg, 1999). Consequently, at a certain flow rate level, there is not enough saliva to wet the various oral surfaces. It has been shown that dryness symptoms become evident when hyposalivation is below 0.1 –0.2 mL/min (Wolff and Kleinberg, 1998), which is in agreement with our findings and also those of others (Wang *et al.*, 1998). In our study, the *very low*, *low* and *normal* groups displayed an increase in unstimulated whole saliva flow rate of 149%, 58% and 20% respectively. The large differences in these increases, when expressed in percent, might be an alternative explanation of the differences found in people's perception of the increase in flow rate. This is in accordance with the suggestion that a saliva flow rate reduction of approximately 40-50% results in symptoms of dry mouth (Dawes, 1987).

The significant differences in the perceived oral dryness between individuals with hyposalivation and individuals with normal saliva secretion might indicate that the present limits for hyposalivation (*very low*  $\leq 0.1$  mL/min and *low* = 0.1-0.2 mL/min) are relevant in relation to subjective symptoms of dry mouth.

## 7 CONCLUSIONS

- A low unstimulated whole saliva flow rate is related to caries activity in women (Study I). This strengthens the view that the unstimulated whole saliva flow rate test could be used in the prediction, prevention and treatment of caries.
- Serum ferritin appears not to be related to caries activity or to the unstimulated whole saliva flow rate (Study I).
- Unstimulated salivary flow rate tests should be performed in a narrow time interval early in the morning in order to obtain an accurate diagnosis of hyposalivation (Study II).
- Individuals with salivary gland hypofunction perceive an increase in saliva flow rate more clearly than those with normal unstimulated whole saliva flow rate (Study II).

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