Hyposalivation and iron stores among individuals with and without active dental caries

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Flink H, Tegelberg Å, Sörensen S. Hyposalivation and iron stores among individuals with and without active dental caries. Acta Odontol Scand 2000;58:265–271. Oslo. ISSN 0001-6357.

The aims of the study were to investigate frequencies of low unstimulated whole saliva (UWS) levels and low serum ferritin (S-f) levels among individuals with active dental caries (ADC) and dental caries inactive (DCI) individuals and to compare the relationship between UWS and S-f levels. In this descriptive study, 48 ADC patients and 48 DCI individuals were compared. The two groups were matched regarding age and sex (30 females and 18 males in each group, age range 15–40 years). In the ADC group, 32 individuals (67%) had low ($\leq$0.20 ml/min) UWS levels compared with 13 individuals (27%) in the DCI group. This difference was statistically significant ($P < 0.001$). The mean values of UWS were significantly lower in the ADC group compared to the DCI group (mean ml/min $\pm$ SD) 0.20 ± 0.13 and 0.33 ± 0.24, respectively ($P = 0.002$). There were significant differences for females but not for males when comparing frequencies of low UWS levels ($P < 0.001$) and mean UWS levels ($P = 0.002$). There was no difference in S-f levels between the two groups. Neither was any correlation between UWS and S-f found. In conclusion, the significant negative relationship found between UWS and ADC indicates that a suppressed defense for dental caries activity could play a more important role in ADC than previously presumed, especially among females. The absence of a correlation between UWS and S-f might indicate that saliva secretion will not be stimulated by iron supplementation. □ *dental caries; iron deficiency; serum ferritin; unstimulated whole saliva; xerostomia*

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There are several symptoms associated with xerostomia (subjective symptoms of dry mouth) and hyposalivation (an objective sign of salivary gland dysfunction evaluated by means of sialometry) (1); one symptom is dental caries. Almost no treatable cause of hyposalivation is known today. The main aim of different treatment modalities is therefore limited to reducing symptoms (2, 3).

A relationship between low iron levels and hyposalivation has been mentioned in some older articles. In 1943, Faber reported that treatment of iron-deficient patients usually resulted in an elimination of symptoms, including hyposalivation (4). Blood examinations related to salivary secretion values by Bertram indicated that low serum iron and reduced salivary secretion might be expressions of the same basal pathologic process (5). One recent study has indicated increased salivation in iron-deficient patients after treatment with iron (6).

On comparing separate studies of hyposalivation and xerostomia with studies of iron deficiency, there are some similarities. Oral symptoms such as glossitis, angular chielitis, stomatitis, dysphagia, and candida infections are all mentioned in studies of patients with xerostomia or hyposalivation as well as in studies of patients with iron deficiency (3, 7, 8). Several studies have shown that xerostomia and hyposalivation are common in the population (20–25%), even among young healthy individuals (9, 10). Twice as many women as men suffer from xerostomia or hyposalivation (9). This might correspond with the fact that iron deficiency is the only remaining nutritional deficiency of any importance in industrial countries, mainly among women (11).

Small iron stores are not unusual in growing children (12). During childhood there are two major periods of growth (13), these periods correspond well with increased caries activity (14). After the last period of growth there is a steady rise to adult iron levels in boys but not for girls. This difference for women remains throughout childbearing age (15). Adolescent girls and women develop dental caries more often than boys and men, even though females have better oral hygiene (16). Treatments with iron supplements have given significantly reduced dental caries activity in animal studies (17).

These background facts made us interested in studying whether or not there is a relationship between iron levels and salivary secretion and if low levels of these factors might increase the risk for dental caries. In this study we have chosen variables that are known to show early signs of hyposalivation and iron deficiency. These are unstimulated whole saliva (UWS) (9, 18) and iron stores, serum ferritin (S-f) (11, 19). The aims of the study were to evaluate the frequencies of low UWS levels and low S-f levels among individuals with and without dental caries activity and to compare the relationship between UWS and S-f levels.

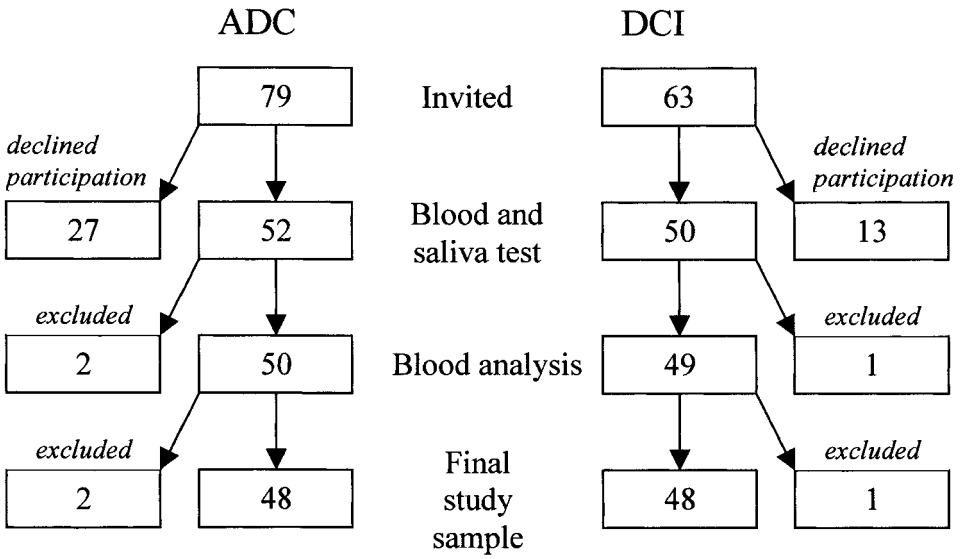

Fig. 1. The number of individuals who were invited, declined participation, underwent testing, were excluded, and finally were included in the two study groups, the Active Dental Caries (ADC) and the Dental Caries Inactive (DCI).

Materials and methods

Subjects

Active dental caries (ADC) group. During 1998 and 1999, patients between the ages of 15 and 40 years who had active dental caries (ADC) and visited the Public Dental Clinic in Sala, Sweden, for their regular dental examination were asked to participate in the study. The clinic, comprising 8 dentists, examines and treats approximately 3000 patients in these age groups regularly (by an individual recall system). Previous epidemiological records from this clinic had shown that about 30% of the patients in these age groups had had manifest dental caries and 10% of the patients had had two or more cavities yearly.

Pregnant patients and patients who were taking any medication with known negative effects on saliva secretion were excluded. Seventy-nine patients were invited to participate in the study. Among these, 52 were tested and 27 declined participating (10 men and 17 women).

Dental caries inactive (DCI) group. After selection and testing of the ADC group, a corresponding group of patients matched regarding sex and age, but dental caries inactive (DCI), was selected. The DCI group was used as a control group. The same exclusion criteria as in the ADC group were used in this group. Sixty-three patients were invited to participate in the study. Among these, 50 patients were tested and 13 did not want to take part in the study (5 men and 8 women). In both groups, the most common reasons for not participating were difficulties in getting time off from work for testing or fear of the needle used in the blood testing.

The numbers of individuals who were invited, declined participation, underwent testing, were excluded, and finally were included, are shown in Fig. 1. Among the 48 in the final study sample, 30 were female and 18 male. Mean ages were (mean years $\pm$ SD) 28.4 ± 7.0 for the ADC group and 29.3 ± 7.9 for the DCI group. The male groups were younger (25.2 ± 7.1 and 25.6 ± 7.6 for the ADC and the DCI groups, respectively) than the female groups (30.3 ± 6.4 and 31.6 ± 7.3, respectively).

Exclusions

After testing, two patients in the ADC group were excluded; one because of incorrect ADC registrations and the other because of pregnancy. One patient in the DCI group was excluded because of medication, for an acute allergic reaction that was taken the day before testing. Three patients were excluded after blood analysis, one patient in both groups because of high human C-reactive protein (CRP) levels and one patient in the ADC group because the blood sample was missing.

Ethics

The ethics committee at the Faculty of Medicine at Uppsala University approved the study. All participants received verbal and written information on the purpose and the content of the study. Participation was voluntary.

Methods

Dental caries. The dental caries activity was judged by clinical and radiographic examination (bite-wings). The

Table 1. Dental caries prevalence in the two study groups: the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group. Prevalence is expressed by decayed, missing, and filled teeth (DMF-T) and decayed, missing, and filled surfaces (DMF-S).

		N	ADC group	DCI group
DMF-T	Female	30	13.7 ± 4.0	6.0 ± 4.2
Score	Male	18	10.5 ± 4.1	3.9 ± 3.8
(0–28)	Total	48	12.5 ± 4.3	5.3 ± 4.1
DMF-S	Female	30	32.2 ± 15.6	8.1 ± 7.2
Score	Male	18	21.6 ± 11.8	4.3 ± 4.4
(0–128)	Total	48	28.2 ± 15.1	6.6 ± 6.5

patient's regular dentist performed the examination. The inclusion criteria used for the ADC group were the development of manifest dental caries in 2 or more teeth since the last examination or the development of manifest dental caries in 1 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 number of dental caries inactive years was counted from the time the patient had had manifest dental caries. This was done by consulting the records of 44 of the patients and by relying on estimations made by 4 of the patients (members of the dental staff) in the final study sample.

In the ADC group, the number of years with ADC was measured from the time the patients had been free from manifest dental caries for more than 3 years. This was possible to trace in the records of 43 of the final 48 patients. The other five patients (not completely traceable) were traced back as far as possible; all of them reported that they had experienced recurrent ADC for many years prior to the first journal registration. To describe the difference between the two groups in dental caries prevalence, decayed, missing and filled teeth (DMF-T) and decayed, missing and filled surfaces (DMF-S) were recorded in all patients. The caries incidence in the ADC group was determined by registering the number of teeth with new manifest dental caries since the last examination (D-T).

Saliva tests. Unstimulated whole saliva (UWS) was collected by asking the patient to drool for 15 min. This registration was made sometime between 7.00 and 9.30 a.m. The patients were instructed not to eat, drink, or use any form of tobacco 1 h before the test and to relax for a couple of minutes before the test. They sat, bent forward, in an ordinary chair during saliva collection. They were told to place the tongue on the lingual surfaces of the upper incisors, keep their mouth open, and remain still, letting the saliva drip into a cup held up to the lower lip. The volume was measured with a 3-ml syringe marked in increments of 0.1 ml. A trained and experienced dental nurse supervised the saliva tests.

The frequencies of very low (≤0.10 ml/min) and low (0.11–0.20 ml/min) UWS levels were compared between the two groups. It took 13 months to test both groups of patients. The majority of the ADC group were tested in the colder months (November–March), and of the DCI group in the warmer months (April–September).

Blood sampling and analyses. Two blood samples were collected (the same morning) at a clinical laboratory after the saliva tests. Venous blood was taken from the patient's arm. The first blood sample was analyzed at the time of the saliva test to determine the level of CRP. The instrument, Cobas® Mira, and the reagents Unimate 3 CRP from Roche® were used in an immunoturbidimetric method. A CRP level >5 mg/l would exclude the individual from the study. The CRP analysis in this study was made to detect inflammations or infections, which can elevate S-f levels (20).

The second blood sample was immediately frozen (−70°C) and stored. After all the patients in both groups had undergone saliva testing, the second blood samples were analyzed to determine S-f levels. An IMX immunoanalyzer (Abbott Laboratories, Chicago, USA) and the MEIA (microparticle enzyme immunoanalyzer) method were used. All samples were analyzed on the same day using the same reagent batch to maximize the correspondence between the S-f levels. The frequencies of very low (≤15 μg/l) and low (15–30 μg/l for female and 15–50 μg/l for male) S-f levels were compared between the two groups. Both CRP and S-f assays were performed according to the manufacturer's instructions.

Postponement of testing. All participants were requested to postpone the date of testing at least 3 weeks if they were having or had recently had any kind of general infection or inflammation.

Statistics

The chi-squared test was used for categorical variables. For continuous variables, the differences between the patients and their controls were calculated and the difference from value = 0 was tested using the *t*-test. The power of correlation between UWS and S-f was tested with Pearson's correlation coefficient. The level of statistical significance (*P* value) is shown if $P < 0.05$; otherwise it is denoted as not significant (N.S.).

Results

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 ADC group was 3.2 ± 2.3 (mean ± SD), and there was no significant difference

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.

	ADC group		DCI group	
	N	(Mean UWS ± SD)	(Mean UWS ± SD)	Significance
Female	30	0.19 ± 0.11	0.35 ± 0.21	$P = 0.002$
Male	18	0.21 ± 0.16	0.32 ± 0.28	NS
Total	48	0.20 ± 0.13	0.33 ± 0.24	$P = 0.002$

between females and males. In the DCI group, the mean number of months free from dental caries was 129.8 ± 68.3 (mean ± SD). The corresponding mean number of months with dental caries activity in the ADC group was 155.1 ± 112.1 (mean ± SD). There were no significant gender differences when comparing these two variables.

Unstimulated whole saliva

The distributions of UWS values were compared between the ADC and DCI groups (Fig. 2). Frequencies of very low and low levels of UWS were compared with frequencies of normal levels in the two groups. In the ADC group, 32 individuals (67%) had very low or low UWS levels 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 frequencies of very low and low levels between the ADC and the DCI groups. The mean values for the groups showed statistically significant differences that were similar (Table 2). The number and frequencies of individuals tested before 8.00 a.m. and between 8.00 and 9.30 a.m. were compared between the two groups (Table 3). The median time for testing was 8.00 a.m. in both groups.

Serum ferritin. The distributions of S-f levels were compared between the ADC and DCI groups (Fig. 3). Frequencies of very low and low values of S-f were compared with frequencies of normal levels in the two groups. In both groups, only the women had very low S-f levels. The mean S-f values were lower in the ADC group but the differences between the two groups were not statistically significant (Table 4). Neither was there any statistically significant difference in the numbers of individuals with very low or low S-f levels between the two groups.

Relationship between unstimulated whole saliva and serum ferritin. A clearly negative relationship was found between UWS and ADC. No correlation, however, was found between S-f and ADC or between UWS and S-f in this study.

Table 3. Time of saliva test in the two study groups: the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group. Number and frequencies of individuals testing saliva before 8.00 a.m. or between 8.00 and 9.30 a.m. Mean values of unstimulated whole saliva (UWS), ml/min.

	ADC group			DCI group			
	N	(%)	(Mean UWS ± SD)	N	(%)	(Mean UWS ± SD)	Significance
Before 8.00 a.m.	20	(42)	0.15 ± 0.09	22	(46)	0.33 ± 0.20	$P = 0.001$
8.00–9.30 a.m.	28	(58)	0.23 ± 0.15	26	(54)	0.34 ± 0.27	NS
Total	48	(100)	0.20 ± 0.13	48	(100)	0.33 ± 0.24	$P = 0.001$

NS = not significant.

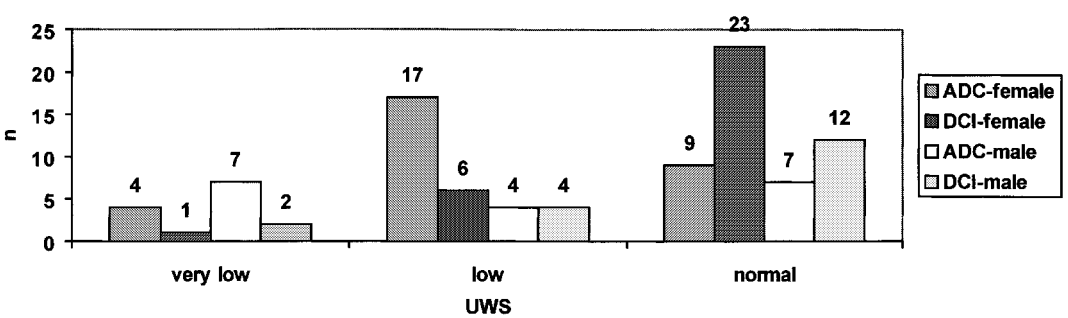

Fig. 2. Distribution of unstimulated whole saliva (UWS) values according to gender in the two study groups: the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group. Values are expressed as very low (≤0.11 ml/min), low (0.11–0.2 ml/min), or normal (> 0.2 ml/min).

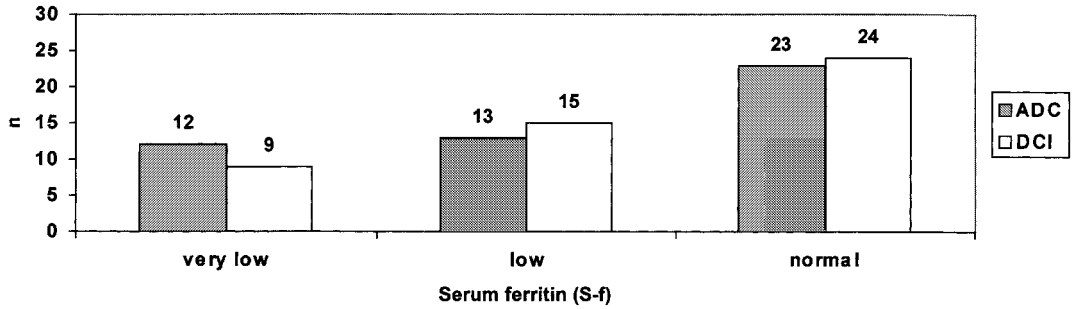

Fig. 3. Distribution of serum ferritin (S-f) values in the two study groups: the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group. Values are expressed as very low (≤15 μg/l), low (16–30 μg/l for female and 16–50 μg/l for male), and normal (>30 μg/l for female and >50 μg/l for male).

Discussion

Dental caries

In this study, the absence and presence of dental caries activity was compared. It was not the intention of the study to control important factors such as size and localization of caries, time between examinations, and prophylactic therapies. No comparisons between different levels of carious activity were made. The DCI and ADC groups were, however, clearly divided by prevalence (expressed by DMF-T and DMF-S), as well as the time free from ADC in the DCI group versus the time with dental caries activity in the ADC group. The dropouts from this study were similar in gender and age in both groups. The main reasons were job interference and anxiety for the needles used for blood sampling. It is our opinion that these factors do not influence the study results.

Unstimulated whole saliva

There is no uniform methodology for assessing UWS, but some factors of concern in the testing procedure are mentioned in other studies. Three factors most often mentioned are the time of day when the testing was performed, the length in time of the testing, and the method used to measure the collected saliva. The time of day testing was performed is discussed below. The length of the testing varied from 5 to 15 min. Dawes has proposed that the minimum time needed to collect a sufficient volume to accurately estimate flow rate is probably 5 min (21). Testing times between 5 and 10 min seem to be the most common (22–28). Our choice of a 15-min testing time is based on the clinical use of the UWS test in the diagnosis of Sjögrens's syndrome (29). Visual measurement of volume has probably been most used in the past, but gravitation methods are increasing (24, 26, 28) and should be used if possible as gravitation methods are more accurate. In clinical use, however, it is likely that the scales needed for these methods are too expensive. In the present study, we used a simplified method to measure volume, with tools (for flushing root canals) frequently found in an ordinary dental clinic. The accuracy of this method compared to weighing saliva has not yet been evaluated. Other factors mentioned include the sitting position (bent forward), the method of collection (drained, drooled, or dripped) and the instructions given to the patient (not to eat, drink or smoke 1–2 h before testing) (22, 25, 29). Occasionally, instructions to relax for 5 min before testing (22, 28) or to perform collection in a relaxed position or location are given (24, 25).

Table 4. Mean values of serum ferritin (S-f), μg/l, according to gender in the two study groups: the Active Dental Caries (ADC) group and the Dental Caries Inactive (DCI) group.

	N	ADC group (Mean S-f ± SD)	DCI group (Mean S-f ± SD)	Significance
Female	30	29.4 ± 24.9	30.9 ± 20.2	NS
Male	18	67.3 ± 31.5	82.6 ± 55.3	NS
Total	48	43.6 ± 32.9	50.3 ± 44.7	NS

NS = not significant.

The values we chose for very low and low UWS levels were those used by Sreebny et al. (9), which they named abnormal and low normal, respectively. The UWS values and means in this study were lower than those reported in other descriptive studies (22, 23). One possible reason for this difference is the time of day the test was performed. This is a factor of major importance in the UWS testing, as UWS has a circadian rhythm with high amplitudes: the peak flow rate occurs in the late afternoon and the lowest flow rate in the early morning (21). Significantly different values were found when one group of individuals was tested first between 7.30 and 8.30 a.m. and then later between 11.00 and 12.00 a.m. (24). The differences increase later in the afternoon (25). In the present study, the median time for testing in both groups was 8.00 a.m. When these results are compared with those in studies where testing was carried out between 9.00 and 11.00 a.m. (22), for example, the mean UWS values found in our study will most likely be lower. This study also found an increased difference in UWS levels between the ADC and the DCI groups when the testing time was early (before

8.00 a.m.). This could indicate that individuals with hyposalivation might be more sensitive to the circadian rhythm. However, it is important to evaluate this finding with caution, as this part of the study was not matched in gender and age. A proper study of circadian rhythm should also be intraindividual in design (the same group of individuals tested at different hours). The circadian rhythm is probably a part of the methodology that needs more attention and investigation.

A circannual rhythm of salivary flow has also been described. These changes have been attributed to dehydration, with minimum flow rates in summer and maximum flow rates in winter. Studies of this relationship have been made on subjects living in climate zones with very hot summer months (21). A recent study has indicated that this fluctuation in flow rates can occur in individuals living in more temperate climates. The variations in salivary flow rates were found to be inversely associated with ambient temperature (26). It is not clear how important the circannual rhythm is. If this factor has influenced the results of the present study, it probably would have narrowed the difference in UWS levels between the two groups since the majority of the ADC group were tested during winter (higher rates) and of the DCI group in the summer months (lower rates).

Relationship between unstimulated whole saliva and active dental caries

In reviews of earlier studies, it has not been possible to demonstrate a clear negative correlation between salivary flow and ADC in general. There is no doubt, however, that a severe impairment in salivary secretion results in a marked increase in the incidence of dental caries (30) and that significant negative correlations have been found between UWS and root surface caries (27, 28) as well as between UWS and DMF-S (23).

With this uncertain background, it is obvious that the significant negative relationship found in this study between UWS flow and ADC needs to be confirmed by further studies. If a negative relationship can be verified, it might indicate that a suppressed defense for caries activity could play a more important role in ADC than previously presumed. In the future, this could be a reason for recommending more extensive measurement of UWS in the clinical examination of ADC patients. The UWS test could be a simple and inexpensive complement to use in the prediction of dental caries and in the choice of prophylactic regime (31). Findings from this study indicate that the therapeutic methods used today to arrest ADC are ineffective, as dental caries activity is recurrent for many years in most of the patients tested.

The significant difference in UWS levels found in females but not in males when comparing the ADC and the DCI groups is difficult to explain, especially as more males (39%) had "very low" UWS values than females (13%) in the ADC group. More females (70%) with ADC were found to have hyposalivation (very low or low UWS values) than males (61%), while hypofunction was more depressed in affected males.

Serum ferritin

Normal S-f levels express a wide range of values (15–300 μg/l). In general, men have higher S-f concentrations than women; mean values are approximately 100 μg/l for men and 50 μg/l for women (19). Iron deficiency is best established by testing for an absence of stainable storage iron in the reticuloendothelial cells in bone marrow smears or a low level of S-f, <15 μg/l (11). In this study, this value was chosen for a very low limit. The definition of low S-f used in this study was based on findings by Osaki et al. (6), who found that the majority of the patients in their study had S-f values less than 30 μg/l and were suspected to have a "latent" iron deficiency (in this study 88% were females). The mean S-f values in the present study were lower in general than those of other studies (19). Only females had very low values, which agrees with findings in other studies. The frequencies of very low values were slightly higher in the ADC group, 40%, but identical in the DCI group, 30%, with those in other studies of women in Nordic countries (11).

Relationship between unstimulated whole saliva and S-ferritin

The absence of correlation between UWS and S-f in this study raises doubts about whether saliva secretion can be increased by iron supplementation. However, if the results presented recently by Osaki et al. (6), which indicate that this is possible, are reproducible, other explanations for the absence of correlation must be found. Multiple deficiencies of different micronutrients are almost always involved in severe deficiencies. If this is also the case in minor deficiencies, there may be other factors affecting the S-f values, which could explain the absence of correlation. For example, one known factor that has a close relationship to iron balance is vitamin B_{12}. In untreated megaloblastic anemia, S-f levels are elevated and fall after vitamin B_{12} therapy (32). This speculation is of interest only if it is possible to increase saliva secretion by iron treatment. So far we have found no controlled study that has clarified whether or not this is possible. As almost no causal treatments of hyposalivation exist today, further studies are important.

Conclusions

In conclusion, the significant negative relationship found between UWS flow and ADC indicates that a suppressed defense for caries activity could play a more important role in ADC than previously presumed, especially among females. No correlation was found between UWS and S-f in this study, which might indicate that saliva secretion will not be stimulated by iron supplementation. However, as

no controlled study has clarified whether or not this is possible, further studies are needed.

Acknowledgements.—This project was supported by grants from the "Colgate Research Fund", Västmanland County, Sweden; the Swedish Patent Revenue Research Fund; and the Swedish Dental Society.

References

1. Nederfors T. Xerostomia: Prevalence and pharmacotherapy, with special reference to β-adrenoceptor antagonists. Swed Dent J 1996;Suppl 116:18.
2. Council on Dental Therapeutics. Consensus: Oral health effects of products that increase salivary flow rate. JADA 1988;116:757–9.
3. Sreebny LM. Working group 10 of the Commission on Oral Health, Research and Epidemiology (CORE), FDI 1991. Saliva: Its role in health and disease. Int Dent J 1992;42:291–304.
4. Faber M. Xerostomi. En ofte overset lidelse. Tandlægebladet 1943;47:177–90 (Danish).
5. Bertram U. Xerostomia: Clinical aspects, pathology and pathogenesis. Acta Odontol Scand 1967;25 Suppl 49:12–30.
6. Osaki T, Ueta E, Arisawa K, Kitamura Y, Matsugi N. The pathophysiology of glossal pain in patients with iron deficiency and anemia. Am J Med Sci 1999;318:324–9.
7. Fergusson MM. Oral mucous membrane markers of internal disease: Part II. In: Dolby AE, editor. Oral mucosa in health and disease. Oxford: Blackwell; 1975.
8. Fletcher J, et al. 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.
9. Sreebny LM, Valdini A. Xerostomia. Part I. Relationship to other oral symptoms and salivary gland hypofunction. Oral Surg Oral Med Oral Pathol 1988;66:451–8.
10. Nederfors T, Isaksson R, Mörnstad H, Dahlöf C. Prevalence of xerostomia in an adult Swedish population-relation to age, sex and pharmacotherapy. Community Dent Oral Epidemiol 1997; 25:211–6.
11. Hallberg L, Rossander-Hulthén L. Nutritional anaemia and iron deficiency. In: Somogyi JC, Hejda S, editors. Nutrition in the prevention of disease. Bibl Nutr Dieta. Basel: Karger; 1989. p. 94–105.
12. Siimes MA, Addiego JE, Dallman PR. Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. Blood 1974;43:581–90.
13. Björk A. Timing of interceptive orthodontic measures based on stages of maturation. Trans Eur Orthod Soc 1972. p. 61–74.
14. Massler M. Teenage cariology. Dent Clin N Am 1969;13:405–23.
15. Burman D. Iron metabolism in infancy and childhood. In: Jacobs A, Worwood M, editors. Iron in biochemistry and medicine. London: Academic Press; 1974. p. 543–62.
16. Stephen KW, Purdell-Lewis DJ. Behavioural aspects of oral hygiene. In: Embery G, Rølla G, editors. Clinical and biological aspects of dentifrices. Oxford Medical Publications; 1992. p. 13–20.
17. Miguel JC, Bowen WH, Pearson SK. Effects of iron salts in sucrose on dental caries and plaque in rats. Arch Oral Biol 1997;42:377–83.
18. Becks H, Wainwright WW. Human Saliva. IX. The effect of activation of salivary flow. J Dent Res 1939;18:447–56.
19. Jacobs A. Ferritin: an interim reviev. Curr Top Hematol 1985;5: 25–62.
20. Birgegård G, Hällgren R, Killander A, Strömberg A, Venge P, Wide L. Serum ferritin during infection. Scand J Haematol 1978;21:333–40.
21. Dawes C. Rhythms in salivary flow rate and composition. Int J Chronobiol 1974;2:253–77.
22. Heintze U, Birkhed D, Björn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age. Swed Dent J 1983;7:227–38.
23. Percival RS, Challacombe SJ, Marsh PD. Flow rates of resting whole and stimulated parotid saliva in to age and gender. J Dent Res 1994;73:1416–20.
24. Nederfors T, Dahlöf C. Effects of the β-adrenoceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. Arch Oral Biol 1992;37:579–84.
25. Rantonen PJF, Meurman JH. Viscosity of whole saliva. Acta Odontol Scand 1998;56:210–14.
26. Kavanagh DA, O'Mullane DM, Smeeton N. Variation of salivary flow rate in adolescents. Arch Oral Biol 1998;43:347–52.
27. Fure S, Zickert I. Root surface caries and associated factors. Scand J Dent Res 1990;98:391–400.
28. Risheim H, Arneberg P, Birkhed D. Oral sugar clearance and root caries prevalence in rheumatic patients with dry mouth symptoms. Caries Res 1992;26:439–44.
29. Haga H-J, Hulten B, Bolstad A, Ulvestad E, Jonsson R. Reliability and sensitivity of diagnostic test for primary Sjögren's syndrome. J Rheumatol 1999;26:604–8.
30. Sreebny LM. Salivary flow and dental caries. Cariology Today. Basel: Kager; 1984. p. 56–69.
31. Melvin JE. Saliva and dental diseases. Curr Opin Dent 1991;1: 795–801.
32. Hussien S, Laulicht M, Hoffbrand AV. Serum ferritin in megaloblastic anaemia. Scand J Haematol 1978;20:241–5.

Received for publication 26 June 2000
Accepted 19 September 2000