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Effect of oral iron supplementation on unstimulated salivary flow rate: a randomized, double-blind, placebo-controlled trial

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BACKGROUND: No treatment is known to permanently increase salivary flow in patients with hyposalivation. The objective of this study was to investigate the effect of iron supplementation on salivary flow rate.

METHODS: A double-blind, randomized, placebo-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 iron orally twice a day for 3 months, while the other half received placebo.

RESULTS: No statistically significant difference was found between the groups after treatment for the unstimulated flow rate and in the subjective assessments of oral dryness. The serum ferritin values increased significantly in the iron group but not in the placebo group.

CONCLUSION: Oral supplementation with iron for 3 months has no effect on salivary flow rate among individuals with hyposalivation and low serum ferritin values.
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Keywords: dry mouth; iron deficiency; serum ferritin; xerostomia

Introduction

The symptom known as xerostomia, the subjective perception of oral dryness, is partly associated with hyposalivation, which is defined as an objectively measured reduction in salivary flow rate (1). It has been shown that xerostomia is often present below an unstimulated whole salivary flow rate of 0.2 ml/min (2, 3). In addition to xerostomia, several other symptoms are associated with hyposalivation; they include thirst, difficulty speaking and difficulty eating dry food (4). If severe, hyposalivation could affect quality of life (5, 6) and lead to dental diseases, such as inflammatory conditions in the oral cavity and dental caries (4).

Hyposalivation is associated with the insufficient intake of food and thereby insufficient nutrition to the salivary glands, for example, in children with malnutrition, inadequate intake in older adults or individuals with eating disorders (7–9). In patients with iron deficiency, as well as in patients with xerostomia and/or hyposalivation, the oral signs are similar; they include glossitis, angular chielitis, stomatitis, dysphagia and candida infections (4, 10, 11). In the 1940s, it was reported that the treatment of iron-deficient patients usually resulted in the elimination of these signs, including hyposalivation (12). A more recent study has suggested that treatment with iron increases salivation in iron-deficient patients and alleviates glossal pain (13). To the best of our knowledge, there are no other studies of the effect of iron on salivary flow in humans. It is unclear how iron deficiency would affect the salivary gland cells. However it is known that all cells require iron, as a constituent of proteins that carry out essential housekeeping functions for cellular metabolism. Cellular iron deficiency arrests cell growth and leads to cell death (14).

As there are a limited number of treatment modalities for hyposalivation, the use of iron supplementation appeared to be a possibility that has not yet been fully investigated. We therefore aimed to investigate whether oral iron supplementation increases salivary flow rate in individuals with hyposalivation and low serum ferritin levels. For this purpose, we performed an explanatory randomized, double-blind, placebo-controlled trial in individuals with both low iron stores, defined as ferritin values of ≤30 μg/l for women and ≤50 μg/l for men and hyposalivation, defined as unstimulated whole salivary flow rates of ≤0.2 ml/min. Our research hypothesis was that iron supplementation would increase salivary flow and alleviate oral dryness.

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Materials and methods

Subjects

The study was performed at the Public Dental Clinic in Sala, Sweden, with approximately 4000 patients in the age group 15–46 years. In this group, 306 patients had a previous or a planned saliva test for the assessment of caries risk. Of these patients, 204 agreed to perform a saliva test (Fig. 1). The exclusion criteria were pregnancy and medication with xerogenic drugs. One hundred and thirty-five individuals met the inclusion criterion of having an unstimulated whole salivary flow rate of ≤0.2 ml/min and were tested for serum ferritin. Forty-four individuals had serum ferritin levels of ≤30 μg/l for females and ≤50 μg/l for males but ⩾10 μg/l and were included in the study, with the exception of one patient who withdrew for personal reasons. Twelve individuals with serum ferritin values of <10 μg/l were referred to a specialist in internal medicine for further investigation. Of these individuals, four were diagnosed as anaemic requiring medical treatment and one individual did not turn up for further investigation. Seven of the referred individuals were therefore included in the study, resulting in a total of 50 subjects with complete baseline data and fulfilling the inclusion criteria. The ordinary health declarations were used to assess health status known to the patients. One subject was diagnosed with Sjögren's syndrome and a second was found to have high blood pressure which was being treated with the drug enalapril + hydrochlorothiazide which has an unclear effect on salivation. There are few reports on dry mouth (<1%) from patients taking this drug (15).

All the subjects gave their written informed consent to participate in the study. For participants below the age

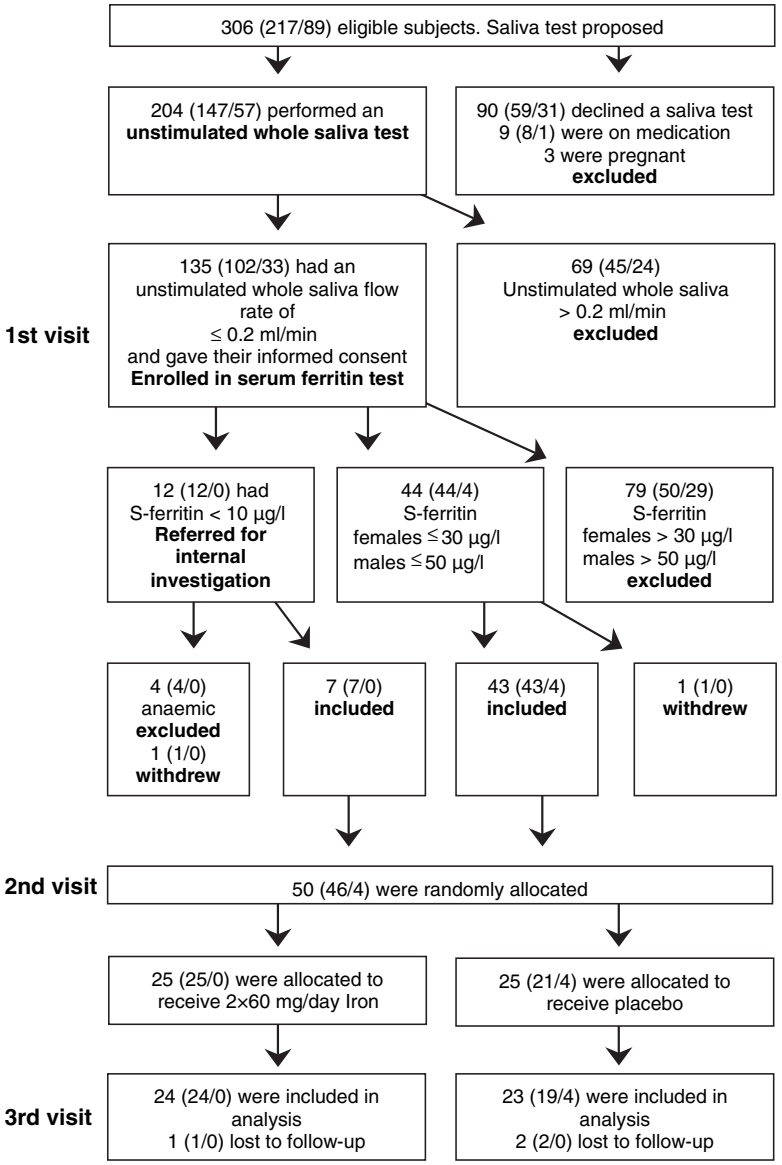

Figure 1 Flow diagram of the randomized, placebo-controlled, double-blind trial of the effect of oral iron supplementation on unstimulated salivary flow rate (female/male).

of 18 years, written informed consent was also given by the parents. The ethics committee at the Faculty of Medicine at Uppsala University approved the study (approval no. 02–024).

Design

The study was an explanatory, randomized, placebo-controlled trial designed to evaluate the effect of oral iron supplementation on the unstimulated whole salivary flow rate. 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 placebo twice a day for 3 months – a total of 180 tablets. The participants were advised to take the tablets first thing in the morning and before bedtime to increase compliance.

Both active and placebo tablets were manufactured by Recip, Haninge, Sweden, under contract from Orion Pharma, Sollentuna, Sweden. In order to make the tablets the same size and colour, each placebo tablet was given an additional content of the colouring components iron oxide (E100), 0.11 mg vs. 0.05 mg in the iron tablet and curcumin (E173), 0.25 mg vs. 0.11 mg. The Swedish Medical Products Agency approved the design and protocol of the study (approval no. 151:912/01). Apoteket Production & Laboratories, Stockholm, Sweden, packed the tablets in numbered identical containers with 200 tablets in each and generated the randomization of the subjects in blocks of eight using a computer random number generator. The randomization was kept sealed one by one in numbered opaque envelopes, as well as in the form of a list, until the end of the study. The intervention code and block size were therefore unknown to the study participants and to the investigators.

Allocation to study groups was performed by the first author on the second visit when the tablets were distributed. The subjects were instructed to reduce the dosage to one tablet a day if they experienced unpleasant side-effects. On the same visit, the participants answered a questionnaire related to xerostomia and symptoms of hyposalivation. After 6 weeks, the patients were contacted by telephone and asked about compliance and side-effects. One week ahead of the third visit, the patients were reminded about their appointment and told to stop taking the tablets. These phone calls were made by the assistant supervising the end-point saliva test. On the third visit, an unstimulated whole saliva test and blood sampling were performed in the same way as at baseline. A second questionnaire relating to symptoms was filled in and the patients were again asked about compliance and side-effects. The remaining tablets were returned and counted and the compliance was estimated as the number of tablets taken/number of tablets that should have been taken in percentages.

Methods

Saliva collection

Both saliva tests for each individual were performed following the same protocol. The saliva collections were performed at 7.30 AM. The subjects were instructed not to eat, drink or use any form of tobacco for 2 h before the collection and to relax for a couple of minutes before the test. At the saliva collection, the subject sat bent forward in an ordinary chair and was told to place his/her tongue on the lingual surfaces of the upper incisors and to hold the mouth open and remain still, letting the saliva drip into a pre-weighed disposable cup held to the lower lip for 15 min. The unstimulated whole salivary flow rate was determined by gravitation, using a scale with an accuracy of 0.01 g (Sartorius, BP310P, Sartorius AG, Goettingen, Germany), presuming that 1 g of saliva is equivalent to 1 ml. All saliva collections were supervised by a specially trained assistant with a long experience of the procedures.

Blood samples

Serum ferritin. Venous blood samples were drawn directly after the saliva test. Serum ferritin was measured on an analyser for heterogeneous immunoassays according to the manufacturer's recommendations (Elecsys 1010, Roche Diagnostics, Mannheim, Germany). For inclusion, freshly drawn samples were analysed immediately. The samples used for comparison between the iron and placebo groups were frozen and analysed after the intervention period on the same day using the same reagent batch.

C-reactive protein. C-reactive protein (CRP) was analysed on the last blood sample in order to detect inflammation or infection (16, 17) using immunoturbidometry (Synchron CX5 Delta, Beckman Coulter, Fullerton, CA, USA).

Questionnaire

At baseline and after treatment, all the participants assessed eleven variables related to the function of the salivary glands and tear glands using visual analogue scales (VAS). The subjects were asked to mark their responses to each item by placing a vertical line on a 100-mm horizontal scale. The eleven VAS items used were as follows.

Rate the difficulty you experience due to dry mouth:

1 During the night
2 During the morning
3 During the day
4 When eating/swallowing
5 When talking
6 From slimy/viscous saliva
7 From smarting pain/burning sensations
8 From taste disturbances
9 From bad breath

Rate the difficulty you experience:

10 From gritty sensations in the eyes
11 From film covering the eyes that you need to blink away

The anchor points of the scale were 'No difficulty at all (0) to Very difficult (100)'.

Statistics

The primary outcome was change in the unstimulated whole salivary flow rate between baseline and after treatment. An initial calculation of sample size based on published data (13) suggested that, if 17 subjects in each group were followed up, it would be possible to use an unpaired *t*-test to detect a difference of 0.1 ml/min in salivary flow rate with a power of 80% and a significance level of 5%, assuming a standard deviation of 0.1 ml/min. Differences in the mean values for salivary flow rate and serum ferritin at baseline and after treatment were analysed between groups using unpaired *t*-test after control of normal distribution. The differences between baseline and after treatment were compared within groups using a paired *t*-test. The Mann–Whitney *U*-test was used for the questionnaire data. Differences in adverse effects were tested using a chi-square test. Correlation was analysed with Pearson's Product Moment Correlation. $P < 0.05$ was chosen as the level for statistical significance.

Analyses of the results were made according to the intention-to-treat (ITT) principle (18). Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS for Windows, Chicago, IL, USA, version 12.0.1).

Results

Of the 50 individuals who were included in the study from October 2002 to September 2004, 47 (94%) completed the trial. Mean compliance during the intervention period was 82%, CI 95% (76, 90) for the placebo group and 71%, CI 95% (61, 82), for the iron group (i.e. resulting in an average daily dose of 85 mg of iron). There was no significant difference between the two groups.

During the treatment period, three patients, all women, withdrew, one in the iron group and two from the placebo group. The patient from the iron group dropped out because of the need for antidepressant treatment. In the placebo group, one patient felt dizzy and wanted to be sure she was receiving iron supplementation and not placebo, while the second lacked the motivation to complete the study (Fig. 1). Furthermore, in the placebo group, one person became pregnant and in the iron group, one patient started medication with the antidepressant drug citalopram hydrobromide, which has a strong xerogenic effect defined as a reported incidence of dry mouth of 10% or more, among patients taking this drug (15). These subjects completed the study protocol and were included in the ITT analyses. Finally, one patient in the placebo group did not have time for the last blood sample but completed the saliva test.

Table 1 Characteristics of the participants at baseline in the iron and placebo group

Characteristic	*Iron group (n = 25), mean (SD)*	*Placebo group (n = 25), mean (SD)*
Gender, female/male	25/0	21/4
Age (year)	34.7 (8.2)	34.0 (10.5)
Unstimulated saliva flow rate (ml/min)	0.090 (0.053)	0.126 (0.055)
Serum ferritin (μg/l)	20.2 (10.5)	23.3 (14.6)

Age was balanced between the two groups at baseline (Table 1), while the four men in the study all ended up in the placebo group. For unstimulated whole salivary flow rate and serum ferritin, the baseline values were lower in the iron group compared with the placebo group. No statistically significant correlation was found between serum ferritin and unstimulated whole salivary flow rate ($r < 0.1$). Values of subjective assesment of salivary and tear function are shown in Table 2.

The unstimulated whole salivary flow rate and serum ferritin values are shown in Table 3. 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 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.

For serum ferritin, a statistically significant difference ($P < 0.001$) was found between the two groups when

Table 2 Characteristics of participants at baseline and after 3 months treatment (end-point) in the iron and placebo group in terms of subjective assessment of salivary and tear gland function registered using a VAS scale (no difficulty at all = 0 to very difficult = 100)

	Iron group[a]		*Placebo group*[a]	
Item in questionnaire	*Baseline (n = 25)*	*End-point (n = 24)*	*Baseline (n = 25)*	*End-point (n = 23)*
Dry mouth, at night	46 (0; 75)	43 (16; 57)	43 (0; 61)	47 (23; 67)
Dry mouth, in the morning	59 (14; 80)	51 (22; 67)	61 (31; 72)	43 (12; 69)
Dry mouth, during the day	23 (5; 51)	34 (4; 50)	28 (8; 40)	25 (0; 42)
Difficulty swallowing	0 (0; 22)	6 (0; 24)	2 (0; 14)	0 (0; 6)
Difficulty speaking	0 (0; 14)	7 (0; 7)	0 (0; 4)	0 (0; 9)
Viscous saliva	0 (0; 20)	8 (0; 36)	24 (2; 39)	10 (0; 36)
Burning sensations	0 (0; 6)	0 (0; 4)	0 (0; 1)	0 (0; 7)
Taste disturbances	0 (0; 6)	0 (0; 5)	0 (0; 1)	0 (0; 11)
Bad breath	8 (0; 34)	16 (0; 26)	17 (0; 37)	14 (2; 42)
Gritty sensations from eyes	10 (0; 58)	13 (0; 43)	3 (0; 29)	8 (0; 32)
Film covering the eyes	5 (0; 17)	4 (0; 15)	0 (0; 7)	4 (0; 12)

[a]Median VAS score, mm (25th; 75th percentiles).

Table 3 Measurement of unstimulated whole saliva flow rate and serum ferritin after treatment with iron or placebo for 3 months

	Iron group (n = 24), mean (SD)	*Placebo group (n = 23), mean (SD)*	*Difference, mean (95% CI)*	*P-value*
Unstimulated saliva flow rate (ml/min)				
Baseline	0.091 (0.054)	0.126 (0.055)		
End-point	0.143 (0.138)	0.196 (0.107)		
Change from baseline	0.053 (0.140)	0.070 (0.114)	−0.018 (−0.093; 0.058)	0.64
Serum ferritin (μg/l)				
Baseline	20.7 (10.5)	24.9 (15.0)[a]		
End-point	33.0 (14.0)	23.4 (15.8)[a]		
Change from baseline	12.4 (12.4)	−1.4 (9.6)[a]	13.8 (7.3; 20.3)	< 0.001

[a]$n = 22$.

comparing the change from baseline (Table 3). A significant increase in serum ferritin was found in the iron group ($P < 0.001$) but not in the placebo group.

In both groups, individuals with an unstimulated whole salivary flow rate of ≤0.1 ml/min (very low) at baseline showed a greater increase in salivary flow between baseline and end-point than the corresponding complete group. A very low baseline salivary flow rate was found in 14 of 24 and 10 of 23 individuals in the iron and placebo groups, respectively. The mean increase in salivary flow rate from baseline to end-point was 0.09 ml/min (SD 0.14 ml/min, $P = 0.029$) and 0.13 ml/min (SD 0.12 ml/min, $P = 0.009$) in these two subgroups.

In the iron group, the 10 iron-depleted individuals (≤15 μg/l) were compared with the 14 individuals with low serum ferritin (>15 μg/l). The change in serum ferritin from baseline differed in a non-significant manner between these groups: 13.8 μg/l (SD 12.5) and 11.4 μg/l (SD 12.5), respectively. The corresponding values for changes in salivary flow rates in these two subgroups were 0.019 ml/min (SD 0.11) and 0.076 ml/min (SD 0.16), a difference that was not significant. The correlation coefficients for unstimulated salivary flow rate at the baseline and after treatment were 0.17 and 0.12 for the iron and placebo group respectively.

Analyses of best and worst case scenarios were performed for the three cases lost to follow-up. No marked differences compared with the complete data set were observed. Analyses per protocol were made without the two cases that completed the intervention but qualified for exclusion because of pregnancy or medication with xerogenic drugs, without influencing the results. Nor was there any change when extending this exclusion to the two cases with Sjögren's syndrome and high blood pressure. Excluding the four men in the placebo group lowered the change from baseline value from 0.070 to 0.048 ml/min for the salivary flow, but it did not change the serum ferritin levels.

The differences in VAS scores from baseline to after treatment with iron or placebo are shown in Table 4. None of the subjective symptoms differed significantly between the iron and placebo group. Three individuals, all in the iron group, had CRP levels of >5 mg/l. Excluding these three individuals altered the change in the salivary flow rate from baseline to 0.028 ml/min (SD 0.095) but did not change the serum ferritin levels. The subject with the highest CRP (21 mg/l) reported symptoms from muscle inflammation in the neck and shoulder region.

Table 4 Differences from baseline after treatment with iron or placebo for 3 months in terms of subjective assessment of salivary and tear gland function registered using a VAS scale

Item in questionnaire	*Iron group[a] (n = 24)*	*Placebo group (n = 23)*	*P-value*
Dry mouth, night	2 (−28; 10)	0 (−15; 4)	0.263
Dry mouth, morning	4 (−2; 22)	11 (−15; 28)	0.966
Dry mouth, during the day	7 (−25; 13)	0 (−12; 19)	0.749
Difficulty swallowing	0 (−20; 0)	0 (0; 12)	0.067
Difficulty speaking	0 (−12; 1)	0 (−4; 0)	0.974
Viscous saliva	0 (−8; 7)	0 (−4; 12)	0.431
Burning sensations	0 (0; 0)	0 (−5; 0)	0.286
Taste disturbances	0 (−2; 3)	0 (−5; 0)	0.281
Bad breath	0 (−10; 27)	0 (−16; 14)	1.000
Gritty sensations from eyes	5 (0; 25)	0 (−9; 10)	0.199
Film covering the eyes	0 (0; 11)	0 (−14; 2)	0.130

[a]Median VAS score, mm (25th; 75th percentiles).

The reported side-effects were mainly nausea, stomach ache, diarrhoea and constipation. Adverse effects in the form of intestinal side-effects were reported by 14 of 24 participants in the iron group at both 6 and 13 weeks of treatment. In the placebo group, three and two of 23 patients reported intestinal side-effects at 6 and 13 weeks of treatment, respectively ($P = 0.003$ and $P = 0.001$).

Discussion

The result for the primary effect variable, unstimulated salivary flow rate, showed no effect from iron supplementation with 120 mg a day for 3 months, in spite of the clear effect on serum ferritin. This was not anticipated, as the secondary findings of Osaki et al. (13) suggested a significant effect by iron supplementation on salivary flow rate. These contradictory results may be explained by the fact that our study design followed the CONSORT recommendations for randomized controlled trials (18), while the study by Osaki et al. (13) did not have a control group. The small increase in unstimulated whole salivary flow rate in both the placebo and iron groups after 3 months treatment found in the

present study may be explained by the statistical phenomenon regression to the mean (19), which could also explain the results reported by Osaki et al. (13).

It does not seem likely that the statistically significant increase in salivary flow in the placebo group could be caused by any component in the placebo tablets *per se*. The addition of the colour components iron oxide and curcumin could have the potential for stimulating the salivary flow rate; however, the amounts that were added were very small. Iron oxide can probably be ruled out, as the additional iron oxide did not affect serum ferritin in the placebo group. The effect of curcumin on salivary glands is not known, but the low daily dose of 0.5 mg most probably has no effect. However, curcumin has been shown to exhibit various effects at cellular level and to have anti-inflammatory properties (20). Even if an effect on salivary flow by curcumin is unlikely, it cannot be ruled out completely.

There is general agreement in the literature about the limit for very low unstimulated whole saliva flow rate being 0.1 ml/min (21, 22). This limit is often regarded as a cut-off value for the definition of hyposalivation. Values between 0.1 and 0.2 ml/min have been suggested as low values, as symptoms of oral dryness become evident in this interval (2, 22, 23). Flow rates >0.2 ml/min have been proposed as normal (22). The choice of a flow rate of ≤ 0.2 ml/min as an inclusion criterion in the present study was based on the findings referred above and the results from Osaki et al. (13) where the mean flow rate before treatment was 0.16 ml/min.

In a group of patients with hyposalivation, there is a risk that some of the subjects will develop a general disease, which may affect the salivary flow rate, such as Sjögren's syndrome. In these individuals, the hyposalivation may be caused by irreversible inflammatory destruction of the glandular parenchyma, resulting in a limited possibility to increase the salivary flow. The risk for this is probably more pronounced in individuals with very low flow rates (24). However, a significant increase in salivary flow rate was found in the subgroups with the very low unstimulated salivary flow rate of ≤ 0.1 ml/min in both the iron and the placebo group. Possible previous inflammation or infection as a result of elevated CRP levels was only detected in three cases of 47. In the present study, only two individuals were diagnosed with chronic diseases and this did not affect the results of the study. But as no medical examinations were performed undiagnosed diseases might have influenced the salivary gland function and serum ferritin levels. In spite of the clear lack of information on risk factors and causes for hyposalivation our intention was to exclude individuals taking drugs with known xerogenic effects. Much more is known about risk factors for iron deficiency, therefore the intention was to exclude only more severe iron deficiency anaemia and changes in iron stores during pregnancy (25).

Serum ferritin levels in a population vary widely. 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 (26). The small number of men included in the study reflects the distribution of iron deficiency in the population (27). This skewed distribution of men in the two groups might have been better resolved by stratification or by including only women in the study. Iron deficiency occurs when iron stores are depleted and has been defined as a serum ferritin concentration of <15 μg/l in both genders (28). The benefit from iron supplementation for individuals with a serum ferritin level above this lower limit has been disputed (13, 29). In the present study, the baseline level of serum ferritin in the iron group did not influence the increase in serum ferritin or unstimulated whole salivary flow rate. The increase in serum ferritin found in the iron group was similar to the results of other studies (13, 30–32) and was an expected effect of the iron supplementation.

Adverse effects in the form of intestinal side-effects have been shown to be related to the daily dose of iron and, surprisingly, they have not reduced compliance (33). This explains the present finding of no difference in compliance between the iron and placebo groups, in spite of the large number of cases with adverse effects. The significant difference in adverse effects between the iron group and the placebo group may reveal the blinding of the study to affected participants and the assistant asking the questions about side-effects. It is, however, unlikely that this lack of complete concealment of the blinding would have had any effect on the salivary flow measurement.

The accurate measurement of salivary flow rate is complicated and dependent on many factors that need to be controlled in order to minimize methodological bias (34). Repeated measurements of unstimulated whole salivary flow rate over periods shorter than 2 weeks have shown high reproducibility with $r = 0.75$–0.91 (23, 35, 36). To the best of our knowledge, there are no data on periods longer than 2 weeks, but it seems likely that the reproducibility decreases with time. In the present study, the correlation between the measurement of salivary flow rate at baseline and after 3 months was much lower. This low correlation might be related to some extent to the regression to the mean and it could have been reduced by repeated baseline saliva tests (19).

One important factor is the circadian rhythm, showing low flow rates during the night and in the morning, increasing to peak values in the middle of the afternoon (37). In the present study, the circadian rhythm was controlled by performing the saliva collection at a fixed time point in the morning (38). Furthermore, a circannual rhythm in salivary flow rate has been described in areas with a warm climate (39, 40). The present data come from a temperate climate where any influence by dehydration is less probable (41). The effect of circannual rhythm was thought to be controlled by the randomized design of the study. The influence of food intake on salivary flow was controlled by restrictions in eating and drinking prior to the tests (42).

The present sampling framework included 4000 young to middle-aged individuals representing the typical clientele at a Swedish public dental clinic. It seems likely that the results from our sample in this explanatory trial could be generalized to apply to most

individuals, or at least to women, in these age groups with hyposalivation and low serum ferritin values without anaemia or other diseases.

In conclusion, this randomized, double-blind, placebo-controlled trial shows no effect by oral iron supplementation for 3 months on unstimulated salivary flow rate in individuals with hyposalivation and low serum ferritin values.

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