

# Salivary variables in relation to tobacco smoking and female sex steroid hormone-use in 30 to 59-year-old women

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Several systemic conditions may have an influence on oral health. Hormone replacement therapy (HRT) has a positive effect on alveolar bone of menopausal women and smoking a negative effect. However, little is known about their effect on saliva. The purpose of this study was to examine the effect of hormone-use and tobacco smoking on the composition of saliva, in particular on the inorganic constituents. Salivary samples were collected from a representative study group comprising 1013 women (30–59 years) participating in a pre-screen referral program for osteoporosis. The participants were divided into 2 subgroups according to age. The younger group ( $\leq 45$  years) comprised 413 women and the older group ( $\geq 50$  years) 600 women. Salivary calcium, magnesium, sodium, potassium, inorganic phosphate, total protein, and flow rate of paraffin-stimulated saliva were measured. In the older age group, female sex steroid users (hormone users) had lower salivary protein concentrations than non-users. Smoking was associated with high salivary calcium, magnesium, and potassium levels in the group of older participants. Neither tobacco smoking nor female sex steroid hormones had any significant effect on the salivary composition in the younger age group. In conclusion, smoking was reflected more clearly than female sex steroid hormone-use in the inorganic composition of saliva in the older age group. The salivary composition was not affected by hormone-use or by smoking among the younger age group. □ *Hormone-use; saliva; tobacco smoking; women*

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Although quite common among postmenopausal women, the effect of hormone replacement therapy (HRT) on the composition of saliva is not clear. Most cross-sectional studies have failed to show any hormone treatment-related effect on the composition of saliva (1, 2). Hietala and co-workers (3) have previously shown that women with HRT had an increased flow rate of paraffin-stimulated saliva together with improved buffer effect. In a longitudinal study, we showed that HRT improves salivary flow rate and buffer effect (4), and temporarily decreases salivary calcium levels when first administered (5). Women on HRT seem to have less calculus than controls of the same age who do not use hormones (6). In addition, postmenopausal women on HRT have less periodontal attachment loss than those who never use hormones (7). The exact mechanism of estrogen effect on bone metabolism is not known, but postmenopausal bone loss can be successfully prevented by HRT (8). Smoking and osteoporosis both have a negative influence on alveolar bone (9). Smoking also affects the mineral composition of saliva. It has been shown earlier (10) that the level of salivary calcium is higher in smokers than in non-smoking counterparts. Magnesium, sodium, and potassium levels of saliva have not been investigated earlier in relation to smoking.

The purpose of this study was to examine the effect of tobacco smoking and female sex steroid hormone-use on

the composition of saliva in two different age groups of women.

## Materials and methods

### Participants

The study group originally comprised 1030 women (age range 30–62 years) participating in a pre-screen referral program for osteoporosis. The screening was carried out at the Public Health Centre of Raisio, Finland, between August and September 1999. The age cohorts invited to the pre-screen program included all women living in the community and born in 1940, 1941, 1943, 1945, 1949, 1954, 1957, 1959, 1964, or 1969. Pregnant women ( $n = 12$ ) and participants ( $n = 3$ ) who were unsure whether they were pregnant or not were excluded. Since there was only one subject of 47 (born in 1948) years of age and another older than 60 (born in 1937) years, these were excluded. The remaining subjects ( $n = 1013$ ) were divided into 2 subgroups according to age. The younger group comprised 413 women ( $\leq 45$  years), while the older group comprised 600 ( $\geq 50$  years). The age cohorts of the participants are given in Table 1.

A brief medical history including medications and smoking habits was recorded using a questionnaire filled

Table 1. Age, number and proportion of women participating in this study originally recruited by the pre-screen referral program for osteoporosis in the Public Health Centre of Raisio, Finland, in August and September 1999

Age	Frequency	Percent
30	68	6.7
35	111	11.0
40	107	10.6
42	1	0.1
45	126	12.4
50	113	11.2
51	1	0.1
52	134	13.2
54	88	8.7
56	87	8.6
58	81	8.0
59	96	9.5
Total	1013	100.0

out by all consenting participants before screening. In the older group, the most prevalent systemic diseases were hypertension (15.6%) and allergies or intolerance for gluten or lactose (8.2%). In younger women, 8.2% reported having allergies and/or intolerance problems and 3.1% hypertension. Altogether 31.2% of subjects had some kind of medication. The use of medications was more frequent in the older age group, but the proportion of medicated subjects did not differ significantly between the following subgroups: non-smokers/smokers, female sex steroid hormone non-users (hormone non-users)/female sex steroid users (hormone users) in either age group.

#### *Smoking and use of female sex steroid hormones*

The participants were classified as non-smokers or smokers. Those who were irregular smokers, those who had recently (<1 year) stopped smoking, and those who could not give exact answers ( $n = 44$ ) were excluded. Current smokers were divided, according to the frequency of smoking, into moderate ( $\leq 10$  cigarettes per day) and heavy smokers ( $\geq 11$  cigarettes per day).

Women receiving no hormone therapy ( $n = 574$ ) were classified as non-users. Irregular hormone-users ( $n = 13$ ) or women who received hormones for treatment of infertility ( $n = 6$ ) were excluded. Transdermal hormones (gels, patches) were used by 1% and 20.2%, and hormones in tablet form by 17.6% (mainly as oral contraceptives) in the younger, and by 30.7% (mainly as HRT) in the older age group, respectively. Oral contraceptives contained ethinyl-estradiol/estradiol in combination with progestin. HRT as patches and gels contained estradiol/estradiolvalerate/estradiolhemihydrate alone or combined with progestin. HRT in tablet form contained estradiolvalerate/estradiolhemihydrate alone, or in combination with progestin. In addition, several different HRT regimens were used, for example, tablets, gels, or patches alone or in combination with oral hormones. A total of 29% of the regular

hormone users did not report the duration of the hormone therapy.

#### *Collection of saliva samples*

The participants refrained from tooth brushing, eating, drinking, and smoking for a minimum of 1 h prior to saliva collection. The saliva was collected by four experienced dentists (AD, SK, ML, LS) and the collection procedure was calibrated before starting the study. Stimulated whole saliva was collected by chewing a piece of paraffin of standardized size (1 g) at habitual pace. After 60 s of prestimulation, saliva was collected for 5 min in graduated disposable plastic cups. The saliva samples were collected in field conditions. No laboratory equipments were available at the site of saliva collection. The flow rate was measured at an accuracy of 1 ml and expressed as ml/min. Immediately after collection, the samples were transferred to test tubes, put on ice, frozen, and stored at  $-20^{\circ}\text{C}$  until further analysis.

#### *Electrolyte and protein analysis*

Calcium, magnesium, potassium, and sodium concentrations were measured by atomic absorption spectrophotometer (Perkin-Elmer Atomic Absorption Spectrophotometer Model 303, Norwalk, USA). Because of the strong affinity of calcium to form complexes with salivary proteins, uncentrifuged whole saliva containing both protein-bound and soluble calcium was used for the assay. A total of 200  $\mu\text{l}$  saliva was mixed with 1760  $\mu\text{l}$  of water and 40  $\mu\text{l}$  of 5% lanthanum oxide. The analyses of magnesium, potassium, and sodium were made from centrifuged saliva (12 000 g, 10 min,  $+4^{\circ}\text{C}$ ) after dilution with ion-exchanged water. Inorganic phosphate was analysed according to Kallner (11) and total protein according to Lowry et al. (12) both from centrifuged saliva. Bovine serum albumin was used as a standard for protein determinations.

#### *Statistical analysis*

The normality of distributions of the response variables was controlled with the Kolmogorov-Smirnov test. Before statistical analyses, logarithmic transformations of the salivary variables were made owing to the skewed distributions.

The younger and older age groups were analysed separately. Hormone-use was dichotomized (yes or no) and tobacco smoking divided into three categories (non-smokers, moderate smokers, and heavy smokers). The interaction of smoking and hormone-use on the saliva was tested with the univariate analysis of variance. If a significant difference was found, multiple comparisons with Tukey's method were made for pairwise comparisons of the groups. Results with a  $P$ -value  $> 0.05$  were considered as non-significant.

A commercial software program (Statistical Package for

Table 2. Salivary variables of 969 women according to age and smoking. P-values were calculated using ANOVA in which the association of tobacco smoking was adjusted with the use of female sex steroid hormones

Salivary variables	Younger women (45 years or less)			Older women (50 years or more)		
	Non-smokers ( <i>n</i> = 296)	Moderate smokers ( <i>n</i> = 40)	Heavy smokers ( <i>n</i> = 56)	Non-smokers ( <i>n</i> = 487)	Moderate smokers ( <i>n</i> = 37) 1–10 cig/day	Heavy smokers ( <i>n</i> = 53) >11 cig/day
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Calcium (mmol/l)	1.32 (0.28)	1.36 (0.26)	1.29 (0.29)	1.60 (0.35)	1.71 (0.47)	1.76 (0.36) ↑—0.007—↑
Phosphate (mmol/l)	3.73 (1.30)	4.05 (1.37)	4.01 (1.38)	4.28 (1.63)	4.97 (2.68) ↓—0.000—↓	4.75 (2.02)
Magnesium (mmol/l)	0.101 (0.061)	0.112 (0.056)	0.108 (0.047)	0.104 (0.055)	0.131 (0.073)	0.147 (0.080) ↑—0.026—↑
Potassium (mmol/l)	22.3 (9.0)	22.8 (6.6)	24.6 (8.6)	19.7 (4.3)	22.1 (5.4) ↓—0.007—↓	21.9 (5.5) ↑—0.013—↑
Sodium (mmol/l)	11.4 (7.5)	10.4 (6.7)	12.8 (8.4)	11.2 (7.7)	9.8 (6.1)	11.8 (8.0)
Protein (g/L)	1.16 (0.34)	1.14 (0.41)	1.10 (0.31)	1.26 (0.41)	1.31 (0.53)	1.27 (0.43)
Flow rate(ml/min)	1.5 (0.7)	1.4 (0.7)	1.5 (0.6)	1.5 (0.7)	1.3 (0.5)	1.5 (0.8)

Social Sciences for Windows, version 9.0, SPSS Inc., Chicago, Illinois, USA) was used to run the statistical analyses.

## Results

In the older group, higher salivary calcium ( $P = 0.007$ ), magnesium ( $P = 0.026$ ), and potassium ( $P = 0.013$ ) values were found in heavy smokers than in non-smokers (Table 2). In moderate smokers, only salivary magnesium ( $P = 0.000$ ) and potassium ( $P = 0.007$ ) concentrations were higher than in non-smokers (Table 2). Subjects who had never smoked did not differ from former smokers by salivary composition when analyzed separately (data not shown).

In the younger age group, there were no hormone- or smoking-related effects on the composition of saliva. In the older age group, the transdermal ( $n = 127$ ) and oral ( $n = 190$ ) hormone users did not differ from each other by salivary composition. These groups were combined for further analyses. We found no differences in salivary flow rate between the hormone-users and the non-users. Mean (SD) salivary protein concentration was 1.22 (0.39) g/L in the hormone users and 1.30 (0.44) g/L in the non-users ( $P = 0.010$ ). The duration of HRT did not have any significant effect on the salivary protein concentrations.

## Discussion

In older women, smoking was associated with higher concentrations of salivary calcium, magnesium, and potassium. Inorganic phosphate concentration was not influenced by smoking. Salivary potassium is known to be independent of salivary flow rate (13). Our finding of higher potassium concentration in smoking women was

probably due to the fact that potassium usually correlates positively with other electrolytes and parallels with changes in them (14). The importance of salivary electrolytes on dental health, especially that of calcium, has been shown earlier (15–18). An association between increased salivary calcium levels and periodontitis susceptibility has also been found (19, 20), and tobacco smoking appears to have an increasing effect on supragingival calculus formation (21).

The lower salivary protein concentrations in women on HRT compared to the non-users was unexpected, since hormones generally increase metabolic activity such as protein synthesis. Although the difference was statistically significant, the protein concentrations were within the normal range in both groups, and the clinical importance of this finding is not yet clear. In contrast to our previous longitudinal study, showing an increased salivary flow rate in response to HRT (4), we found no association between HRT and salivary flow rate. In the present study, several different hormone replacement therapy regimens were used, the amount of hormones varied, and both opposed and unopposed estrogen were used. We did not know the exact menopausal status of these women, contrary to those studied in our previous report (4). Although the effect of hormones on bone is not affected by the route of administration, the condition in saliva may be different (22). HRT has the greatest effect on bone when first administered (23). This is in line with our previous study concerning the effect of HRT on salivary calcium concentrations showing a transient reduction in salivary calcium concentration after the first 3 months of therapy (5). Neither was there any difference in flow rates between the younger and older women. Previous studies (24) have shown that there is a tendency toward decreased flow rate after menopause. It is possible that we failed to show minor differences between the groups owing to the crudeness of our

salivary flow rate measurements carried out with an accuracy of 1 ml as compared to that of 0.5 ml of Parvinen & Larmas (24).

In conclusion, smoking seems to increase the levels of salivary calcium, magnesium, and potassium while HRT seems to decrease the salivary protein content of saliva in older women. Salivary composition is not affected by smoking or female sex steroid hormone-use smoking among the younger age group.

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