Salivary buffer capacity in relation to menarche and progesterone levels in saliva from adolescent girls: a longitudinal study

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The aim of this study was to investigate the relationship between salivary buffer capacity and menarche, and to explore any association with levels of the sex hormone progesterone in stimulated whole saliva in adolescent girls. The material comprised 162 girls, 12 years of age at baseline in the 6th grade, who were followed for 3 years. Every 4th month, a stimulated whole saliva sample was collected, secretion rate and buffer capacity were determined, and information was gathered on menarche, ongoing menses, and caries increment. Once yearly, the salivary concentration of progesterone was determined with an enzyme immunoassay kit. The results showed a significantly impaired salivary buffer capacity over the years ($P < 0.05$). Low buffer capacity was significantly correlated with low secretion rate ($r = 0.42$; $P < 0.001$) and DMFT increment ($r = 0.20$; $P < 0.05$). Pre-menarche buffer capacity did not differ from the post-menarche scores. The concentration of progesterone in saliva increased with age but displayed no significant relationship to buffer capacity, flow rate, or caries increment. In conclusion, the findings of this study suggest that the salivary buffer capacity may be impaired over the adolescent years in females, but the reason remains unclear. □ *Adolescents; buffer capacity; menarche; progesterone; saliva*

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It is a common clinical observation that females exhibit increased caries activity during their teenage years. Several explanations have been offered, the most likely being lifestyle changes and new eating patterns. Several recent studies have shown that food habits can change significantly during adolescence along with other lifestyle changes (1, 2). An additional explanation has been offered by Söderling et al. (3), who reported a diminished salivary buffer capacity in adolescent girls, but not in boys, in a series of data from two cohorts covering the pre- and post-puberty years, and speculated that the impaired buffer capacity was due to hormonal changes. Progesterone is readily detected in human saliva and concentrations are thought to mirror the ovarian function (4). To our knowledge, a possible relationship between progesterone and buffer capacity has not been explored in a true longitudinal study setting during adolescence. The aim of this longitudinal study was therefore to investigate the salivary buffer capacity in adolescent girls and to explore the possible relationship between salivary buffer capacity and menarche and any association with levels of the sex hormone progesterone in stimulated whole saliva. In addition, the impact of salivary buffer capacity and salivary progesterone levels on caries increment was investigated.

Materials and methods

The material consisted of all females ($n = 185$) in the 6th grade who were listed as recall patients at the Public Dental Clinic in Falkenberg, a small town located on the west coast of Sweden. The girls were 12 years of age at baseline and the selected cohort represented approximately 50% of all female 12-year-old subjects in the 35,000 inhabitant community. All participants signed a consent form together with their parents, and the study protocol was approved by the ethics committee of Lund University. Twenty-three girls (12%) dropped out due to relocation and 162 subjects could be followed for 3 years, in the 7th, 8th, and 9th grades, as described below.

Salivary sampling and assays

Samples of paraffin-stimulated whole saliva were collected over 5 min every 4th month during the study period. The secretion rate was calculated and expressed as mL/min and the buffer capacity was immediately determined using the Dentobuff test (Orion Diagnostica, Helsinki, Finland) according to Ericson and Bratthall (5). Once each year, the numbers of salivary mutans streptococci and lactobacilli were estimated with the Dentocult SM (6) and Dentocult LB (7), respectively. The strips were evaluated and scored with the aid of a stereo-microscope according to Twetman and Frostner (8). The following scores were used: **0** = 0–9 colony forming units (CFU); **1** = 10–99 CFU; **2** = 100–499 CFU; **3** = ≥500 CFU. For lactobacilli, $\geq 10^5$ CFU/mL saliva was considered as a high count. The concentration of salivary progesterone was determined in duplicate with the aid of a commercial enzyme immunoassay kit from Salimetrics (State College,

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Pa., USA) and expressed as pg/mL. Because of technical failure of a refrigerator at our laboratory, the saliva samples from the final sampling in the 9th grade could not be analysed with respect to progesterone. On each saliva sampling occasion the girls were asked if they had passed the menarche and whether or not they actually had menses on the day of the sampling. Their present oral hygiene routines and dietary and smoking habits were disclosed by the interview and these findings will be presented elsewhere.

Caries experience and increment

All subjects were thoroughly examined by their regular dentist at baseline and after 3 years in accordance with the Public Dental Service's guidelines slightly modified from the World Health Organization recommendations (9). The evaluations included 2–4 bitewing radiographs and caries experience was expressed as DMFT and DMFS. Proximal lesions within the enamel were scored from the radiographs as ECa. Caries increment was calculated as the difference in caries prevalence between examinations. The regular dentist carried out any required preventive and restorative dental treatment during the study period based on the need of the individual.

Statistical methods

All data were processed with the SPSS software (version 11.5, Chicago, Ill., USA). The data was subjected to one-way analysis of variance for repeated measures and correlations were calculated with the Pearson correlation coefficient. Pre- and post-menarcheal values were pairwise compared with two-sided *t* tests. The buffer capacity scores for each grade were expressed as the median of three samples. A P value <0.05 was considered statistically significant.

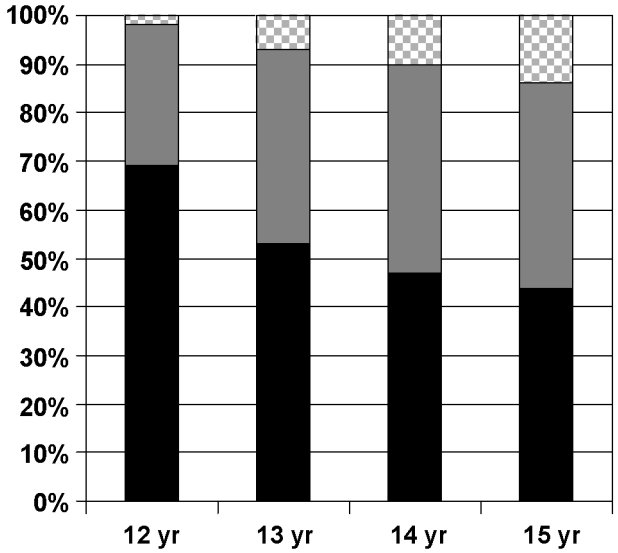

Fig. 1. Buffer capacity in stimulated whole saliva at baseline and after 1, 2, and 3 years in adolescent girls. The buffer capacity was assessed using the Dentobuff test (5) disclosing: ■ = normal buffer capacity (pH ≥6.0); ▩ = impaired buffer capacity (pH 4.5–5.5); ▩ = poor buffer capacity (pH ≤4.0).

Table 1. Mean secretion rate (mL/min) and concentration of progesterone (pg/mL) in stimulated whole saliva at baseline (6th grade) and at the first follow-up in each grade in 162 adolescent girls

Variable	Secretion rate Mean ± *s*	Progesterone Mean ± *s*
12 yr (6th grade)	1.1 ± 0.5	17.54 ± 19.21
13 yr (7th grade)	1.1 ± 0.5	29.22 ± 42.53*
14 yr (8th grade)	1.1 ± 0.5	41.71 ± 58.99*
15 yr (9th grade)	1.2 ± 0.5	Not analysed

* The mean value is significantly higher than baseline, $P < 0.05$ (ANOVA).
s = standard deviation.

Results

The distribution of salivary buffer capacity scores at baseline (12 years) and in the following years is presented in Fig. 1. A clear tendency towards impaired buffer capacity was disclosed and the salivary buffer capacity was significantly lower ($P < 0.05$) in the 8th and 9th grades (14–15 years) compared to the baseline. However, no relationship was found with the individual time of menarche. The buffer capacity values obtained during the period immediately before menarche did not differ from those obtained within a year after the menarche.

The secretion rate and the salivary concentrations of progesterone are given in Table 1. The mean flow rate was 1.1 mL/min in the 6th grade and remained stable throughout the study, while the mean progesterone values increased significantly ($P < 0.05$) from baseline during the first and second years. The individual pre-menarche and post-menarche concentrations are compared in Table 2, indicating that the post-menarche concentrations were significantly higher in the 7th and 8th grades. The salivary progesterone concentrations were slightly lower during menses, but the differences were not statistically significant (data not shown). No relationship was disclosed between

Table 2. Pre-menarche versus post-menarche concentrations of salivary progesterone (pg/mL) at baseline and the designated follow-ups in 162 adolescent girls

	Pre-menarche		Post-menarche		
	n	Mean ± *s*	*n*	Mean ± *s*	*P*
12 year (6th grade)	89	16.43 ± 17.47	73	18.63 ± 21.07	NS
13 year (7th grade)	36	15.72 ± 16.75	126	32.46 ± 46.07	$P < 0.01$
14 year (8th grade)	12	25.00 ± 23.30	150	42.37 ± 59.99	$P < 0.05$

NS = not significant.
s = standard deviation.

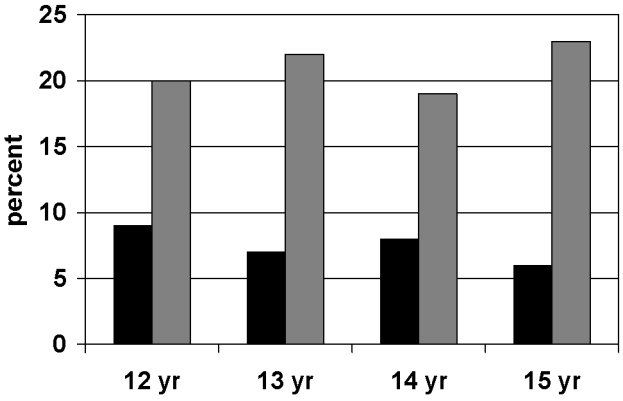

Fig. 2. Prevalence of high counts of mutans streptococci (MS) ■ and lactobacilli (LB) ▒ in stimulated whole saliva at baseline and after 1, 2, and 3 years in adolescent girls. High counts for MS were defined as scores 2 and 3 (≥100 CFU) on the Strip mutans (Dentocult-SM) and $\geq 10^5$/mL CFU of LB on Dentocult-LB.

the salivary progesterone concentrations and salivary buffer capacity or flow rate at any age investigated. On the other hand, a significant relationship between the salivary secretion rate and buffer capacity score was evident on all sampling occasions ($r = 0.42$; $P < 0.001$) indicating that a low saliva secretion was associated with a low buffer capacity.

The numbers of salivary mutans streptococci and lactobacilli were investigated, the results showing that fewer than 10% of the subjects harbored high counts of salivary mutans streptococci at baseline; the corresponding value regarding salivary lactobacilli was 20%. Although some variations were noted, no statistically significant changes of the bacterial scores appeared during the study period (Fig. 2).

The caries scores at baseline as well as the 3-year increment are presented in Table 3. At baseline, 44% of the girls were caries-free and after 3 years the corresponding value had decreased to 27%. Proximal lesions within the enamel were more prevalent than manifest lesions both at baseline and at the final examination. The majority experienced new manifest (62%) and/or proximal enamel lesions (57%) during the study period, while only 19% were totally caries-inactive. When studying the association between buffer capacity and caries increment, there was a

Table 3. Mean caries experience (±*s* and range) at 12 years of age (baseline) and 3-year increment in a cohort of 162 adolescent girls

	Baseline		3-year increment	
Caries index	Mean ± *s*	Range	Mean ± *s*	Range
DMFT	1.5 ± 2.3	0–10	1.1 ± 1.6	0–15
DMFS	1.7 ± 2.9	0–16	2.1 ± 3.0	0–24
ECa	2.3 ± 3.5	0–15	1.6 ± 2.9	0–22

s = standard deviation.

Table 4. Mean 3-year caries increment (±*s*) in relation to salivary buffer capacity during the first year of the study in 162 adolescent girls

		Caries increment		
Buffer capacity	*n*	No	Yes	Δ DMFT
Normal (pH ≥6.0)	80	52%	48%	0.9 ± 1.2
Impaired (pH 4.5–5.5)	72	46%	54%	1.2 ± 1.9
Poor (pH ≤4.0)	10	30%	70%	2.0 ± 2.7

statistically significant ($r = 0.20$; $P < 0.05$) correlation between low buffer capacity during the first study year and DMFT increment (Table 4). No statistically significant correlations between the salivary secretion rate or progesterone and caries were found. High counts of salivary mutans streptococci but not lactobacilli were significantly ($P < 0.01$) associated with caries increment (data not shown).

Discussion

Previous reports have suggested that adolescent girls may exhibit irregularities or a temporary drop in their buffer capacity around the age of 13 years (3, 10, 11) and one reason could be due to hormonal changes in puberty. The aim of this study was therefore to longitudinally investigate the salivary buffer capacity in relation to menarche and the sex hormone progesterone in the saliva from adolescent girls. Our study certainly confirmed that the salivary buffer capacity was significantly impaired in the 8th and 9th grades compared to the 6th grade, but we found no relationship to the individual time of menarche or to the individual levels of the female sex hormone progesterone. However, our findings do not rule out the possibility that other steroids, such as estrogens and growth hormones, may be involved in the process, and this warrants further study. The fact that our buffer capacity findings were obtained by a simple chair-side method was noteworthy, since this evaluation can easily be incorporated in the everyday routines of the general clinician.

The salivary progesterone analysis is thought to reflect the concentration of serum and to provide an attractive and non-invasive alternative to serum measurements monitoring ovarian function; this is of great benefit especially in longitudinal studies (4, 12). The levels of estrogen and progesterone in saliva are low during menses and both reach a broad peak during the lutheal phase (13, 14) and during pregnancy (15). Salivary progesterone levels are not substantially influenced by secretion rate, food intake, and dental care (16, 17). However, in agreement with previous findings, the great inter-individual variations, although stable within subjects, should be pointed out (18).

The impaired salivary buffer capacity may well be of clinical importance for caries development during the

teenage years, especially in light of the current concept suggesting an ecological plaque hypothesis (19). According to this, prolonged acid conditions may select for an overgrowth of aciduric microorganisms in the plaque, promoting a microbial community with the ability to demineralize tooth enamel. It has to be kept in mind that the permanent molars, premolars, and canines normally erupt between the ages of 10 and 13 years, and that these teeth may be susceptible, or even caries-prone, during the first post-eruptive years. In this study, a low buffer capacity was associated with a low secretion rate and increased caries activity, which reinforces findings from previous trials (20, 21). In the 6th grade, two-thirds of the girls exhibited a normal buffer capacity compared to 47% and 44% in the 8th and 9th grades, respectively. It can be speculated that the impaired buffer capacity may be due to the impaired dietary situation, since teenage girls often abandon their regular and prepared meals in favor of irregular small eating and snacking or even dieting in order to lose weight (22, 23). This was definitely also the case in our material, as described elsewhere (24). Another, but perhaps less possible, explanation may be the prevalence of regular smokers among the study group, which increased from 0% to 13% during the study period (data not shown).

In conclusion, the findings of this study suggest that salivary buffer capacity may be impaired over the adolescent years in girls, but that the reason for this remains unclear. No individual relationship was found to menarche or the levels of the female sex hormone progesterone. The impaired salivary buffer capacity may very well be of clinical importance for caries process, especially in the light of the current concept of an ecological plaque hypothesis.

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