Effect of low-dose glucocorticoid treatment on dentin formation and dentinal caries in rats

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The effect of a low dose of glucocorticoids on dentin formation and progression of dental caries was studied in young rats. The animals were inoculated in the mouth with *Streptococcus sobrinus* and fed ad libitum a high-sucrose, a high-starch, or a reference diet. The cortisone or placebo pellet was implanted subcutaneously in the back of the necks of the animals. After a 6-week experimental period the areas of dentin formation and dentinal caries were quantified planimetrically. Schiff's staining was used to classify caries. Cortisone reduced the dentin formation both in high-starch and reference diets, but it did not further affect the reducing effect of the high-sucrose diet on dentin formation. Cortisone did not affect the number or severity of dentinal carious lesions. □ *Caries; dentinogenesis; glucocorticoids; rats*

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A high-sucrose diet has been shown to reduce dentin formation and increase dental caries in the molars of growing rats (1–4). A high-starch diet also reduced dentin formation slightly in growing animals when compared with a standard laboratory rat diet without sucrose (4). High (19 ppm) fluoride concentration in drinking water further reduced dentin formation induced by dietary sucrose, but in contrast to sucrose it also reduced dentinal caries progression (5). Xylitol had the same kind of effect as fluoride on dentin and dental caries when some of the sucrose in the diet was replaced by it (6). These observations led to the paradox that a high-sucrose diet reduces dentin formation and induces dental caries, even though caries should stimulate reparative dentin formation (7).

Because there is a negative correlation between caries progression and the initial phase of secondary dentinogenesis, with a rapid rate of dentin formation in the same tooth, a cause–effect relationship should be possible, although not necessary (8). Therefore experiments reducing dentin formation without cariogenic challenge would shed light on the problem of the possible connections between reduced dentin formation and increased caries activity.

High doses of glucocorticoids are known to reduce dentin formation (9). Sweeney & Shaw (10), however, did not find any significant differences in the initiation or progression of dental caries with a high dose of cortisone medication in rats, indicating that no cause–effect relationship exists between reduced dentin formation and progression of dental caries.

As no data are available about the effect of a low-dose concentration of glucocorticoids on dentin formation or dental caries, we conducted this study to ascertain the effect on these variables, with and without cariogenic challenge.

Materials and methods

Weanling female Sprague–Dawley rats were randomly divided into 2 groups with 30 in each, weighed, marked, and given an intraperitoneal tetracycline injection (Terramycin®, 30 mg/kg; Pfizer, Belgium) to mark dentin formation at the start of the experiment. At the same time the rats were anesthetized with midazolam (Dormicum®), Roche, Switzerland; fentanylfluanisone (Hypnorm®), Janssen Pharmaceutica, Belgium; sterile water, 1:1:2, 0.2 mg/100 of rat weight, given intraperitoneally), and a cortisone or placebo pellet (Innovative Research of America, Toledo, Ohio, USA) was implanted subcutaneously in the back of the neck. Each cortisone pellet held 25 mg of cortisone for 60 days' release, resulting in a release of 0.42 mg/day.

The cortisone and placebo groups were further divided into three different diet subgroups, as shown in Table 1. The diet compositions are defined in Table 2. The first subgroups received a modified Stephan–Harris high-sucrose diet. The second subgroups received a starch diet similar to the first diet except that sucrose was replaced with potato flour. For reference, the third subgroups were fed a powdered standard laboratory rat diet (Brood Stock Feed for Rats and Mice

Table 1. Grouping and treatment of the rats in the experiment

Group	Diet	Medication	No. of rats
1	Sucrose	Cortisone	10
2	Sucrose	Placebo	10
3	Starch	Cortisone	10
4	Starch	Placebo	10
5	Reference	Cortisone	10
6	Reference	Placebo	10

Table 2. *Composition of the diets*

Diet	Ingredient	Amount (%)
Sucrose diet	Sucrose	43
	Wheat flour	22
	Skim milk powder	32
	Vegetable oil	1
	Liver powder	2
Starch diet	Potato flour	43
	Wheat flour	22
	Skim milk powder	32
	Vegetable oil	1
	Liver powder	2
Reference diet	Oat flour	28
	Wheat products	50
	Soya	7
	Fish powder	7
	Fodder yeast	3
	Minerals	3
	Fat	3
	Minerals and trace elements	

R3, Ewos Ab, Södertälje, Sweden). All groups received distilled drinking water and food ad libitum.

For the cariogenic challenge the rats were inoculated orally with *Streptococcus sobrinus* (ATCC 27351) on the 2nd and 3rd day of the experiment and once a week thereafter for 6 weeks.

The animals were housed two or three per cage at a temperature of 21°C and subjected to the same lighting regimen (12 h light and 12 h dark) and the same frequency of human handling and noise. Weight gains were recorded weekly and food and water consumption were recorded at intervals of a few days throughout the experiment.

After the 6-week experiment the rats were anesthetized and killed by decapitation. The stability of cortisone and placebo pellets was examined by inspecting them from a surgical incision.

The mandibles were defleshed and sectioned sagittally (11). The areas of dentin formation and dentinal caries lesions under the middle fissure of the first molar and the mesial one of the second molar were determined by photographing them on Kodak Ektacrome daylight film, 1000 ASA, in ultraviolet light (460 nm) and measuring the areas from video images by outlining them as they appeared on the monitor with a serial mouse connected to a PCVision frame grabber and an Image measure computer program, which calculated the enclosed area (12, 13).

The sections were also stained with Schiff's reagent, and the intact fissures and the fissures with enamel or dentinal lesions of all three molars were counted by means of a modification of the method of König et al. (14).

Statistical analyses were performed by using SPSS for MS WINDOWS Release 6.0. Means, standard deviations, and 95% confidence intervals for means were calculated for dentin formation and dentinal caries progression. To analyze the effect of diet on dentin formation and dentinal caries progression, one-way analysis of variance with Tukey's honestly significant difference test was used to determine whether differences within cortisone and placebo groups existed and to determine the differences in the weight gains between

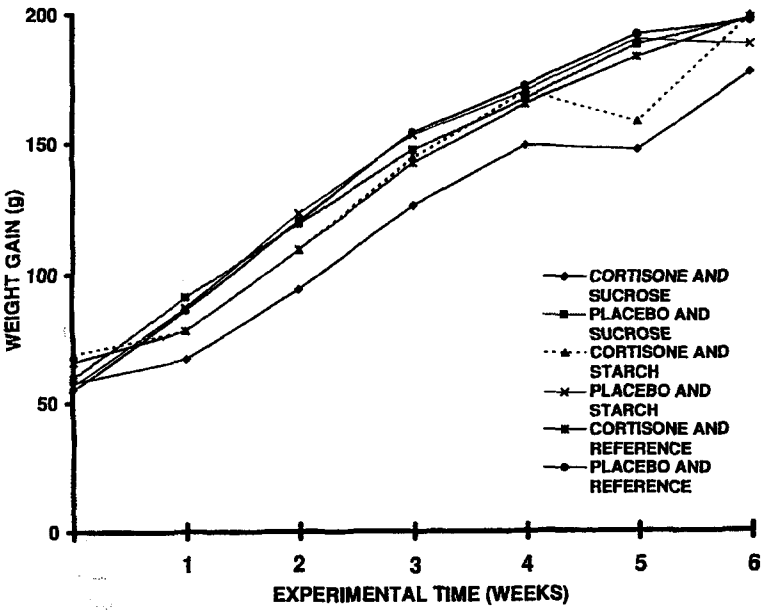

Fig. 1. The average weight gain from the start of the experiment until death.

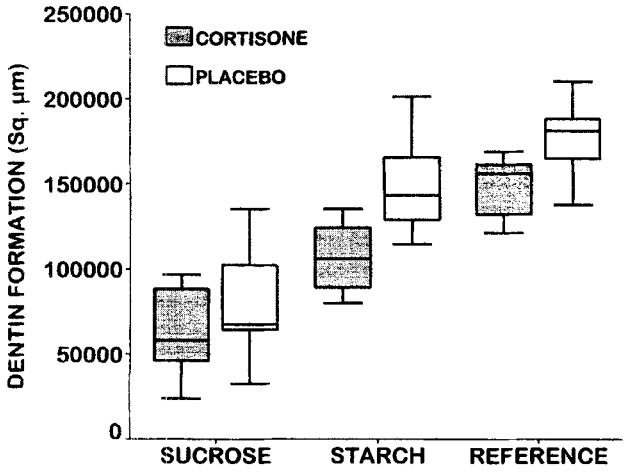

Fig. 2. The areas of dentin formation in the first mandibular molars in square micrometers, illustrated as 'box and whiskers'. The lower boundary of the box represents the 25th percentile, and the upper one the 75th percentile; therefore 50% of the values are located within the box. The horizontal line inside the box is the median, and the whiskers show the lowest and the highest values.

the groups. To analyze the effect of cortisone within each diet, the Mann–Whitney U-test was used. Because the Gaussian curves and Kolmogorow–Smirnoff tests showed that the curves in some groups did not follow the normal distribution very well, the non-parametric test was supposed to give more certain results. The pre-chosen significance level was set at $P < 0.05$.

Two-way ANOVA was used to analyze the interaction between the diet and medication on the general growth, the dentin formation, and the area of dentinal caries.

Results

All animals survived the experiment, and they seemed healthy and lively in all the groups. The location of the cortisone and placebo pellets was stable in all the rats during the entire experiment. Without cortisone, the different diets alone did not alter weight gain. No differences in food or water consumption between the groups were present (data not shown). Two-way ANOVA showed an interaction between the diet and medication, which slightly reduced the weight gain in the cortisone groups with starch and sucrose diets at the measurements in the 4th and 5th weeks (Fig. 1).

Cortisone reduced the dentin formation in the first molars in the starch and reference diet groups. The difference was significant. Non-significant reduction was seen in the second molars in these groups. Cortisone had no significant effect on dentin formation with the high-sucrose diet (Fig. 2 and Table 3). The starch diet also markedly reduced dentin formation in comparison with the reference diet in both molars (Table 3).

The areas of dentinal caries lesions were significantly larger in the high-sucrose diet groups, and cortisone did not alter this effect (Table 4). In the starch and reference diet groups practically no dentinal caries lesions were seen.

In the high-sucrose groups the number of the carious fissures was significantly greater (50% in the cortisone and 47% in the placebo groups) than in other diet groups, in which practically all the fissures (97–100%) were intact, as shown with Schiff's reagent. In the high-sucrose group cortisone did not induce any further

Table 3. The area of dentin formation (μm^2, $\times 10^3$): means and standard deviations (s) and 95% confidence intervals (CI) of the mean. The statistical analyses between diets were performed with ANOVA with Tukey's honestly significant difference test, and the analyses between cortisone and placebo with the Mann–Whitney U-test

Group	Mean	s	95% CI
First mandibular molar			
Sucrose + cortisone	78.0*†	36.6	47.4; 108.6
Sucrose + placebo	87.7*†	49.0	50.0; 125.3
Starch + cortisone	110.3†‡	19.8	97.7; 122.9
Starch + placebo	149.5	29.7	128.2; 170.7
Reference + cortisone	157.3[c]	24.5	139.8; 174.8
Reference + placebo	176.3	19.0	164.8; 187.8
Second mandibular molar			
Sucrose + cortisone	66.3*†	31.1	40.4; 92.3
Sucrose + placebo	54.3*†	17.6	40.7; 67.8
Starch + cortisone	80.6†	23.4	65.7; 95.5
Starch + placebo	98.5†	16.6	86.6; 110.4
Reference + cortisone	118.5	27.4	98.9; 138.1
Reference + placebo	121.4	17.9	110.6; 132.3

* Significantly different from starch diet.
† Significantly different from reference diet.
‡ Significantly different from placebo.

Table 4. The area of dentinal caries lesions (μm^2, $\times 10^3$): mean ± standard deviation (s) and 95% confidence intervals (CI) of the mean. Statistical analyses and abbreviations as in Table 3

Group	Mean	s	95% CI
First mandibular molar			
Sucrose + cortisone	49.6*†	29.7	24.7; 74.4
Sucrose + placebo	32.3*†	32.4	7.4; 57.3
Starch + cortisone	2.4	8.4	−2.9; 7.7
Starch + placebo	0		
Reference + cortisone	0		
Reference + placebo	0.6	2.0	−0.7; 1.8
Second mandibular molar			
Sucrose + cortisone	57.1*†	48.6	16.4; 97.8
Sucrose + placebo	41.9*†	40.3	10.9; 72.8
Starch + cortisone	0		
Starch + placebo	0		
Reference + cortisone	0		
Reference + placebo	0		

* Significantly different from starch diet.
† Significantly different from reference diet.

caries (Table 5). No synergistic effect was seen between diet and medication either on dentin formation or dental caries.

Discussion

The two-way ANOVA showed an interaction in weight gain between the diet and medication in the 5th week measurement in cortisone groups with the sucrose and starch diets. The diet itself did not affect weight gain. The onset of the weight gain was delayed in the sucrose and starch diet groups. In the cortisone group with a high-sucrose diet the weight of the other groups was never quite reached. Cortisone slightly reduced the weight gain with starch and clearly with sucrose. This might be explained with the altered glucose metabolism of the rats. Glucocorticoids increase the blood glucose level and might in that manner retard the growth of animals, in the same manner as diabetes in animals (15).

As the difference in the final weight between the groups did not vary markedly, we suggest that the difference in dentin formation between the groups is independent of the final weight. This supports earlier findings that in spite of a marked difference in the weights between the sexes, no differences exist in dentin formation (4).

Johannessen (9) has reported results similar to ours: a depressed dentinogenesis in the mandibular first molars of the young rat after administration of cortisone in the diet. In his investigation the cortisone concentration was high enough to markedly retard the general growth in addition to the depressed dentin formation.

We assume that the reduced dentinal growth with the cortisone was caused by the inhibition of protein metabolism in the odontoblasts, resembling the effects of glucocorticoids on bone cells (16). Steroid treatment affects the calcium balance of the body by reducing the intestinal calcium absorption and increasing the calcium excretion from kidneys and thus, in at least two ways, results in a negative calcium balance (17). A high-sucrose diet is also known to induce alterations in calcium balance in humans (18, 19) and bone composition in animals (20). The effect on dentin may be mediated via similar mechanisms as in bone, because both are in many ways regulated in a similar fashion (21).

Table 5. Number of carious fissures analyzed with Schiff's reaction. All fissures analyzed together (percentage within parenthesis)

Group	Intact	Enamel lesion	Dentinal lesion
Sucrose + cortisone	54 (50)	38 (35)	16 (15)
Sucrose + placebo	57 (53)	32 (30)	19 (17)
Starch + cortisone	100 (97)	8 (7)	0
Starch + placebo	106 (98)	2 (2)	0
Reference + cortisone	108 (100)	0	0
Reference + placebo	105 (98)	3 (2)	0

It has previously been observed that the high-sucrose diet reduced the rate of dentin formation (4, 6). The reducing effect of sucrose on dentin formation is so great that it masks the reducing effect of cortisone alone. In all other diet groups cortisone clearly reduced the dentin formation. Two-way ANOVA did not support the synergism of the effects of the diet and medication on dentin formation or dentinal caries progression, suggesting that both of them act individually.

Cortisone did not at this concentration modify the cariogenic effect of a high-sucrose diet. This confirms a previous report (10), in which cortisone did not affect the severity or the number of carious lesions even with higher doses. Its salivary effect does not modify caries activity either.

Because cortisone reduced dentin formation without an effect on caries progression, this further supports the view that there is no cause–effect relationship between caries progression and retarded dentin formation, both of them being different entities.

Our results point out the effect of both sucrose and cortisone on dentin, probably via retarded formation, altered structure and/or some other change in the growth and development of dentin.

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