

# The effect of two sucrose diets on formation of dentin and predentin in growing rats

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The effect of two high-sucrose diets on dentinal caries, dentin formation, and the predentin width was studied in Sprague-Dawley rats. Rats were weaned at the age of 3 weeks and for 4 weeks fed a non-cariogenic commercial rat food (R36) for control, a high-sucrose Stephan-Harris (S-H) diet, or a new high-sucrose (sR36) diet in which most of the barley and wheat flour of the control R36 diet were replaced by sucrose. The areas of dentinal caries, the areas of dentin formation, and the width of predentin and dentin were quantified. Both high-sucrose diets induced dentinal caries, and both reduced dentin formation and increased the width of predentin compared with the control diet. Moreover, rats fed the S-H high-sucrose diet showed significantly greater progression of caries and reduction of dentin formation relative to rats fed the new high-sucrose diet, sR36. The high-sucrose diet thus was a substrate for caries-inducing microbes and a significant, but possibly not the exclusive, substrate for host modulation of odontoblast function.

□ *Dental caries; food; odontoblasts; physiologic calcification*

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Dental caries is a dietary-conditioned infectious disease, profoundly affected by the presence of sucrose in the diet (1). The Stephan-Harris (S-H) high-sucrose diet is well demonstrated to be caries-inductive in young and old rats (2-7). It has, in addition, been shown to both reduce the formation of dentin and increase the width of predentin in young rats (7, 8).

Physiologic dentin is described as primary and secondary, depending on whether it is formed before or after completed root formation. A third form, reparative or reactive dentin, is formed in response to caries or other outer influences (9).

Dentin formation represents the secretion and subsequent mineralization of collagen matrix. In healthy teeth the ratio between the deposition of the non-mineralized portion, predentin, and the formation of mineralized physiologic dentin is amazingly stable (10). The rate of predentin formation under normal conditions accordingly reflects the rate of dentin formation and vice versa (10). The width of the predentin, on the other hand, reflects the rate of mineralization.

Progressing from enamel into dentin, the carious process advances from non-cellular hard tissue into hard tissue, where cellular processes, from odontoblasts and possibly pulpal fibroblasts, are involved. There is increasing evidence that the progression of dentinal caries is cell-mediated (11). The suppressing effect of a high-sucrose diet on primary dentin formation and its increasing effect on the width of predentin described above are both considered to be mediated via the odontoblasts (3, 7). The problem remains open whether the effects are due the high-sucrose content itself or the

other ingredients of the S-H diet. The present study was carried out to clear up this problem, using both the S-H diet and a new high-sucrose diet, both similar in sucrose content but differing in other elements, recording the effects by progression of caries, formation of primary dentin, and the width of predentin.

## Materials and methods

Thirty rats of both sexes were weaned on day 21, weighed, marked, and divided into three different groups. One group of rats was fed a powdered commercial standard non-cariogenic control diet specially produced for growing rats and mice (Brood Stock Feed for Rats and Mice R36, Finnewos Aqua Oy, Turku, Finland). This control diet contains 34.0% barley flour, 43.0% wheat flour, 4.0% wheat grains, 4.5% vitamins and trace elements, 5.0% soya, 4.0% fish powder, and 4.5% other ingredients.

The second group of rats was fed a cariogenic S-H diet containing 41% sucrose plus 22% wheat flour, 32% milk powder, 2% liver powder, and 3% corn oil and has previously been tested in our laboratory.

The third group was fed a new high-sucrose diet in which the sucrose was added to the control R36 diet so that the energy value of the new diet remained practically the same as that of the control R36 diet. The new sucrose diet (sR36) contained 10.1% barley flour, 10.3% wheat flour, 41.0% sucrose, and 5.8% casein. The casein was added to compensate for the loss of protein when the wheat and barley flour content was reduced. The other ingredients were the same. The

Table 1. The percentage distribution of the diets (%)

Nutritive substance	Control diet	Stephan-Harris diet	New sucrose diet
Protein	19	14	16
Fat	4	4	3
Carbohydrates	56	72	67
Filaments	3	1	3
Ash	6	3	5
Moisture content	12	6	6

nutritional values of the diets are calculated in Table 1. Food and drink given as distilled water were available ad libitum.

All the animals were given an intraperitoneal injection of oxytetracycline hydrochloride (30 mg/kg; Terramycin, Pfizer Corp., Brussels, Belgium) at the age of 21 days and 2 days before death at the end of the experiment to mark the dentin formed during the test period. For cariogenic challenge and stabilization of the oral flora all rats were inoculated with a fresh suspension of *Streptococcus sobrinus* (ATCC 27351 K 1 Fitzgerald).

At the age of 49 days the animals were weighed and then decapitated under carbon dioxide. The jaws were defleshed and preserved in 40% ethanol. The molars of the hemimandibles were sectioned sagittally by the method of Keyes (12). Dentin formation and caries lesions were quantified by examining the first and second molars under an Orthoplan Ploemopak microscope equipped with incident fluorescent light (emission wavelength, 460 nm), which showed the carious lesions (13) and the tetracycline stripes marking the dentin formed during the experiment. The area under the main central transverse fissure of each molar was photographed with Kodak Ektachrome daylight film (400 ASA). The dental carious lesions, the tetracy-

cline-marked dentinal zones, and the thicknesses under the main fissure were measured planimetrically from video images by circumscribing their respective areas on a monitor, as described previously (2, 13). The first and second molars were analyzed together. All tissue sections were coded and randomized before measurements.

Molars from the upper jaws were fixed in 10% formalin before being decalcified in 5% formic acid. After decalcification for 4-5 weeks, the jaws were processed with glycolmethacrylate (GMA) and were then embedded in blocks and cut into sections. The sections were stained with toluidine blue (STB). The width of the predentin was measured under a microscope at six sites under the main fissures of the first and second molars, and the mean was considered to represent the thickness of predentin for that particular animal (7).

The statistical analysis were performed using SPSS MS Windows Release 6.0. One-way ANOVA with Tukey's honestly significant difference (HSD) test with a significance level of 0.05 was used to compare dentin formation, dentinal caries, and the predentin zone between the groups.

## Results

No statistically significant differences in the areas of dentinal carious lesions, in the area of formed dentin, or in the width of predentin were found between the first and second molars within each group, and thus the results for both teeth were combined when the mean values were presented. However, when statistical analysis was performed, the number of animals was used (*n*).

There were no significant differences in weight gains or between water and food consumptions (data not shown).

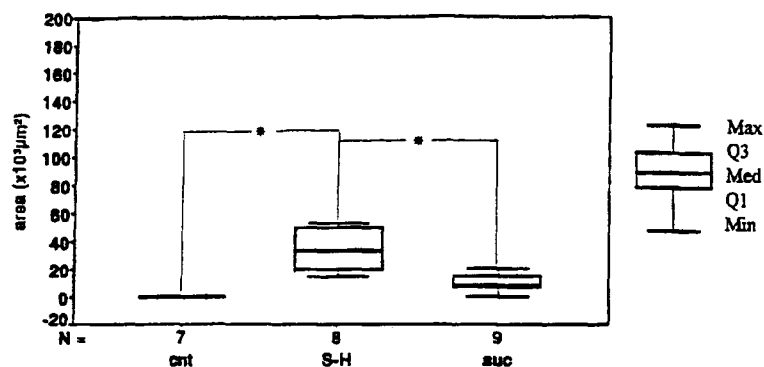


Fig. 1. The areas of dentinal carious lesions. Abbreviations: cnt = the control diet; S-H = the Stephan-Harris sucrose diet; suc = the new sucrose diet. Min = minimum; Q1 = 25% quartile; Med = median; Q3 = 75% quartile; Max = maximum. \*Indicates a statistically significant difference (ANOVA with Tukey's HSD test).

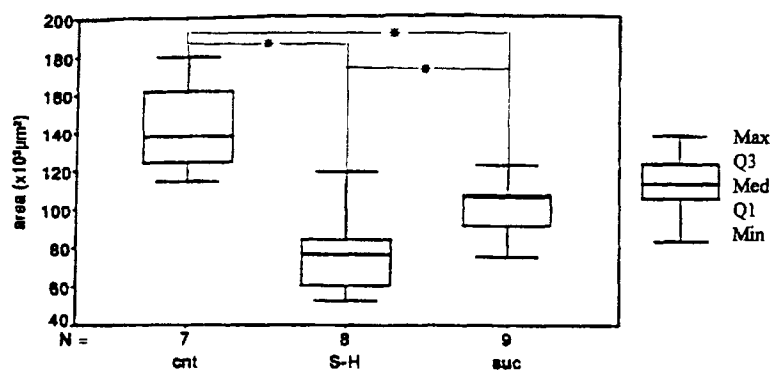


Fig. 2. The areas of dentin formation during the experiment. Abbreviations as in Fig. 1.

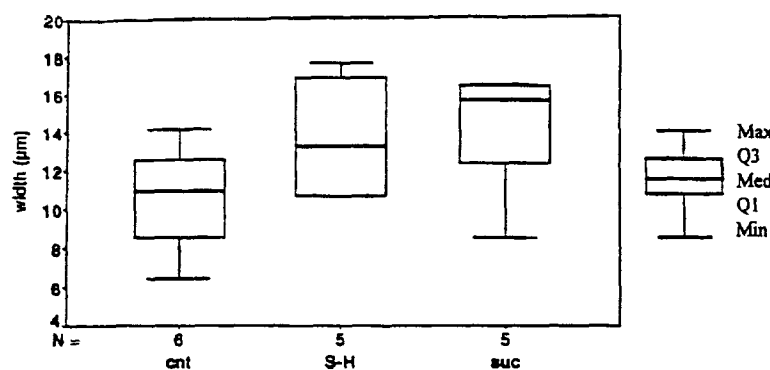


Fig. 3. The width of the predentin in micrometers. Abbreviations as in Fig. 1.

Both high-sucrose groups showed dentinal caries. The areas of dentinal carious lesions were greater in the S-H group than in the new sucrose group ( $P < 0.05$ ). The control group did not show dentinal caries (Fig. 1).

Both sucrose groups reduced significantly the area of dentin formed ( $P < 0.05$ ) when compared with the control group; however, the effect in the S-H group was more pronounced ( $P < 0.05$ ) (Fig. 2).

The dentin thickness in both sucrose groups was also smaller than in the control group. The differences were statistically significant ( $P < 0.05$ ) between the S-H and control group (data not shown).

There was a slightly wider predentin zone in both sucrose groups than in control group (Fig. 3), although the difference was not statistically significant (Table 2).

## Discussion

The rats remained healthy and were housed similarly, and the weight gains did not differ significantly, which supports the concept that the rat adjusts its food intake to meet energy demands (14).

Both sucrose diets reduced dentin formation when compared with the control diet, which is in line with previous studies (3–8, 15), in which a high-sucrose S-H

diet caused reduced dentin formation. The results support the concept that it is the sucrose in the diet that disturbs dentin formation. However, the more pronounced reduction in dentin formation with the S-H diet indicates that there may also be some nutritional deficiencies or some other ingredients in this diet which further enhance the effect.

In our study the areas of dentinal carious lesions were smaller with the new sucrose diet, and the dentin reduction was not so pronounced as with the S-H diet, which may indicate that disturbed dentin formation may also be connected with accelerated caries progression, although it was not so inevitably connected in previous studies (3, 7).

The formation of dentin involves secretion of matrix proteins by odontoblasts, before the deposition of apatite crystals. These matrix proteins probably play a role in the transformation of predentin to dentin (16). Proteoglycans of dentin have been implicated in cell regulation and in inhibition and initiation of mineralization. The organic matrix of circumpulpal dentin is, with the exception of a minor serum protein content, produced entirely by the odontoblasts (9).

Biochemical and metabolic changes in the organic matrix may be partly responsible for the irregular mineralization (17) and cause the widening of pre-

Table 2. Means, standard deviations (*s*), and 95% confidence intervals of the areas of dentin formation and caries lesions, and widths of predentin zone of various groups

	Mean	<i>s</i>	95% CI
Areas of caries lesions ( $\times 1000 \mu\text{m}^2$ )			
Control diet	0	0	0
Stephan-Harris diet	40.5*	29.6	15.7; 65.2
New sucrose diet	9.7†	7.0	9.4; 15.1
Areas of dentin formation ( $\times 1000 \mu\text{m}^2$ )			
Control diet	142.9	24.4	120.4; 165.5
Stephan-Harris diet	76.9*	21.1	59.3; 94.5
New sucrose diet	101.7*†	14.7	90.4; 113.1
Width of predentin zone ( $\mu\text{m}$ )			
Control diet	10.6	2.9	7.6; 13.6
Stephan-Harris diet	13.8	3.3	9.7; 17.9
New sucrose diet	13.8	3.4	9.6; 18.1

\* Significantly different from the control group.

† Significantly different from the Stephan-Harris group.

dentin. Couve (18) postulated that the predentin may change its thickness as a response to metabolic disorders. There are also studies (19–21) in which low-calcium diets and vitamin D deficiency increased the predentin width. In our study the cariogenic diets increased the width of predentin, as also observed in earlier studies (8, 9) indicating disturbed mineralization and matrix synthesis. This disturbance of mineralization may be caused by metabolic disorders caused by nutritional deficiencies or by some other ingredients or by sucrose, which may indicate that the sucrose in the diet is a substrate both for the caries-inducing microbes causing carious lesions and for the host, modulating dentin formation by odontoblast function. Further studies are needed to solve the relation.

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