High-sucrose diet reduces defensive reactions of the pulpo-dentinal complex to dentinal caries in young rats

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The significance of systemic dietary effects on the response of the pulpo-dentinal complex to dentinal caries was examined. Weanling rats were divided into high sucrose or control diet groups both with and without cariogenic bacterial inoculation. At the onset, tetracycline was injected to mark the dentin formation during the experiment. After 5–6 week, mandibular molars were sectioned sagittally. The areas of dentin formed during the experiment and those of dentinal caries were quantified separately in the first and second molars. In the control diet groups the area of dentin was significantly greater under carious fissures, whereas in the high sucrose diet groups the area of dentin formed did not differ between intact and carious fissures. The high sucrose diet resulted in a significantly smaller area of dentin formation than did the control diet. The high sucrose diet with cariogenic bacterial inoculation resulted in the greatest area of dentinal caries. With the control diet a positive response against dentinal caries occurs, but the high dietary sucrose content impairs the defensive reactions of pulpo-dentinal complex against dentinal caries. These findings add further evidence of the importance of the local endogenous factors of caries progression.
☐ *Caries; dentin; diet; pulpo-dentinal complex; rats*

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In addition to its cariogenic effect, a high concentration (43 g/100 g) of dietary sucrose has been shown to reduce both dentin formation (1–3) and mineralization of dentin (4) in the molars of young rats. Detrimental effects of dentinal caries lesions on cell function is not likely, since no differences were detected in the amount of dentin under intact and carious fissures in the high-sucrose group (1). This observation is also paradoxical to the present concept of the effect of caries on dentin. Caries has been supposed to activate the deposition of dentin in the form of reparative dentin (5) regardless of the manner of caries induction.

The caries process advances from non-cellular enamel to dentin. Dentin containing an inorganic component closely associated with an organic extracellular matrix is connected to living cells—that is, odontoblasts and other pulpal cells. The pulpo-dentinal complex is capable of responding to caries even in its very initial stage (5–8). The possibility of the systemic factors modulating this response (2) challenges the traditional opinion that caries is regulated only by factors affecting the external surface of the teeth.

Dietary carbohydrates and the virulence of cariogenic bacteria are considered the most important factors for caries initiation in enamel. The defence mechanisms of the pulpo-dentinal complex in the regulation of caries progression is not known, but the progression of the lesion in dentin is probably modulated not only by the intensity of bacterial challenge or by endogenous factors within the dentin but also by systemic factors through dental pulp. Indeed, the negative correlation between dentin formation and dentinal caries progression suggests that deprivation of the systemic defence reactions by the high sucrose diet might enhance the dentinal caries progression (1, 3). However, these assumptions are based on a fairly small data set. To clarify further the roles of external and internal factors affecting the dentinal caries progression, we collected a larger data set to test the relationship between the dentin formation and dentinal caries progression in the molar teeth of young rats fed either a control or a high-sucrose diet with and without cariogenic bacterial inoculation.

Materials and methods

Animals, animal care, and collection of the data

The areas of dentin formation and dentinal caries from five separate experiments were combined to form two larger data sets (see later). The aim of this approach was to collect a data set large enough to diminish the effect of the variation between individuals on the results and to assess more reliably the association between dentin formation and dentinal caries progression. The experiments were similar in terms of all the factors affecting the results: a) all the experiments were performed in our laboratory with Wistar or Sprague–Dawley rats; b) the timing of the start of the experiment (at 21 days of age) and the duration of the experiment (5–6 weeks) were similar; c) the diets were

Table 1. The area of dentin formed in the first and second mandibular molars of rats fed a high-sucrose or a control diet with and without cariogenic bacterial inoculation for 5–6 weeks*

Group	*n*	Dentin area ($\mu m^2 \times 10^3$)
First mandibular molar		
Sucrose with bacteria	36	111.4 ± 52.1^a
Sucrose without bacteria	55	127.4 ± 31.8^a
Control with bacteria	38	177.7 ± 33.2^b
Control without bacteria	52	199.4 ± 43.8^c
Second mandibular molar		
Sucrose with bacteria	36	87.9 ± 46.9^a
Sucrose without bacteria	55	101.9 ± 27.2^a
Control with bacteria	38	138.7 ± 34.8^b
Control without bacteria	52	150.7 ± 39.8^b

* Values are means ± standard deviation. Values with different superscript letters differ significantly ($P < 0.05$).

Table 2. The area of dentinal caries lesions in the first and second mandibular molars of rats fed a high-sucrose or a control diet with and without cariogenic bacterial inoculation for 5–6 weeks*

Group	*n*	Caries area ($\mu m^2 \times 10^3$)		
		Min	Med	Max
First mandibular molar				
Sucrose with bacteria	36	0.0	35.6^a	130.6
Sucrose without bacteria	55	0.0	12.5^b	43.4
Control with bacteria	38	0.0	0.0^c	44.0
Control without bacteria	52	0.0	0.0^c	30.9
Second mandibular molar				
Sucrose with bacteria	36	7.6	51.7^a	127.3
Sucrose without bacteria	55	0.0	14.7^b	46.4
Control with bacteria	38	0.0	0.0^c	70.5
Control without bacteria	52	0.0	0.0^d	25.6

* Values are minimum, median, and maximum. Values with different superscript letters differ significantly ($P < 0.05$).

the same in all experiments. The high-sucrose diet contained 43% sucrose, 22% wheat flour, 32% milk powder, 2% whole liver powder, and 1% corn oil. Control diets were standard laboratory diets containing 28%–34% oat flour, 50%–48% wheat products, 5%–7% soya, 4%–7% fish powder, and 8% other products (Brood Stock Feed for Rats and Mice R3 and R36, Ewos AB, Södertälje, Sweden). All the diets satisfied fairly well the requirements of the National Research Council (9). The free fluoride concentration of the sucrose diet was 0 ppm and that of the control diets 0.05 ppm; the total fluoride concentration was 5 and 50 ppm, respectively. In the preliminary analysis there were no differences in dentin formation or caries progression between the control diet groups, so they were combined for the analyses. Only the groups fed the sucrose or control diet with and without bacterial inoculation but without any additional experimental procedures were included from each experiment, and food and water were freely available; d) animal care was similar (housing, handling, feeding, and noise); and e) specimen processing was identical (the cutting of the mandibles and measuring the dentin formation and dentinal caries). At the beginning of each experiment all the animals were given an intraperitoneal injection of oxytetracycline hydrochloride (30–40 mg/kg, Terramycin®, Pfizer Corp., Brussels, Belgium) to mark the mineralizing dentin at that time. All experimental procedures were approved by the Animal Care and Use Committee, University of Oulu, Oulu, Finland.

The first data were collected to investigate the role of rapid caries progression in the defensive mechanisms of the pulpo-dentinal complex as evaluated by the amount of dentin formed during the experiment. The rats were inoculated with a fresh suspension of *Streptococcus sobrinus* (ATCC 27531 K 1 Fitzgerald) on days 2 and 3 of the experiment and then weekly to ensure the cariogenic challenge. In these combined data there were 36 animals in the sucrose group and 38 in the control group.

The second data set was collected to evaluate the importance of the systemic dietary factors for the defence reaction of the pulpo-dentinal complex with minimal cariogenic challenge. For this reason the rats were not inoculated with cariogenic bacteria. There were 55 rats in the sucrose group and 52 in the control group.

At the end of the experiments, the animals were anesthetized either with ether or with a combination of fentanyl-fluanisone (Hypnorm®, Janssen Pharmaceutica, Brussels, Belgium) and midazolam (Dormicum®, Roche, Basel, Switzerland), and killed by decapitation. The mandibles were dissected, defleshed, and sectioned sagittally by the technique of Keyes (10). To measure the amount of dentin formation, the first and second molars were each examined under an Orthoplan Ploemopack microscope (Leitz, Westlar, Germany) equipped with fluorescent light (detector wavelength, 460 nm), with which the tetracycline stripes surrounding the newly formed dentin during the experiment could readily be seen (11). The main central transverse fissure of the first mandibular molar and the mesial one of the second molar were photographed with Kodak Ektacrome daylight film (400 ASA; Kodak, UK). The tetracycline-marked zones of dentin formation were measured planimetrically from video images by circumscribing their respective areas on a monitor (Salora 445 A RGB, Salo, Finland; camera, Hitachi VKM 96 E, Tokyo, Japan) using a serial 'mouse' connected to a PCVision Frame Grabber (Imaging Technology, Woburn, Mass., USA) (11). The size of the dentinal caries lesion, seen as a change of fluorescence (12) under the fissure, was also measured planimetrically as described above.

Statistical methods

Statistical analyses were performed using the SPSS statistical package (SPSS Version 7.1, SPSS, Chigago, Ill., USA). Means and standard deviations were calculated for the areas of dentin formation. Median values with minimums and maximums were calculated for the areas of dentinal caries because these data were not normally

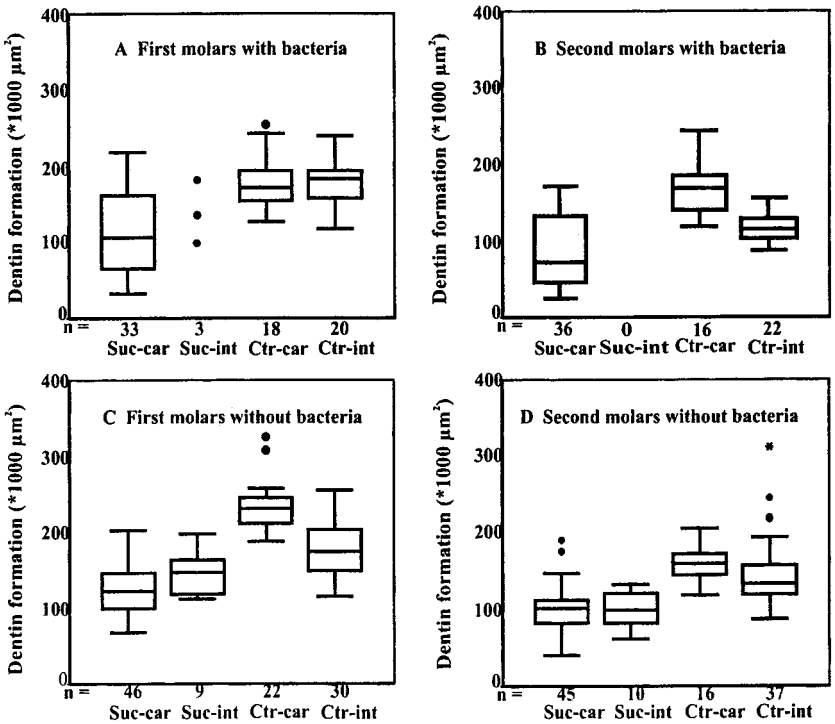

Fig. 1. The areas of dentin under intact (int) and carious (car) fissures in the molars of rats fed a high-sucrose (Suc) or control (Ctr) diet with (A and B) and without (C and D) cariogenic bacterial inoculation. There were no differences between intact (Suc–int) and carious (Suc–car) fissures in the sucrose diet groups. In the control diet groups a significantly greater area of dentin formation was observed in the first molars without cariogenic bacteria and in the second molars with cariogenic bacteria under carious (Ctr-car) fissures than in intact (Ctr-int) fissures. The box shows the 1st and 3rd quartiles with the median value inside, and the whiskers show the minimum and maximum. The dots and asterisk represent the values higher than the statistically calculated maximal value in the 'box and whiskers'. In the sucrose diet group there were only three intact fissures in the first molars, and the three dots represent these individual values; in the second molar no intact fissures were observed.

distributed. One-way ANOVA with Tukey's honestly significant difference test was used to identify differences in the area of dentin formation between the groups. To analyze the differences between the groups in the area of dentinal caries, the non-parametric Kruskal–Wallis ANOVA was used to determine whether an overall difference existed among the groups. If a difference was detected, the Mann–Whitney U-test was used to determine differences among the groups. After selection of fissures into intact and carious within the groups, an independent samples *t* test was used to determine differences in the areas of dentin within each group. The level of statistical significance was set at $P < 0.05$.

Results

No differences were found in the weight gain of the rats or in food or water consumption between the groups (data not shown).

The high-sucrose diet with and without bacterial inoculation significantly reduced dentin formation in the first and second molars compared with the control diet. The formation rate of dentin was higher in the control diet group without bacterial inoculation than in the respective diet group with cariogenic bacteria in the first molars (Table 1).

Rats fed the sucrose diet and inoculated with cariogenic bacteria showed the largest dentinal caries lesions in both molars. Even though the inoculation of cariogenic bacteria increased the area of dentinal caries in the control group, the sucrose diet without cariogenic bacteria caused an even greater increase the area of dentinal caries than the control diet with bacteria in both molars (Table 2).

When intact and carious fissures were analyzed separately in different groups, the area of dentin was significantly greater under carious fissures than under intact fissures in the control diet group in the second molars with cariogenic bacteria (Fig. 1B) and in the first molars without cariogenic bacteria (Fig. 1C). In the control diet group in the second molars without cariogenic bacteria the area of dentin was also greater under carious fissures (Fig. 1D), but statistical significance was not reached. In the high-sucrose diet groups no differences

in the area of dentin formation was detected under intact or carious fissures (Fig. 1).

Discussion

As we collected the data from several experiments, two rat strains were included in the analyses. In caries experiments different rat strains have been used. Although there may be differences in caries susceptibility between different strains, there is evidence that individual variation is also marked (13, 14). In this experiment we did not find any obvious strain differences with regard to either caries progression or dentin formation. Furthermore, in our data the analysis of the dentin formation and caries progression was done tooth by tooth. Thus we think that the use of two different strains of rats did not have an influence on the results or conclusions from the findings.

The finding that the effect of a high-sucrose diet reduces dentin formation under both intact and carious fissures is in accordance with our previous study (1), in which we showed that the critical concentration with regard to the dentin formation was between 30% and 43% of dietary sucrose. Notably, in most animal caries experiments the concentration of dietary sucrose has been between 43% and 70% (15). In those experiments the external factors causing the initiation of enamel caries have been considered to be of importance also in caries progression in dentin. Although there is much evidence that the pulpodentinal complex responds differently to slowly and rapidly progressing caries, possibly regulated by the growth factors released from carious dentin (16), the systemic effects modifying the defence reactions have not previously been considered to be of importance.

Rats fed the control diet showed significantly smaller caries lesions than those fed the sucrose diet. This indicates that without dietary sucrose, even the inoculation of cariogenic bacteria does not result in rapid dentinal caries progression. This supports the previous finding that the relationship between the bacterial counts and caries is not very strong, the relationship between dietary sucrose and dental caries being stronger (17).

Whereas progression of caries into dentin resulted in the formation of a greater amount of dentin in the control diet group, no such difference was observed in the teeth of the sucrose diet group, even though the traditional view holds that the increase in caries progression should increase the dentin formation to prevent pulpal exposure (5). It seems that the teeth of rats fed the high-sucrose diet are not able to defend against caries attack. The reduction of dentin formation by the sucrose diet irrespective of the dentinal caries further emphasizes the role of the systemic effect on dentinogenesis suggested earlier (1–3). With regard to humans, these results offer a possible explanation of the clinically familiar situation in which an active caries lesion rapidly penetrates deeply into the dentin without activation of adequate defensive mechanisms resulting in reparative dentin formation or physiologic sclerosis of dentin. On the other hand, the finding of increased dentin formation under carious fissures in the control diet group corresponds to two previously known phenomena. First, small caries lesions cause an increase both in the predentinal collagen formation and in matrix mineralization (18). Second, enzymatic changes in the pulpal border of dentin occur (8, 19, 20). Under deep lesions several enzyme activities along with the collagen formation rate are decreased (18). It has been concluded that, whereas under small lesions reparative dentin formation is induced, dentin collagen maturation and matrix mineralization are seriously disturbed under deeper lesions. The only enzyme showing a slight increase in activity under deep lesions is acid phosphatase, indicating cell deaths in the odontoblastic layer (18). In progressive acute lesion the defensive reaction may be overtaken by the caries process itself, and pulpal exposure may occur after a relatively short time of caries development (5).

With regard to the severity of caries, the amount of sucrose has previously not been believed to be as critical as the frequency of its intake (21). This might well be true with regard to the number of carious fissures, but the data from previous (1) and this experiment indicate that in the progression of caries in dentin, the impairment of the systemic defensive mechanisms in the dentin-pulp complex caused by a high total intake of sucrose is even more important.

In addition to the reduction in dentin formation with the sucrose diet, it has been shown that the dentinal fluid flow is reduced (22–24). The diet-induced reduction of dentin formation and dentinal fluid flow could be related. Impaired function of odontoblasts and/or pulpal cells could interfere with physiologic defensive reactions, such as increased outward flow of dentinal fluid, sclerosis of dentin, and neutralization of acids in dentinal tubules. In active lesions the pH of the carious dentin is markedly lower and the acid profile differs from that of chronic lesions (25, 26), and this could be caused by a lowered dentinal fluid flow. Alternatively, the diet-induced alteration in odontoblast metabolism may result in a decreased response to the growth factors liberated from dentin during caries progression. This may, at least partially, contribute to the reduced dentin formation. There are studies showing that implantation of bioactive molecules in the base of unexposed cavities can lead to stimulation and up-regulation of cellular activity in the odontoblasts underlying the cavity (16, 27).

Only young rats still undergoing active secondary dentin formation were included in these studies. Therefore, this kind of depression of the odontoblast function may be characteristic of metabolically highly active odontoblasts. In older rats with 10% of the dentin formation rate in young rats (28, 29) the high-sucrose diet does not alter dentin formation (28). Although there are apparent species differences, this could also be the case for humans, explaining the higher caries susceptibility of young individuals.

In conclusion, this study provides evidence that the

pulpo-dentinal complex is able to defend against caries independently of the presence of cariogenic bacteria, so long as the exposure to dietary carbohydrates is within reasonable limits. However, the systemic effect of a high concentration of dietary sucrose abolishes the ability of the dentin-pulp complex to induce the defensive reactions against dentinal caries. The acknowledgement of the existence and importance of the endogenous regulation of the dentinal caries progression offers an alternative approach to the understanding of the mechanisms of caries.

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References

1. Huumonen S, Tjäderhane L, Larmas M. Greater concentration of dietary sucrose decreases dentin formation and increases the area of dentinal caries in growing rats. J. Nutr 1997;127:2226–30.
2. Larmas M, Kortelainen S, Bäckman T, Hietala E-L, Pajari U. Odontoblast-mediated regulation of the progression of dentinal caries. Proc Finn Dent Soc 1992;88 Suppl I:310–20.
3. Tjäderhane L, Hietala E-L, Larmas M. Reduction in dentin apposition in rat molars by a high-sucrose diet. Arch Oral Biol 1994;39:491–5.
4. Tjäderhane L. The effect of high sucrose diet on dentin minerals measured by electron probe microanalyzer (EPMA) in growing rat molars. In: Shimano M, Maeda T, Suda H, Takahashi K, editors. Dentin/pulp complex. Tokyo: Quintessence Publishing Co., Ltd; 1996. p. 293–6.
5. Massler M. Pulpal reactions to dental caries. Int Dent J 1967;17: 441–60.
6. Björndal L, Darvann T, Thylstrup A. A quantitative light microscopic study of the odontoblast and subodontoblastic reactions to active and arrested enamel caries without cavitation. Caries Res 1998;32:59–69.
7. Brännström M, Garberoglio R. The dentinal tubules and the odontoblast processes. A scanning electron microscopic study. Acta Odontol Scand 1972;30:291–311.
8. Läikkö I, Larmas M. Phosphomonoesterase activity in dentine of sound and carious human teeth. Caries Res 1979;12:148–58.
9. National Research Council. Nutrient requirements of the laboratory rat. In: Committee on Animal Nutrition, ed. Nutrient requirements of laboratory animals. 2nd ed. Washington (DC): National Academy of Science; 1972. p. 56–93.
10. Keyes PH. Dental caries in the molar teeth of the rat. J Dent Res 1958;37:1088–99.
11. Larmas M, Kortelainen S. Quantification of the areas of dentinal lesions and secondary dentin in fissures of rat molars. Caries Res 1989;23:32–5.
12. Hietala E-L, Tjäderhane L, Larmas M. Dentin caries recording with Schiff's reagent, fluorescence and backscattered electron image. J Dent Res 1993;72:1588–92.
13. Larson R, Goss B. Diet as a limiting factor in the transmissibility of caries activity between rats of different strains. Arch Oral Biol 1976;12:1085–93.
14. Marthaler T. Versuchsfehler und steungszerlegung in der medizinischen experimentelen forschung. Helv Odontol Acta 1965:9:1–71.
15. Shaw JH. A summary of the relationship of dietary carbohydrates to experimental dental caries. In: Tanzer JM, editor. Proceedings of Symposium on Animal Models in Cariology. Microbiol Abs 1981:287–97.
16. Tziafas D, Smith AJ, Lesot H. Designing new treatment strategies in vital pulp therapy. J Dent 2000;28:77–98.
17. Mundorff-Shrestha SA, Featherstone JDB, Eisenberg AD, Cowles E, Curzon MEJ, Espeland MA, et al. Cariogenic potential of foods. II. Relationship of food composition, plaque microbial counts, and salivary parameters to caries in the rat model. Caries Res 1994;28:106–15.
18. Karjalainen S. Metabolic alterations in the odontoblast-predentine region during the propagation of caries. A biochemical study on human teeth [thesis]. Turku, Finland: University of Turku; 1979.
19. Larmas M. Response of pulpo-dentinal complex to caries attack. Proc Finn Dent Soc 1986;82:298–304.
20. Larmas M, Kantola S. A histochemical study of arylaminopeptidases and alkaline phosphatases in sound and carious human teeth. Acta Odontol. Scand 1973;31:179–85.
21. König KG, Schmid P, Schmid R. An apparatus for frequency-controlled feeding of small rodents and its use in dental caries experiments. Arch Oral Biol 1968;13:13–26.
22. Leonora J, Tieche J-M, Steinman RR. The effect of dietary factors on intradentinal dye penetration in the rat. Arch Oral Biol 1992;37:733–41.
23. Steinman RR, Leonora J. Relationship of fluid transport through the dentin to the incidence of dental caries. J Dent Res 1971;50: 1536–43.
24. Tieche J-M, Leonora J, Steinman RR. High-sucrose diet inhibits basal secretion of intradentinal dye penetration-stimulating parotid hormone in pig. J Appl Physiol 1994;76:218–22.
25. Hojo S, Takahashi N, Yamada T. Acid profile in carious dentin. J Dent Res 1991;70:182–6.
26. Hojo S, Komatsu M, Okuda R, Takahashi N, Yamada T. Acid profiles and pH of carious dentin in active and arrested lesions. J Dent Res 1994;73:1853–7.
27. Rutherford B, Spanberg L, Tucker M, Charette M. Transdentinal stimulation of reparative dentine formation by osteogenic protein-1 in monkeys. Arch Oral Biol 1995;40:681–3.
28. Hietala E-L, Larmas M. The effect of ovariectomy and high-sucrose diet on dentine formation and caries in growing rats. Arch Oral Biol 1994;39:973–8.
29. Kortelainen S, Larmas M. Effect of fluoride on the rate of dentin apposition and caries progression in young and old Wistar rats. Scand J Dent Res 1994;102:30–3.

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