

The effect of early weaning on dentin formation and dentinal caries in rats

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Hietala E-L, Autio J, Larmas M. The effect of early weaning on dentin formation and dentinal caries in rats. *Acta Odontol Scand* 1997;55:201-205. Oslo. ISSN 0001-6357.

The effect of early weaning on caries progression, dentin formation, and dentin mineralization was examined in four groups of rats. Two groups received a normal diet and were weaned on day 18 or 21, and another two received a sucrose-rich diet and were weaned on day 18 or 21. At age 35 days the lower molars were sectioned sagittally, and the areas of dentin formation and of the dentinal caries were quantified. The width of the predentin zone was measured from histologically stained sections of maxillary molars. Early weaning reduced dentin formation in the group on the high-sucrose diet only the first days; later this effect was partially caught up with. A high-sucrose diet significantly increased caries frequency and extension of caries lesions compared with a normal diet in both early weaned and normally weaned groups. The effect of early weaning on caries frequency and extension in the high-sucrose group was insignificant compared with the normally weaned group on a high-sucrose diet. The predentin zone was wider in the sucrose groups than in the control groups at the end of the experiment. These results indicate that the effect of sucrose on dentin formation was dependent on the stage of physiologic dentin formation, but early weaning as such did not affect this. □ *Dentin mineralization; diet; predentin*

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Previously, we have noted that the rate of caries progression in experimental animals is dependent on the rate of dentin formation. In adult, fully formed teeth (during secondary physiologic dentin formation), the area of carious lesion during the same cariogenic challenge is less than 1/10th of that in growing teeth during primary physiologic dentin formation (1).

Recent clinical findings that a considerable portion of the population develops fissure caries to a stage where also a filling decision is made in the year of their eruption, termed as 'immediate posteruptive step' in caries curves (2), also emphasize the importance of the very early stages of tooth development for its caries resistance.

There are also reports that early weaning of rat pups (at day 18 or 19 instead of 21) tends to increase the severity of dental caries if the cariogenic challenge starts at weaning (3). In contrast, in an earlier study a delay in the cariogenic challenge to 25 days of age significantly reduced the severity of caries as compared with a challenge started on day 19 (4).

Both experimental and clinical studies thus emphasize the importance of tooth eruption time in caries development in terms of both its initiation on enamel surface and its progression, which probably is host cell-mediated (5). There is some evidence in the literature that the enamel of newly erupted teeth needs further maturation, which is accomplished by contact of the enamel surface with the oral fluids (6, 7). This process makes the enamel attain its final hardness some time after eruption. If the enamel of newly erupted tooth elements is challenged by a cariogenic diet, it is to be expected that caries will start and progress faster in these teeth than in more mature teeth. When caries lesions are serious, the lesion will extend downwards

through the enamel, and subsequently also the dentin will be affected.

In addition to the local factors involved in the cariogenic challenge by sugar at the plaque-enamel interface, it has been shown recently that a high-sucrose diet causes reduced dentin formation at the pulp-dentin border in rat molars (8-11). Dentin formation is regulated by odontoblasts, so that they first secrete an extracellular matrix to form an unmineralized predentin, which is then transformed to dentin (12). The rate of mineralization of predentin as it is transformed to dentin is equal to the rate of synthesis and secretion of the extracellular macromolecules making up predentin (12). Our hypothesis was that during an early stage in the development of the tissue the cariogenic challenge interferes with the dentin formation and thereby accelerates caries progression in dentin so that the rate of progression is host cell-mediated (5). To determine this, we evaluated the effect of early cariogenic challenge on dentin formation and caries progression during primary physiologic dentin formation in growing rat teeth and the possible cause-effect relationship between these phenomena. We also tested the hypothesis that a high-sucrose diet interferes with the mineralization, which would be seen as a possible change in the width of predentin (13).

Materials and methods

Forty Sprague-Dawley rats were weaned on day 18 (early weaning group) or on day 21 (normal group). They were then weighed, marked, and divided into groups of 10. Otherwise the animals were treated as described earlier (8, 11, 14, 15). An intraperitoneal

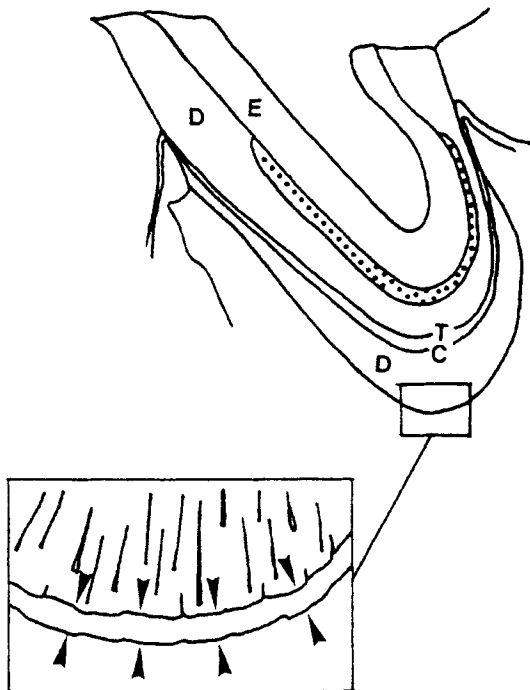


Fig. 1. A schematic presentation of a rat molar, showing the areas of dentin formation (lined by tetracycline and calcein), dentinal caries (dotted area), and the measurements of the predentin width (between the arrowheads). E = enamel, D = dentin, T = tetracycline line, and C = calcein line.

injection of oxytetracycline hydrochloride (30 mg/kg, Terramycin[®], Pfizer Corp., Brussels, Belgium) was given to all of them on day 18 to mark the dentin formed at that time, and another intraperitoneal injection of calcein (10 mg/kg, Chemical Co., St. Louis, Mo., USA) was given to all the groups on day 21.

The animals were divided into four groups. Early

weaning group A (five males and five females) received a modified Stephan-Harris high-sucrose diet (sucrose, 41%; wheat flour, 22%; skim milk powder, 32%; vegetable oil, 3%; and liver powder, 2%) on age 18 days and onwards, and early weaning group B (five males and five females) received a commercial non-cariogenic powdered standard diet specially produced for growing rats and mice (Brood Stock Feed for Rats and Mice R36, Finnewos Aqua Oy, Turku, Finland). The control diet contained barley flour, 34%; wheat flour, 43%; wheat grains, 4%; vitamins and trace elements, 4.5%; soya, 5%; fish powder, 4%; and other ingredients, 4.5%. Normal weaning group C (five males and five females) received a high-sucrose diet from age 21 days onwards, and normal weaning group D (four males and six females) the same non-cariogenic control diet as group B. All the groups received distilled water, and food and drink were available ad libitum.

At the age of 35 days the animals were weighed and then decapitated under carbon dioxide. The jaws were defleshed and preserved in ethanol. The lower jaws were sectioned sagittally by the method of Keyes (16). Dentin formation and caries lesions were quantified and measured as described earlier (17, 18) (Fig. 1). The first and second molars were analyzed together, and the daily dentin formation for each group was calculated from the mean for the group. The mean of the dentin formation formed between days 18 and 21 was divided by 3 days, dentin formation between days 18 and 35 by 17 days, and dentin formation between days 21 and 35 by 14 days. The distances between tetracycline/calcein lines were also measured from video images.

All three molars in the sections were stained with Schiff reagent, and the number of intact fissures and fissures with enamel lesions and early and advanced dentinal lesions was scored by a modification of the method of König et al. (19). The teeth were scored by number and severity of fissure caries, classified in terms

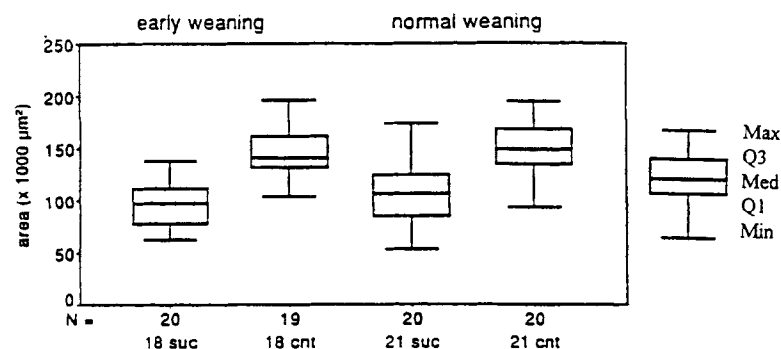


Fig. 2. Box plot comparison of dentin formation between sucrose and control diets in early (18 days of age) or normally (21 days of age) weaned groups. Abbreviations: 18suc = weaning at 18 days of age with sucrose diet, 18cnt = weaning at 18 days of age with control diet, 21suc = weaning at 21 days of age with sucrose diet, 21cnt = weaning at 21 days of age with control diet. The box plot presents maximum (Max) and minimum (Min) values, and median (Med) with upper and lower quartiles (Q3, 75% quartile and Q1, 25% quartile) in each group.

of intact fissures, enamel lesions, and dentinal lesions. The maximum potential caries scores would be 6 for the number of lesions (all fissures) and 12 for severity (0 for intact, 1 for an enamel lesion, and 2 for a dentinal lesion) (9).

Molars from the upper jaw were fixed in 10% formalin before being decalcified in formic acid. After decalcification for 4–5 weeks, the jaws were processed with glycolmetacrylate (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 for the particular group (20).

The statistical analyses were performed using SPSS MS WINDOWS Release 6.0. Anova with Tukey's HSD test with a significance level of 0.05 was used to compare dentin formation, caries progression, and the predentin zone between the groups. Independent-samples *t* tests were used to compare differences in daily dentin formation.

Results

The high-sucrose Stephan–Harris diet reduced the area of dentin formed during the experiment (Fig. 2). The difference between the sucrose and control diet was statistically significant ($P < 0.05$) in early and normal weaning groups.

The high-sucrose diet also reduced the calculated dentin formation per day from day 18 to 21 (Fig. 3). The difference was significant ($P < 0.005$) when compared with the control diet. Early weaning to control diet did not reduce daily dentin formation between days 18 and 21 (Fig. 3). Dentin formation calculated per day between days 18 and 21 was significantly greater than

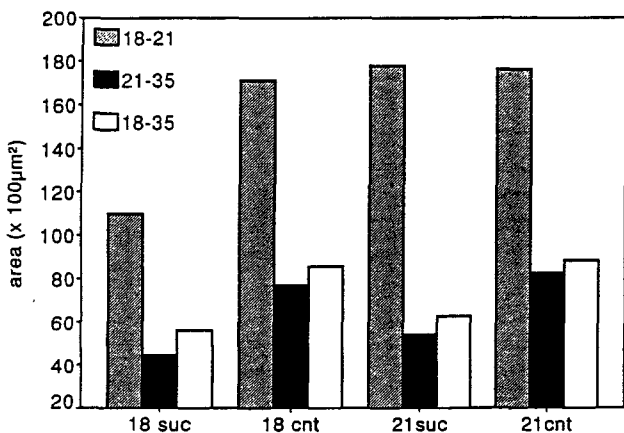


Fig. 3. The daily dentin formation in different periods: from day 18 to 21, 21 to 35, and 18 to 35. See Fig. 2 for definition of groups.

Table 1. Caries scores in different groups. Number, mean value (standard deviation) of carious fissures per rat (maximum, 6). Severity, mean (standard deviation) severity index calculated from the penetration depth of carious lesion per rat (maximum, 12)

Group*	Number	Severity
18 suc	5.6 (0.5)	8.7 (1.9)
18 cnt	4.2 (1.7)	4.5 (2.1)
21 suc	5.3 (0.8)	7.7 (1.6)
21 cnt	4.0 (2.0)	4.0 (2.0)

* See Fig. 2 for definition of groups.

that between days 21 and 35 in all the groups ($P < 0.005$). No significant differences in daily dentin formation were seen between the early and normally weaned sucrose groups in the period from day 21 to day 35.

The thickness of the dentin layer formed between days 18 and 21 was smaller in the early weaning sucrose group, but the difference was not statistically significant. Between days 21 and 35 too the thickness of dentin was smaller in the sucrose groups, but not statistically significantly.

The severity of the carious lesions, as visualized by Schiff staining, was higher in the sucrose groups than in the control groups (Table 1) and higher in the early weaning sucrose group than the one on normal weaning. The percentage of early dentinal caries lesions was higher in the early weaning sucrose group (49%) than in the normal weaning sucrose group (31%) (Table 2). There were no significant differences in the fluorescence areas denoting dentinal caries between the sucrose groups, but these were in both cases greater than in the controls ($P < 0.05$), in which practically no dentinal lesions were seen (Fig. 4).

The predentin width was greater in sucrose groups than in control groups. The difference was statistically significant ($P < 0.05$) between the sucrose and control diets in both early and normal weaning groups (Fig. 5).

No significant differences in weight gain were found between the groups at either the beginning or the end of the experiment.

No significant differences in dentin formation or the

Table 2. Number of fissures in relation to the depth of caries penetration (percentages in parentheses)

Group*	Intact fissures	Enamel lesions	Early dentinal lesions	Advanced dentinal lesions
18 suc	5 (8)	21 (36)	29 (49)	4 (7)
18 cnt	16 (28)	37 (65)	4 (7)	0 (0)
21 suc	5 (9)	29 (50)	18 (31)	6 (10)
21 cnt	17 (30)	40 (70)	0 (0)	0 (0)

* See Fig. 2 for definition of groups.

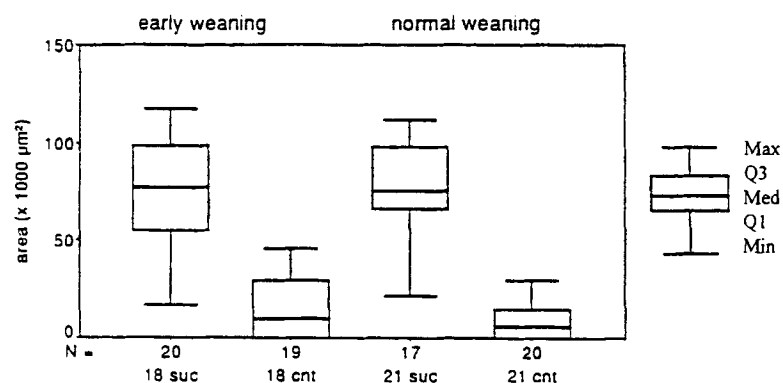


Fig. 4. Box plot comparison of the areas of dentinal carious lesions in molars between sucrose and control diets in early (18 days of age) or normally (21 days of age) weaned groups. See Fig. 2 for definition of abbreviations.

areas of dentinal caries lesions were found between the first and second molars within each group, and thus the results for both teeth were combined when mean values were presented. When statistical analysis was performed, the number of animals was taken as *n*.

Discussion

Early weaning with or without an early onset of cariogenic challenge did not disturb the normal growth of the rats, nor has a high-sucrose diet per se previously been found to have any adverse effects on the growth of rats after weaning at the normal age of 21 days (9). As the weight gains were alike, and all the animals appeared to be healthy, the diets were regarded as nutritionally sufficient.

The results are presented in terms of surface area per day instead of the thickness of the mineralized dentin layer formed per day, because we believe that the area under the pulp chamber represents the increments of dentin in sound teeth during dentin formation, whereas

the thickness of dentin under the lesion represents also the dentinal response (reparative/tertiary dentin).

The reducing effect of a high-sucrose diet on dentin formation in rats is well documented (1, 8–11, 14, 15, 20); however, the weaning age has been 21 days in these studies. In the light of the present study the reduction was significant even for the very short period between days 18 and 21 when we study the daily dentin formation. The reduction in daily dentin formation was partly compensated for between days 21 and 35, indicating that the effect is more pronounced during the early stages of dentin formation (Fig. 3). Our results were in line with the findings of Johannessen (21), who observed the largest increments per day before weaning (21st day) in the dentin of the first molars of albino rats, while they were smaller between 21 and 41 days. The rate of dentin formation was not associated with the increments in body weight, nor were there any statistically significant sex differences (21), and again our results pointed to the same conclusions. We therefore concluded that the early weaning as such did not affect dentin formation.

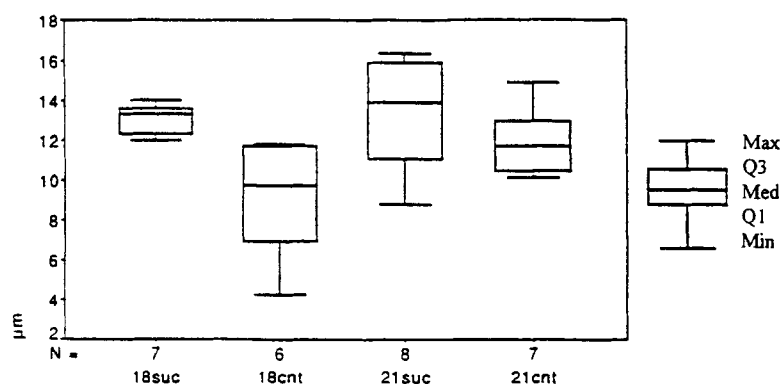


Fig. 5. Box plot comparison of the width of predentin in molars between sucrose and control diets in early (18 days of age) or normally (21 days of age) weaned groups. See Fig. 2 for definition of abbreviations.

The amount of dentin reflects the rate of secretion of dentin matrix proteins and their mineralization (22), and the width of the predentin mainly reflects its mineralization capacity (22). The fact that the predentin zone was wider in the sucrose groups, in which dentin formation was also smallest, indicates both disturbed mineralization and matrix synthesis and supports previous findings (10).

Measurements of predentin were made from upper molars, and measurements of dentin formation from lower molars, because the number of teeth analyzed was so much greater. Our laboratory has ascertained that dentin formation in both jaws correlate. However, it has been claimed that the width of predentin in intact teeth may vary greatly from tooth to tooth and from one morphologic area to another (23). That may explain the variation between animals in our study.

After lactation a high-sucrose diet from day 18 or 21 onwards induced caries as compared with the control diet. Early weaning onto a high-sucrose diet increased the severity of caries, as shown by Schiff staining, but the difference was not significant with the fluorescent method at 35 days, even though the cariogenic challenge had been 3 days longer. These observations emphasize the importance of time of tooth emergence for caries susceptibility: delay of cariogenic challenge by 4 days from day 21 significantly reduces carious effect (4), whereas starting 3 days earlier results in an accelerated attack.

Although it has been suggested that the weaning age, the time when the cariogenic challenge was established, and the type of diet offered before this challenge may be critical to the severity and reproducibility of caries in the rat (4), the weaning age as such was not critical for caries in our experiment, whereas only early weaning onto a high-sucrose diet was.

The teeth studied were erupting and still undergoing primary physiologic dentin formation during weaning, so that early weaning and early onset of the cariogenic challenge posed by a sucrose diet disturbed the function of the odontoblasts, as reflected in reduced daily dentin formation. The period was long enough to reduce the rate of dentin mineralization (wider predentin), but the possible cause-and-effect relationship between increased caries and decreased dentin formation remains an open question. This study supports the clinical findings that young teeth are more susceptible to caries attack than more mature teeth.

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