Effects of a high sucrose diet and intragastric sucrose feeding on the dentinogenesis, dental caries, and mineral excretion of the young rat

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Previous studies show that a high sucrose diet reduces the rate of primary dentinogenesis and increases dental caries, although their cause–effect relationship is still obscure. The purpose of this study was to explore whether the effect of sucrose load on the dentinogenesis and dental caries of young rat molars is mediated by systemic (intragastric) or by systemic and local (dietary) factors. At weaning (19 days), animals were randomized into the control, intragastric sucrose, and dietary sucrose groups for 4 weeks. The areas of dentin appositions and dentinal caries lesions were measured planimetrically. Caries was also determined with Shiff's staining and the width of predentin by histology. Urinary Ca, K, and Na levels were measured by flame photometry, urinary P levels using an UV method, and serum insulin levels using radioimmunoassay. Systemic and local sucrose load reduced dentin appositions and intragastric sucrose increased urinary Ca excretion. No differences in the width of predentin were noticed. Only dietary sucrose enhanced the occurrence and progression of caries. The present findings show that sucrose load reduces dentinogenesis by impairing the synthesis of dentin matrix, but also point out the crucial importance of the local sucrose challenge in the initiation of dental caries. □ *Caries; dentinogenesis; intragastric; rat; sucrose*

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A high sucrose diet reduces the rate of dentin formation during primary dentinogenesis (1–4) in a dose-dependent manner (5). This is in line with observations of the deteriorating effect of a high sucrose diet on bone metabolism (6–9). Bone and dentin alterations can be explained by the observation that sucrose feeding increases calcium excretion and decreases phosphorus, potassium, and sodium excretions to urine (10). However, it does not affect mineral levels of serum or the width of the predentin layer (10), i.e. it does not reduce the speed of dentin mineralization. Moreover, the reductive effect of a high sucrose diet on the dentinogenesis of young rats is predisposed by the sucrose feeding of their dams during the preweaning period (11), just as is early weaning of rat pups (12). Although all previous results suggest that the effect of the diet is mediated via the response of the host during primary dentinogenesis, the mechanism by which a high sucrose diet reduces dentin formation is still unknown.

It is known that a high sucrose diet causes sucrose-induced insulin resistance, which leads to hyperinsulinemia (13–15) and deterioration of glucose tolerance (13, 16) in the rat. Elevated insulin concentration induces calciuria (17) and reduces urinary phosphorus excretion (18), although part of the antiphosphaturic effect of insulin has been suggested to be caused by the antagonism of PTH action (19). It has also been speculated that sucrose feeding impairs dentinal fluid movement, resulting in a lower dye penetration rate into dentinal tubules (20).

The purpose of this study was to explore the effects of high sucrose feeding (systemic intragastric or local and systemic dietary) on dental caries and dentinogenesis. Our working hypothesis is that both intragastric and dietary sucrose feeding reduce the dentinogenesis of the young rat, but also induce calciuria. The specific hypothesis was that the odontoblast responds to the high sucrose load by reducing dentin matrix formation, which leads to a reduced rate of dentin apposition. Intragastric sucrose feeding was hypothesized to exclude the local cariogenic challenge in the oral cavity, and dietary sucrose feeding to enhance it. The possible cause–effect relationship between reduced dentinogenesis and dental caries progression is evaluated.

Material and methods

Animal feeding and handling

Twenty-eight Sprague-Dawley rats (Møllegård Ltd, Ejby, Denmark) were weighed, marked, and distributed among 3 dams on the day of birth in order to obtain litters as similar in weight and number of pups as possible during the lactation period. The animals were weaned at the age of 19 days and randomized into the 3 diet groups. The control group (CNT) of 9 pups (5M, 4F) and the intragastric sucrose group (IGS) of 9 pups (5M, 4F) were fed a standard rodent diet (Lactamin R 36; Ewos, Stockholm, Sweden) containing 34% barley flour, 43% wheat flour, 5.0% wheat grains, 4.5% vitamins and trace elements, 5.0% soya, 4.0% fish powder, and 4.5% other ingredients (12.60 MJ/kg). The animals of the IGS group were exposed to sucrose by intragastric sucrose feeding

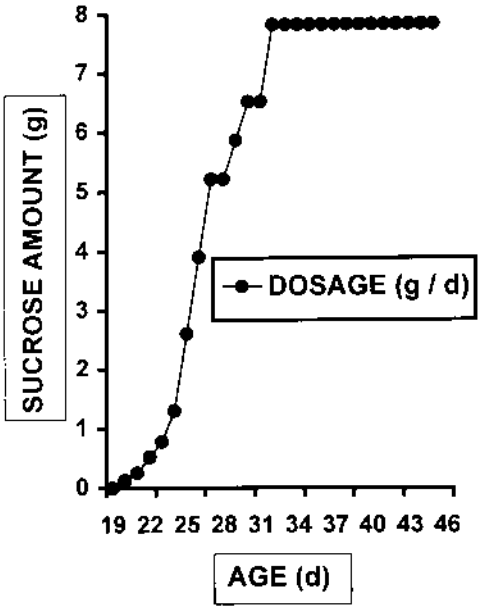

Fig. 1. The sucrose load of the intragastric sucrose feeding group (IGS) during the experiment period (concentration of sucrose solution 0.65 g/mL).

(0.2–12 mL; 0.65 g/mL), resulting in increasing sucrose load during the experiment, which was started one day after the weaning and ended on the day of termination. A detailed description of the intragastric sucrose dosage is explained in Fig. 1. The dietary sucrose group (DIS) of 10 animals (6M, 4F) received a diet containing 41% sucrose, 10.1% barley flour, 10.3% wheat flour, and 5.8% casein, which was added to compensate for the loss of protein when the wheat and barley flour content was reduced (Ewos R 642; Ewos, Stockholm, Sweden) (13.53 MJ/kg). The CNT and DIS groups were fed intragastrically with tap water using the same procedure and amount of liquid as with the IGS group, otherwise the food and tap water were available *ad libitum*. Both diets were in the form of powder and have been used previously (4, 10, 11). Detailed compositions of the diets are presented in Table 1.

The animals were housed 2–3 per cage under normal athmospheric conditions at 21°C and subjected to the same light/dark cycle (12 h of light and 12 h of dark), the same times of feeding, handling, and noise level. To mark the areas of dentin apposition, each animal was given an intraperitoneal injection of oxytetracycline hydrochloride (30 mg/kg); Terramycin®; Pfizer, Brussels, Belgium) upon weaning and 1 day before termination of the experiment, on day 46. At ages 29, 33, 36, 40, and 43 days, the animals were individually housed in metabolic cages for a 12 h nocturnal period and the urine excreted in that time was collected in Erlenmeyer bottles (to minimize evaporation) and measured. Food and water intakes were measured during the entire experimental period. Four weeks after weaning the animals were weighed and anesthetized using a mixture of midazolam (Dormicum®; Roche, Basel, Switzerland), fentanyl-fluanisone (Hypnorm®; Jansen Pharmaceutica, Brussels, Belgium), and aqua (1:1:2 at 0.3 mL/100 g, i.p.). Feeding was ended 3 h prior to anesthesia. All experimental procedures were approved by the Experimental Animal Committee of the Medical Faculty, University of Oulu, Oulu, Finland.

Sample preparation

The blood from each pup was drawn by cardiac puncture and the animals were decapitated. After an incubation period of 10 min, the blood was centrifuged

Table 1. Micro and macro components of the diets with comparison to the RDA

	Standard	Sucrose	RDA
Calcium (g/kg)	9.8	9.8	5.6
Phosphorus (g/kg)	7.5	7.2	4.4
Potassium (g/kg)	6.0	5.5	2.0
Sodium chloride (g/kg)	7.0	7.0	6.0
Copper (mg/kg)	30.0	24.6	5.6
Ferric (mg/kg)	190.0	190.0	38.9
Manganese (mg/kg)	100.0	57.9	55.6
Zinc (mg/kg)	110.0	105.9	13.3
Iodine (mg/kg)	2.0	2.0	0.17
Retinol (mg/kg)	0.36	0.36	0.67
Vitamin D (IU/kg)	1500	1500	1111
Vitamin K (mg/kg)	0.25	0.25	0.06
Thiamine hydrochloride (mg/kg)	13.0	5.9	1.4
Riboflavin (mg/kg)	13.9	12.6	2.8
Niacin (mg/kg)	111.9	62.3	16.7
Calcium panthotenate (mg/kg)	21.6	13.6	8.9
Pyridoxine hydrochloride (mg/kg)	10.4	5.6	7.8
Cobalamin (mg/kg)	0.02	0.02	0.0056
Choline chloride (mg/kg)	1000	1000	833
dl- α-tocopherol (mg/kg)	80.5	68.0	39.0

RDA = Recommended Daily Allowance (26).

Table 2. Mean ± SD urine and urinary mineral excretions of the experimental groups

	CNT ($n = 9$)	IGS ($n = 9$)	DIS ($n = 10$)
Urine excretion (mL/12 h)*			
	3.9 ± 0.7	6.0 ± 1.3[A]	5.3 ± 1.3
Urinary mineral excretions (mg/12 h)**			
Calcium	0.4 ± 0.1	0.9 ± 0.4[A]	0.7 ± 0.3
Phosphorus	4.0 ± 1.0	3.0 ± 0.8	3.2 ± 1.2
Potassium	15.2 ± 3.7	9.5 ± 2.6[A]	13.9 ± 2.4
Sodium	7.1 ± 1.5	5.5 ± 1.1	6.5 ± 1.6

Urine excretion levels are presented as a mean of the area under the urine excretion curve of all animals in a particular group. **Mineral excretions were counted from the daily urine excretions using the mineral levels of urine and the atomic weight of the particular mineral as determinative factors. [A] Significantly different from the CNT group ($P < 0.05$) (Tukey's HSD t test). CNT = Standard diet with intragastric water, IGS = Standard diet with intragastric sucrose, DIS = Sucrose diet with intragastric water.

and serum stored by freezing. The jaws were defleshed, right hemimandibles were preserved in absolute ethanol and sectioned sagittally (21). Left hemimandibles were fixed in 10% formalin before being decalcified in 5% formic acid. After decalcification for 4 weeks, the jaws were processed with glycomethacrylate (GMA) and then embedded in blocks and cut into sections. The sections were stained with toluidine blue (STB).

Measurements

Serum insulin levels (ng/mL) were determined using a radioimmunoassay (Rat Insulin RIA Kit, Linco Research Inc., St. Charles, USA) which utilizes an antibody made specifically against rat insulin. Urinary inorganic phosphorus (mmol/L) levels was measured using the UV method (UV test for phosphate, SYS 1, Boehringer Mannheim/Hitachi 704/911, Cat. no. 1489348) modified for Cobas Fara II centrifugal analyzer (F. Hofmann-La Roche Ltd, Diagnostic Division, Basel, Switzerland). Calcium, potassium, and sodium levels of urine (mmol/L) were determined by flame photometry (Eppendorf Efox 5053, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany).

In order to measure the amount of dentin apposition ($\mu m^2 \times 10^3$) and the areas of dentinal caries lesions ($\mu m^2 \times 10^3$), the main central transverse fissure of the mandibular first and second molars was measured planimetrically using a microscope (Leica DMRB, Leica Mikroskopie und Systemic GmbH, D 35530 Wetzlar, Germany) equipped with a computer-linked video image analyser (Leica Q 500 MC, Leica Cambridge Ltd, UK) and fluorescent light with which the tetracycline stripes surrounding the formed dentin could be seen (22) (magnification ×5). The areas of dentinal caries lesions, seen as a change of fluorescence, were measured planimetrically as described above. Occurrence and progression of dental caries, seen as classic Shiff's reactions (23, 24), were determined under the main fissures. The width of the predentin layer (μm) was measured under a microscope (magnification ×40) at 6 sites under the main fissures of the molars, and the mean was considered to represent the thickness of predentin for that particular animal (3).

Statistics

The statistical analyses were done with SPSS for Windows Release 7.5. The differences in mean weights, mean weight gains, food intake, dentin apposition, and predentin layer between the diet groups were determined using one-way ANOVA with Tukey's honestly significant difference (HSD) t test. Daily urinary excretion and daily mineral excretion rates were determined by counting the area under the urine excretion and urinary mineral level curve for each animal and mineral, where the arithmetic mean urine and mineral excretion values of the groups were calculated (25). Mineral excretion rates were counted using the atomic weight of the particular mineral as a determinant and the differences in urine and mineral excretions among diet groups were determined using Tukey's HSD t test. Differences in the areas of dentinal caries and serum insulin levels were determined using the non-parametric Kruskal-Wallis ANOVA, because the data did not meet the assumption of homogeneity of variances. If the significant differences were detected, the differences between two groups were determined using the non-parametric Mann-Whitney U-test.

Results

Sucrose exposure of the animals reduced the rate of their dentin apposition ($\mu m^2 \times 10^3$) (Fig. 2). Dentin appositions (mean ± SD) in the IGS (117.2 ± 6.8) and DIS (92.5 ± 16.0) groups were significantly reduced ($P < 0.05$) compared to controls (138.9 ± 16.5), and dentin apposition was more reduced in the DIS than in the IGS group ($P < 0.05$). No differences in width of the predentin (μm) were noticed among the experimental groups (CNT 9.6 ± 1.6, IGS 9.5 ± 1.3, and DIS 8.9 ± 1.7).

The mean (±SD) insulin levels (ng/mL) in the DIS (1.8 ± 2.0) group were elevated about 60%, although the differences to the CNT (1.1 ± 0.6) or IGS (1.1 ± 0.8) groups were non-significant.

Urine excretion (mL) and urinary calcium excretion (mg) were increased in the IGS group compared to the CNT group (Table 2). The differences were statistically significant ($P < 0.05$). Potassium excretion of the IGS

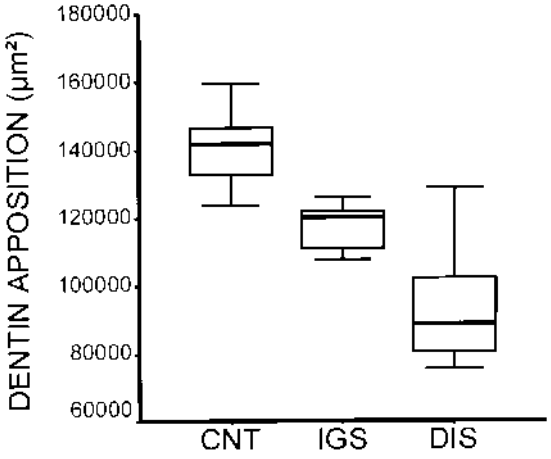

Fig. 2. The areas of dentin appositions illustrated as box plots for each experimental group. The box reveals the 1st and 3rd quartiles with the median value inside, and the whiskers show the lowest and the highest values. CNT = Standard diet with intragastric water, IGS = standard diet with intragastric sucrose, DIS = sucrose diet with intragastric water.

group was significantly reduced ($P < 0.05$) compared to the CNT group.

Dietary sucrose feeding enhanced the occurrence and progression of caries. In the DIS group, only 10% of the molars remained sound, whereas 25% and 33% of the teeth remained sound in the CNT and IGS groups. On the other hand, in the DIS group, 25% of teeth had dentinal lesions, whereas the corresponding percentage in the CNT and IGS groups was 11% (Fig. 3). The mean (± SD) areas ($\mu m^2 \times 10^3$) of dentinal caries lesions in the DIS group (15.6 ± 11.3) were 30 to 80 times larger than in the CNT (0.5 ± 0.4) or IGS (0.2 ± 0.4) groups and the differences were statistically significant ($P < 0.001$).

The mean (± SD) food intake (g/d) in the IGS group

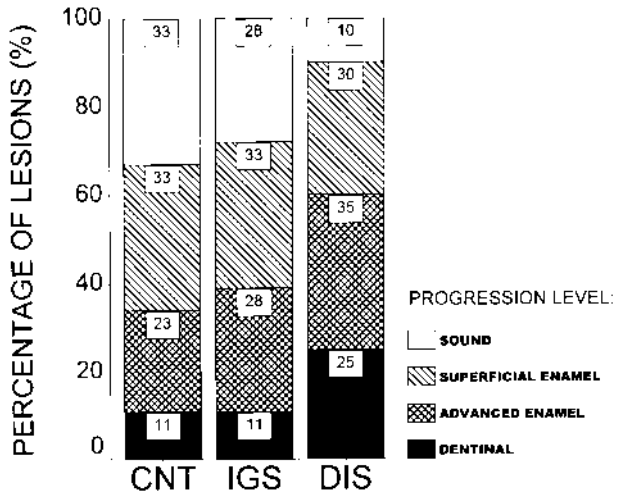

Fig. 3. The occurrence of caries lesions and sound teeth as a percentage (%) of all examined teeth. Abbreviations as in Fig. 2.

(17.4 ± 1.6) was reduced compared to the CNT (21.3 ± 3.2) or DIS (19.3 ± 0.1) groups ($P < 0.05$). Despite differences in food intake, no differences in the mean (± SD) weights (g) at the end of the experiment (CNT 187.4 ± 27.9, IGS 179.9 ± 23.5, and DIS 186.8 ± 16.1) or in weight gains (g/d) during the experimental period (CNT 5.3 ± 1.0, IGS 5.0 ± 0.9, and DIS 5.2 ± 0.6) were noticed. Mean (± SD) sucrose load in the IGS group was 6.1 g/day (± 0.0) and in the DIS group 7.9 g/day (± 0.0).

Discussion

The results support our assumption that a high sucrose load reduces dentin apposition regardless of route of administration. It can thus be regarded as a response of pulpa-dentin complex and especially by odontoblasts to the sucrose load. Huumonen et al. (5) suggested that the high sucrose effect is dose-dependent, which explains the more reduced dentinogenesis in the DIS group (7.9 g/day) compared to the IGS group (6.1 g/day), because the sucrose load in the DIS group was higher than the load of the IGS group. This difference was due to the calculation that the maximum intragastric dosage of liquid was 6 mL twice a day (12 mL/day); thereby the maximum sucrose load was 7.8 g/day (Fig. 1), which was presumed to be the upper limit of welfare of the animals during the experimental period. The lower mean (g/day) food intake of the IGS group can be explained by a lower dietary energy need, caused by the intragastric sucrose feeding, because the rat can regulate food intake to meet energy demands (26). However, the borderline concentration of the reducing effect of sucrose on dentinogenesis is 30% in the diet (5), which was calculated to be exceeded in both the DIS (41%) and IGS (35%) groups in the present series of experiments. As the width of the predentin layer was not widened, we hold to our present concept that the reduction of dentin apposition, caused by high sucrose feeding, was the outcome from impaired synthesis of dentin organic matrix rather than the result in retarded mineralization of predentin (10).

In the high sucrose animals, calcium excretion to urine was induced and phosphorus, potassium, and sodium excretions reduced (Table 2), which is in line with our previous results (10, 11) although the habituation of the animals to the sucrose load could be seen as well as in the mineral excretion rates and serum insulin levels, especially in the DIS group. This was probably the consequence of the longer test period used here, enabling a better adaptation to the high sucrose diet. Reduced dentin appositions (Fig. 2), with reduced rates of dentin matrix synthesis, support our working hypothesis and show that sucrose feeding reduces dentinogenesis of the young rat, which is in good accordance with our previous results (10).

The problem of the possible cause–effect relationship between the increased incidence of caries and reduced primary dentinogenesis remains unresolved. Sucrose load through intragastric feeding did not induce caries, which is in good accordance with earlier observations (27),

although it did reduce dentinogenesis. In rats, the amount of sucrose is less important in caries initiation than is the frequency of use, as analysed by automatic feeding machines (28, 29). This is indirect support for the observation that a high sucrose load directly to the stomach did not induce considerable caries in these experimental animals. A high local sucrose challenge resulted in highly increased caries progression, but it also further decreased dentin apposition compared to IGS rats. This would suggest that the reduction of dentin apposition in DIS animals might be partly due to the deteriorating effect of dental caries on dentin apposition during the primary dentinogenesis, although we have not found evidence of this in our earlier studies (4). In adult animals, with closed dental root apices, the situation might be different: the reduced dentin apposition in adults, compared to young animals (30), could be stimulated by caries as a consequence of reparative dentin formation and so the reduction of dentin apposition might be caught up with age.

In conclusion, the results support our previous findings and show that reduced dentinogenesis, caused by sucrose load, is the outcome of reduced synthesis of dentin organic matrix. A direct causal relation between sucrose intake and reduced dentin matrix formation remains to be proven.

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