The effect of ovariectomy on dentin formation and caries in adult rats

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Since acute osteoporosis is known to enhance bone remodeling and osteoid formation, it may also affect dentin apposition. We induced osteoporosis in 15-week-old Wistar rats by ovariectomy and dental caries by *Streptococcus sobrinus* infection in the presence of either a high sucrose (cariogenic) diet or a non-cariogenic diet. Intact animals with the same diets served as controls. After 99 days of cariogenic challenge the rats were killed, and the success of ovariectomy was confirmed by failure to detect ovarian tissue and observation of marked atrophy of the uterine horns. Areas of dentinal apposition during the experiment and carious lesions were quantified with a tetracycline method. Ovariectomy activated dentin formation significantly in rats fed either a high-sucrose or a non-cariogenic diet, indicating enhanced odontoblast function. The rate of dentinal apposition in adult rats was smaller than reported in young animals. The effect of ovariectomy on caries remained negligible. □ *Dentinal apposition; odontoblast; osteoporosis*

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Ovariectomy is known to induce osteoporosis in rats, a conclusion based on the histomorphometric observation that it induces accelerated bone metabolism (1). Wronski et al. (2) also found that the initial rapid phase of bone loss in ovariectomized rats is coincident with the maximal increase in bone turnover. The observation of marked bone loss in ovariectomized rats despite increased bone formation indicates that the increment in bone resorption exceeds the increment in bone formation (2).

Not much is known about the effect of ovariectomy on metabolism of odontoblasts, possibly because the normal life cycle of the tooth is at a steady state at the time when osteoporosis normally appears and there is no remodeling in the dentin, although new dentin is secreted throughout the individual's lifetime. As osteoblasts and odontoblasts are both cells that secrete a collagenous extracellular matrix involved in mineralized tissues and are both of mesenchymal origin, it is reasonable to think that the functions of these two cells may be regulated partly by the same mechanisms—for example, disturbances in calcium and phosphorus homeostasis (like rickets and osteoporosis).

We found recently that response of the cells to these challenges may also vary, as shown in a case of vitamin D-resistant rickets in which dental manifestations were not totally corrected by the treatment correcting the skeletal alterations (3, 4). In acquired rickets dental manifestations follow the time course of the vitamin D deficiency and normal dentin is apposited when the deficiency is supplemented (3). Osteoporosis induced by ovariectomy represents another, experimental disturbance in calcium and phosphorus homeostasis.

Osteoporosis and dental caries have some epidemiologic characteristics in common. Both caries (5, 6) and osteoporosis (7) are more common in women than in men and there is less caries (8–10) and osteoporosis (7, 11) in the south than in the north in the northern hemisphere. Black people are less susceptible to both caries (12–14) and osteoporosis (15) than white people.

The aim of this work was to determine whether ovariectomy as such or combined

with a cariogenic diet modifies (1) the rate of dentin apposition; (2) the number of caries lesions; (3) and in this or some other way (e.g. a salivary effect) the rate of progression of caries in dentin.

Materials and methods

Ovariectomy (O+) was carried out by the dorsal approach in 15-week-old female Wistar rats. The caries experiment was started after a recovery period of 1 week. Untreated animals of the same age served as controls.

Rats

A total of 45 Wistar rats from the stocks of the University of Kuopio, Finland, were used. Twenty-two were subjected to ovariectomy; of these, 11 were maintained on a cariogenic sucrose diet (O+/suc) and 11 on a non-cariogenic, powdered normal pellet diet (Ewos R3) (O+/pel). Twenty-three rats were used as controls; 12 were on a non-cariogenic control diet (cnt/pel) and 11 on the sucrose diet (cnt/suc).

Housing

Before the experiment the rats had been housed in groups of 10 in large cages (Makrolon IV). For the experiment they were moved to smaller cages, two or three rats to a cage (Makrolon III), on a bedding of European aspen shavings, with a 12/12-h dark/light regimen, a room temperature of 21 °C, and 40–60% humidity.

Feed

The rats were weaned at 22 days of age. Before the experiment (from 22 days to the age of 16 weeks) they were fed a normal commercial pellet diet (Ewos R3). During the experiment the sucrose groups were fed ad libitum on a modified Stefan–Harris diet with 43% sucrose and distilled water. The control diet groups had the same commercial pellet diet as before the experiment, but now powdered, with no added sucrose.

Ovariectomy

The rats were ovariectomized at the age of 15 weeks by the dorsal approach while under anesthesia with fluanisone-fontanyl and midazolon (Hypnorm®, Roche, and Dormicum®, Jensen, respectively, 1:1:2 water, 0.2–0.4 ml/100 g intraperitoneally). A recovery period of a week was permitted before the beginning of the caries experiment. No medicaments were needed after the operation.

Caries experiment

To induce caries, human *Streptococcus sobrinus* was administered to the animals orally once a week during the experiment.

Tetracycline labeling

Tetracycline, 40 mg/kg (Terramycin®, 100 mg/ml oxytetracyclin hydrocloride, Pfizer), was injected intraperitoneally 2 days before the beginning of the dietary experiment (a week after the operation) to mark the dentin mineralization at that time.

Duration

Because osteopenia is known to be induced in 100 days (2), the rats were killed with ether anesthesia at that time.

Preparation of the specimens

The success of the operation was confirmed by failure to detect ovarian tissue and in terms of atrophy of the uterine tube and arteries. Induction of the osteoporosis was confirmed from femurs. The jaws were prepared and hemisected sagittally in the mesiodistal plane (16).

One fissure from each mandibular molar was microphotographed (Kodak Ektacrome daylight film, 400 ASA) under an Orthoplan Ploemopak microscope (×16 magnification) equipped with ultraviolet light (460-nm detector) to identify the mineralization areas synthesized after the tetracycline labeling. The fissures studied were the middle one in the first molar and the mesial one in the second and third molar. Dentinal apposition under each photographed fissure during the

experiment was determined from the black and white video image by drawing the areas encircled by the tetracycline fluorescence as it appeared on the monitor (Salora 445 A RGB, Camera Hitachi VKM 98 E) via a serial mouse connected to a PC Vision frame grabber (Imaging Technology, Woburn, Mass., USA) and the Image Measure computer program (Microscience, Washington, D.C., USA). The hemisected jaws were stained with Schiff's reagent, and the progression of carious lesions was estimated by the method introduced by König et al. (17). Caries was also determined from the change in the dentin fluorescence in a manner similar to that for dentinal apposition (18).

Before the experiment a pilot study with 35 rats was carried out. In this pilot study the dose of tetracycline used for labeling the mineralizing dentin was 30 mg/kg, but this was observed to give only a weak fluorescence in some rats. In the actual experiment the dose was increased to 40 mg/kg to give a more intense and thus more exact fluorescent line. The principal results of the two experimental runs were the same, but because of some adjustments made in the measurement procedure after the pilot study, the absolute areas are not comparable. The results of the actual experiment are reported here because of the more precise determination of the area of dentinal apposition. Nine fissures were left out of the analyses because of failure in the hemisection or failure to distinguish the tetracycline line.

Table 1. Daily consumption of food and water per rat (mean) during the experiment and weight gains expressed as percentages of initial weight

	Food (g/day)	Water (ml/day)	Weight gain %	Weight gain SD
O+/pel	22.3	25.9	33.8	5.8
O+/suc	17.1	23.9	40.1	15.5
Cnt/pel	22.7	29.5	8.3	9.6
Cnt/suc	18.8	29.0	12.0	3.7

O+/pel = ovariectomized rats fed a non-cariogenic diet; O+/suc = ovariectomized rats fed a cariogenic diet; Cnt/pel = intact animals fed a non-cariogenic diet; Cnt/suc = intact animals fed a cariogenic diet.

Because of the skewness of the measurement data, statistical analysis was carried out with the Wilcoxon test and Fisher's exact test (two-tailed) for the Schiff reagent scores.

Results

The ovariectomized rats weighed more at the time of death than did the control animals (Table 1), and the sucrose rats in both groups

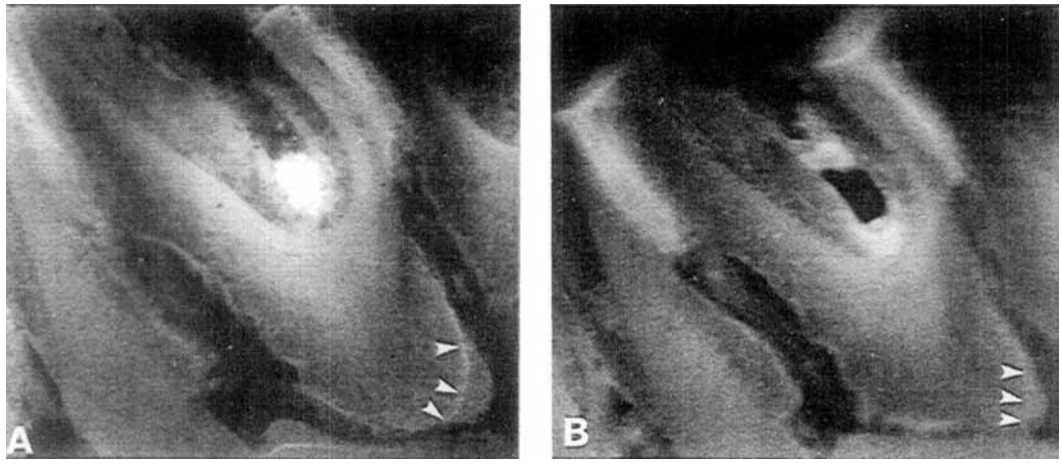

Fig. 1. Photomicrograph of the second mandibular molar of an ovariectomized rat (A) and control animal (B). The tetracycline line is marked with arrows.

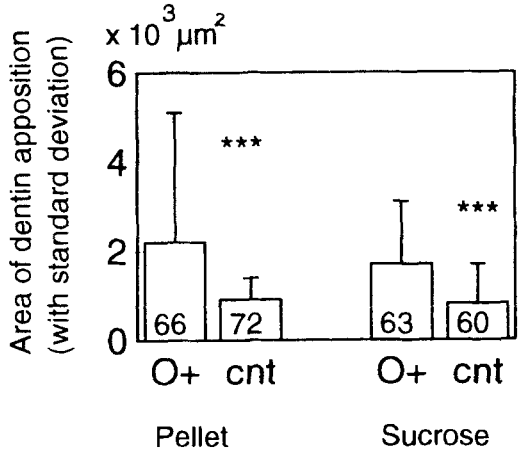

Fig. 2. Mean areas of dentin apposition with standard deviations under fissures ($\times 10^3$ µm^2) in the pellet and sucrose groups (all molars analyzed together; number of fissures in bars). O+ = ovariectomized rats; cnt = intact animals. *** Statistical significance.

were heavier than those fed a pellet diet. The weight gain (expressed as a percentage of initial weight) was 40.12% (SD = 15.53) in the ovariectomized sucrose group, 33.83% (SD = 5.8) in the ovariectomized pellet group, 12.04% (SD = 9.61) in the intact sucrose group, and 8.31% (SD = 9.61) in the intact pellet group. Induction of osteoporosis was obvious, according to the analyses of the femoral cross section (22).

Food consumption was greater in the pellet groups than in the sucrose groups but with no differences between the ovariectomized and control rats. The consumption of water was slightly higher among the control rats than among the ovariectomized ones, and slightly higher in the pellet groups than in the sucrose groups.

Dentinal apposition (Fig. 1) in the molars (all molars analyzed together) was greater in the ovariectomized rats than in the intact animals ($p = 0.0001$) regardless of diet ($p = 0.0006$ in the pellet groups and $p = 0.0003$ in the sucrose groups) (Fig. 2). There were differences between the molars when they were analyzed separately. In the first molars, $p = 0.0021$ in the pellet groups and 0.017 in the sucrose groups. In the second molars, $p = 0.3101$ in the pellet group and 0.0008 in the sucrose groups, and in the third molars $p = 0.0544$ and 0.7782, respectively (Table 2).

The number of carious lesions was greater in the sucrose groups than in the pellet groups. More initial (T lesions) and advanced (B lesions) dentinal lesions were seen in the intact sucrose group than in any other. The number of intact fissures was

Table 2. Dentin apposition in various groups (in square micrometers)

		Max	Q3	Med	Q1	Min	No. of fissures
Dentin apposition in sucrose groups							
MI	O+/suc	3656	1990	1285	794	0	21
	Cnt/suc	5025	1087	506	249	0	20
MII	O+/suc	4241	3308	2176	1138	11	22
	Cnt/suc	3460	1196	551	0	0	20
MIII	O+/suc	7072	1299	716	6	0	20
	Cnt/suc	2069	1106	764	154	0	20
Dentin apposition in pellet groups							
MI	O+/pel	8020	3276	1356	317	0	22
	Cnt/pel	2866	536	130	0	0	24
MII	O+/pel	4090	2496	1540	7	0	22
	Cnt/pel	5107	2232	343	0	0	24
MIII	O+/pel	16360	5300	1178	17	0	22
	Cnt/pel	7535	1905	0	0	0	24

Max = maximum; Q3 = 75% quartile; Med = median; Q1 = 25% quartile; Min = minimum; MI = first molar; MII = second molar; MIII = third molar. Other abbreviations as in Table 1.

Table 3. Intact fissures, enamel lesions (A), initial dentinal lesions (T), advanced dentinal lesions (B), and cavitations (C) in various groups, as percentages of all fissures (by König's method)

		Intact	A	T	B	C	No. of teeth	No. of fissures
O+/pel	MI	75.8	22.7	1.5	0.0	0.0	22	66
	MII	70.5	27.3	2.3	0.0	0.0	22	44
	MIII	72.7	27.3	0.0	0.0	0.0	22	22
O+/suc	MI	47.6	30.2	19.0	3.2	0.0	21	63
	MII	47.7	38.6	13.6	0.0	0.0	22	44
	MIII	45.0	55.0	5.0	0.0	0.0	20	20
Cnt/pel	MI	68.1	27.8	4.2	0.0	0.0	24	72
	MII	66.7	33.3	0.0	0.0	0.0	24	48
	MIII	83.3	16.7	0.0	0.0	0.0	24	24
Cnt/suc	MI	33.3	36.7	26.7	3.0	0.0	20	60
	MII	22.5	55.0	15.0	7.5	0.0	20	40
	MIII	75.0	20.0	5.0	0.0	0.0	20	20

Abbreviations as in Tables 1 and 2.

greatest in the ovariectomized rats fed a non-cariogenous pellet diet. The Schiff reagent did not show any cavitations (Table 3). The difference was statistically significant only for the rats fed a sucrose diet in the second molars ($p = 0.036$).

The mean area of caries lesions was greater in the animals on a sucrose diet, both ovariectomized and intact, than in those on a pellet diet (Fig. 3). There were no statistically significant differences in the areas of dentinal carious lesions between ovariectomized and control animals.

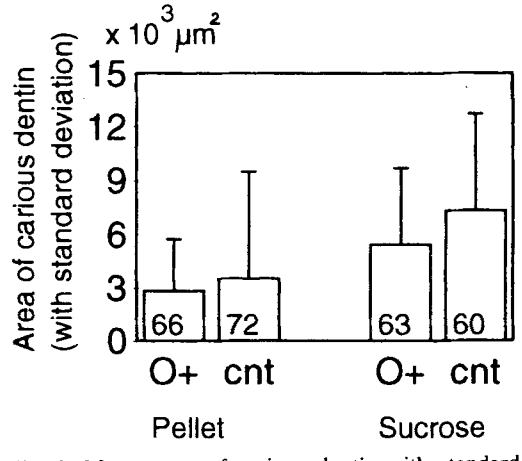

Fig. 3. Mean areas of carious dentin with standard deviations ($\times 10^3$ µm^2) in the pellet and sucrose groups (all molars analyzed together; number of fissures in bars). Abbreviations as in Fig. 2.

Discussion

Although the food consumption of the ovariectomized and intact animals was the same, the weight gains of the ovariectomized rats were greater, indicating that ovariectomy as such enhances weight gain in rats (1, 2, 19–22).

During the experiment enhanced dentinal apposition was obvious in the ovariectomized rats regardless of the diet and led us to the hypothesis that the metabolism of odontoblasts (or perhaps pulpal fibroblasts (23)) is accelerated. This finding is in line with observations on osteoblasts (2, 22). The rate of dentinal apposition observed here in adult rats over 99 days is a 10th of that noted in young animals in 35 days (24). Odontoblast activity diminishes once tooth development is completed (25). In contrast to the reducing effect of a sucrose diet on dentin apposition in developing teeth (26), its effect on dentinal apposition in adult rats was negligible in the present experiment.

The great variation in the dentinal apposition is partly methodologic. The Keyes method of hemisecting the rat teeth (16) is somewhat inaccurate in adult animals

because of the curvature of the mandibula. It is impossible to get all molars in the same line, and one of the molars, most probably the third one, gets out of the section line. In addition, the teeth curve lingually, making the transversal hemisection inaccurate.

Devlin & Ferguson (27) found no evidence of any effect of ovariectomy on dentin apposition in the incisors of rats. The continuous growth of the rat incisors consists of primary dentinogenesis, but dentinogenesis in adult rat molars consists of secondary dentin formation. This may explain the difference, indicating that primary and secondary dentinogenesis may be different entities.

The cariogenic challenge time of 99 days, the 'prestabilized' phase of postovariectomy osteoporosis (1), was obviously not long enough for caries to penetrate deep into the dentin or to produce cavitations, although it was long enough for the development of osteoporosis, and an increase in secondary dentin apposition could readily be detected. The mean areas of carious lesions were smaller than those reported in young animals after 35 days of cariogenic challenge (24), even though the present experiment lasted 99 days. The teeth of adult animals are more able to resist cariogenic challenge than developing teeth. This may be due to the completion of posteruptive maturation and/or dentinogenesis. The effect of ovariectomy on caries initiation seems to be negligible. Since the number of dentinal lesions was very small in this experiment, no profound conclusions can be reached with regard to the effect of ovariectomy on dentinal caries progression.

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