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Fluoride and hard cheese exposure on etched enamel in neck-irradiated patients *in situ*

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ABSTRACT

Objectives: The effect of a fluoride mouth rinse with hard cheese exposure was investigated on rehardening of an etched tooth enamel surface in subjects with radiation-induced hyposalivation *in situ*.
Methods: Ten patients, six males and four females of mean age 48 years, irradiated with 30 Gy per week for neck and head cancer, volunteered for the present intraoral study. The unstimulated saliva flow rate varied between 0.01 and 0.15 ml min^{-1}. Enamel slabs, approximately 2 mm × 2 mm in size, cut from human molar teeth were embedded in self-curing acrylic resin to fit a microhardness tester. Hardness measurements were carried out on the polished and subsequently etched enamel surface, rinsed for 1 min in the mouth with 10 ml Meridol (GABA INT.), containing 0.025% F as amine fluoride and stannous fluoride, and exposed alternatively to mastication of 20 g cheddar cheese for 5 min.
Results: Surface erosion of the enamel slabs decreased the mean hardness to a similar degree in all samples. The difference between the mean increased degrees of enamel microhardness following fluoride, fluoride and hard cheese, or repeated fluoride–cheese exposures was significant compared to the etched enamel values. The rate of rehardening derived from a second fluoride–cheese treatment was found to be improved significantly. It seems that the reduced saliva flow in xerostomic patients is sufficient to release bound calcium and phosphate from cheese products.
Conclusions: It is suggested that for xerostomic patients frequent exposures to low-fluoride solutions combined with hard cheese consumption may prevent and remineralize initial demineralization. Copyright © 1996 Elsevier Science Ltd.

KEY WORDS: Irradiation, Fluoride, Hard cheese, Enamel

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INTRODUCTION

Recently, the cariostatic properties of cheese have been the subject of intensive research[1–6]. Chewing of cheese stimulates saliva flow. Saliva is important for the preservation and maintenance of oral health[7]. Silva *et al.*[3], using the intraoral cariogenicity test, suggested that calcium and phosphorus content of cheese seems to be a factor in the cariostatic mechanisms of cheese.

Radiotherapy to the head and neck for the treatment of cancer may result in a marked decrease in salivation[8]. Hyposalivation induced by radiation may be accompanied by frequent consumption of foods high in carbohydrate, which increases the caries challenge[9]. The reduced enamel rehardening capacity of calcium and phosphate ions in saliva of irradiated patients, due to a decreased flow, may be supplemented by cheese[6]. In irradiated patients the rehardening effect on tooth enamel through cheese consumption was not necessarily mediated by stimulation of saliva; it seemed that even a reduced saliva flow was sufficient to release the bound calcium and phosphate in cheese products[5,6]. Krobicka *et al.*[10] demonstrated that in rats with a severely limited salivary function, cheese exerts a protective effect against coronal and root-surface caries.

The successful use of topically applied fluorides to prevent dental caries in patients suffering from radiation-induced hyposalivation has been reported[8,11]. The enhancement of remineralization is currently considered to play an important role in the mechanism of the action of fluoride[12]. In normal subjects it was found that, as expected, a fluoride rinse containing 0.025% F increased the rehardening effect on softened enamel by the mastication of hard cheese[5].

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AIM

The aim of the present investigation was to assess the effect of a fluoride mouthrinse and hard cheese consumption on enamel rehardening in patients with radiation-induced hyposalivation.

MATERIALS AND METHODS

In the experimental design, enamel surfaces of human teeth were etched by exposure for 30 min to an acidic medium of pH 3.4, followed by intraoral rehardening[5,6]. The changes in the enamel surfaces were monitored by measurements of enamel microhardness.

Ten patients wearing prosthetic devices and suffering from head and neck cancer were treated with 30 Gy irradiation per week, which is known to cause pronounced hyposalivation[6]. The patients volunteered for this study. They were asked not to drink or eat anything during the last hour before the measurements of saliva flow and the intraoral test. Unstimulated saliva was collected for 10 min in a graduated tube in the morning. The saliva flow rate varied from 0.01 to 0.15 ml min^{-1}, as expected in xerostomic patients[6]. Six patients were male and four were female; the mean age was 48 years.

Sound human molar teeth were used for the preparation of enamel slabs. The crowns of the teeth were separated from the roots, cleaned and washed with 70% ethanol for 1 h. Longitudinal buccal surface sections, of middle enamel of approximately 2×2 mm size, were cut perpendicular to the enamel prisms and subsequently embedded in self-curing acrylic resin to fit in the Leitz microhardness tester[5,6]. After embedding, the sections were ground with 1000 grade carborundum paper under running water. A final polishing was accomplished with diamond paste on felt cloth. Microhardness was measured with a Vickers diamond under a 300-g load, always advanced perpendicular to a horizontal plane on the enamel surface. With the accurate standardization of the diamond indenter on the test surface and with the microscope reading on the indentation, quantitative hardness measurements could be carried out[5,6]. The average microhardness for each specimen was determined from four to six indentations performed close to the original indentation. The individual readings were within 5% of the average value.

Each enamel section was exposed to 25 ml orange beverage (pH 3.4, ppm F, Goldsun, Tel-Aviv, Israel) for 30 min *in vitro* to induce enamel pre-etching. Prior to the microhardness re-examination for the degree of etching, the enamel slab was rinsed with water and dried. The enamel slab was placed in each prosthetic appliance in the line of direct occlusion to enable hard cheese to be masticated actually on the enamel slab.

After introducing the prosthetic appliance with one inserted etched enamel slab into the mouth, the patient was instructed to place 10 ml of a fluoride pre-rinse into his/her mouth. The fluoride mouthrinse used, Meridol (kindly supplied by GABA INT., Basle, Switzerland), pH 4.9, contained 0.0125% F as amine fluoride and 0.0125% F as stannous fluoride. The patient was instructed to move the mouth rinse about within his/her mouth for 1 min. After expectorating the mouth rinse, the fluoride-exposed enamel slab was recovered and retested for microhardness (Group 1). In Group 2, after the introduction of the prosthetic appliance with an inserted etched enamel slab into the mouth and after the mouth rinse had been expectorated, the same patient was asked to chew for 5 min a 20-g piece of hard cheese (Cheddar; per 100 g: 25 g protein, 33 g fat, 40 g water, 720 mg Ca, 500 mg P, based on manufacturer's data). He/she was instructed not to swallow it for 5 min, but to chew it continuously over that period. Actual chewing of 20-g cheese for 5 min was found to be satisfactory from the rehardening experiments in our previous investigations[5,6]. In Group 3 repeated fluoride pre-rinse and cheese exposures were carried out at times identical to those for Group 2.

The degree of removing and rehardening an outer enamel layer was defined as the alterations between initial and experimental microhardness of the enamel surface. The same ten patients were available in the three experimental series. The length of time of treatment or time between treatment and remeasurement of microhardness was similar for all patients.

Statistical analysis

The difference between the three treatments was examined by Friedman two-way ANOVA since the variables were not normally distributed. In case the Friedman two-way ANOVA test was significant, the Wilcoxon matched pairs rank test was performed between each of two treatments. The significance level was $P < 0.05$.

RESULTS

The results are summarized in *Table I*. The mean microhardness of the baseline (starting point) enamel slabs was not significantly different between the three groups. Exposure of the enamel slabs to an acidic medium for surface erosion decreased the mean hardness to a similar degree. The difference between the mean degrees of enamel microhardness following fluoride, fluoride and hard cheese, or repeated fluoride–cheese exposures respectively was significant ($P = 0.027$) compared to the etched enamel values. The rehardened mean difference from the respective mean baseline hardness between the repeated fluoride–cheese or fluoride rinse alone exposed enamel, Groups 1 vs. 3 (*Table I*), was significant ($P = 0.022$). A significant change was found for the difference between

mean baseline and returned hardness following the three respective exposures ($P = 0.045$).

DISCUSSION AND CONCLUSIONS

This investigation was conducted in a group of patients where the dynamics of de- and remineralization of incipient acid-etched enamel was influenced by the pathogenicity of microbiota and by complex host responses. Since the major salivary glands were included in the radiotherapy, salivary secretion was decreased.

Satisfactory results for prevention of xerostomia-related dental caries in irradiated cancer patients using extensive programmes with high doses of topical fluoride have been reported[13,14]. According to Johansen and Olsen[13], the extensive use of fluorides may, however, be considered a health risk since the use of high-concentration raises plasma fluoride levels[15]. They recommended both short-term intensive use of a super-saturated solution of calcium phosphate with respect to apatite presumably and fluoride (0.1%) to provide nucleation sites for mineral growth[13]. In the present study, the use of a mouthwash rinse containing 0.025% F in irradiated patients consuming hard cheese, was investigated on rehardening degrees of pre-etched enamel slabs. The induced rehardening differences between the three exposures were significant (*Table I*). In fact the rate of rehardening derived from the second fluoride–cheese exposed enamel was found to be improved significantly ($P = 0.022$), as compared to that achieved by the fluoride rinse only. This is most probably due to the supplementary source of rehardening provided by the Ca and PO_4 ions derived from the cheese[5,6]. It seems that even the reduced saliva flow in our xerostomic patients was sufficient to release bound calcium and phosphate from the cheese product[10].

From our previous investigations we observed that saliva secretion rehardened etched tooth enamel in non-irradiated and irradiated subjects, the latter suffering from radiation-induced hyposalivation[5,6]. A fluoride mouth-pre-rinse (10 ml Meridol containing 0.025% F) increased the enamel rehardening potential in normal subjects[5]. Tooth enamel exposure in the mouth of cancer patients, who have received therapeutic radiation to the head and neck, to a single hard cheese chewing for 5 min, induced a supplementary rehardening to that of the saliva effect alone, in spite of the known saliva dysfunction in xerostomic patients[6]. According to previous observations by Reynolds *et al.*[16], many proteins, in particular casein-related peptides, bind to mineral and are absorbed directly to an etched enamel surface, where they may play a role in calcium deposition inhibiting apatite growth. The second F-cheese exposure (Group 3) returned much closer to the initial level of hardness than the F pre-rinse alone (Group 1). It could be suggested that rehardening would have increased more with frequent and longer exposure periods (*Table I*). For ethical reasons, due to the health condition of our patients, we were obliged to abstain from longer trials.

To date, many investigators propose that the dynamic calcium phosphate coatings in various areas of etched enamel surface can be interpreted in terms of remineralization phenomena[4–6,17,18], regardless of its

Table I. In situ rehardening of etched human enamel with fluoride and hard cheese exposures in neck-irradiated patients (Vickers Hardness Number)

		F	*F plus cheese*	*F plus cheese + F plus cheese*	*Friedman two way ANOVA* (P value)	*Wilcoxon matched pairs rank test*
Group number		1	2	3		
Baseline enamel	Mean (SD)	303.1 (25.3)	299.72 (19.5)	308.3 (13.1)	NS	—
Etched enamel	Mean (SD)	265.8 (20.4)	268.3 (12.7)	279.7 (13.4)	NS	—
Rehardened enamel	Mean (SD)	282.0 (23.3)	287.7 (14.2)	299.6 (12.1)	0.027	1 vs 2: NS 2 vs 3: NS 1 vs 3: 0.022
Difference from baseline	Mean (SD)	21.1 (13.0)	12.0 (9.0)	8.7 (9.6)	0.045	1 vs 2: NS 2 vs 3: NS 1 vs 3: 0.022

Each group consisted of the same ten patients: Group 1 was treated with fluoride mouth rinse, Group 2 with fluoride mouthrinse and cheese, Group 3 was treated similarly to Group 2 followed by a second fluoride mouthrinse and cheese exposure.
Data are expressed in Vickers Hardness Number: mean (SD).

crystalline degree of organization, substantially increased in the presence of fluoride[12]. It has been found that remineralization in human enamel is always incomplete[17,18]. Generally, the remineralization pattern is not in accordance with the surface morphology of untreated enamel[6].

Several factors have been suggested to be responsible for caries inhibition by hard cheese consumption. Hypothesized mechanisms include protection derived from cheese fat[19] and the promotion of food clearance by saliva stimulated by textural and/or gustatory stimuli present in cheese[20]. Prevention of demineralization and/or promotion of remineralization by casein, calcium lactate and ionizable calcium and phosphate present in cheese[21] with concomitant low concentration of fluoride exposures, may be proposed to cancer patients treated with radiotherapy to the head and neck, as a non-complicated prophylactic dental procedure.

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References

1. Schachtele CF, Jensen ME, Harlander SK, Halambeck SM, Morris HA. Cheese as a paradigm for evaluating human plaque pH changes. *J Dent Res* 1982; **61**: (abstr 789).
2. Jensen ME, Harlander SK, Schachtele CF, Halambeck SM, Morris HA. Evaluation of the acidogenic and antacid properties of cheeses by telemetric monitoring of human dental plaque pH. In: Hefferen JJ and Koehler HM, eds. *Foods, Nutrition and Dental Health*. Chicago: ADA, 1984: 31–48.
3. Silva MFA, Jenkins GN, Burgess RC, Sandham HJ. Effects of cheese on experimental caries in human subjects. *Caries Res* 1986; **20**: 263–269.
4. Gedalia I, Ionat-Bendat D, Ben-Mosheh S, Shapira L. Tooth enamel softening with a cola type drink and rehardening with hard cheese or stimulated saliva. *J Oral Rehabil* 1991; **18**: 501–506.
5. Gedalia I, Davidov I, Lewinstein I, Shapira L. Effect of hard cheese exposure, with and without fluoride prerinse, on the rehardening of softened human enamel. *Caries Res* 1992; **26**: 290–292.
6. Sela M, Gedalia I, Shah L, Skobe Z, Kashket S, Lewinstein I. Enamel rehardening with cheese in irradiated patients. *Am J Dent* 1994; **7**: 134–136.
7. Mandel ID. The function of saliva. *J Dent Res* 1987; **66**: 623–627.
8. Meyerowitz C, Featherstone JDB, Billings RJ *et al.* Use of intraoral model to evaluate 0.05% sodium fluoride mouthrinse in radiation-induced hyposalivation. *J Dent Res* 1991; **70**: 894–898.
9. Brown LR, Dreizen S, Handler S *et al.* Effects of radiation induced xerostomia on human oral microflora. *J Dent Res* 1975; **54**: 740–750.
10. Krobicka A, Bowen WH, Pearson S, Young DA. The effects of cheese snacks on caries in desalivated rats. *J Dent Res* 1987; **66**: 1116–1119.
11. Mushanoff O, Gedalia I and Daphni L. Fluoride acquisition by surface enamel of human teeth *in vivo* following toothbrushing with an amine-fluoride toothpaste. *J Dent* 1981; **9**: 144–149.
12. White DJ. Reactivity of fluoride dentifrices with artificial caries. I. Effects of early lesions: F uptake, surface hardening and remineralization. *Caries Res* 1987; **21**: 126–140.
13. Johansen E, Olsen TO. Topical fluorides in the prevention and onset of dental caries. In: Johansen E, Taves D and Olsen TD, eds. *Continuing Evaluation of the Use of Fluorides*. Boulder, CO: Westwood, 1979; 61–110.
14. Horiot JC, Schraub S, Bone MC *et al.* Dental preservation in patient irradiated for head and neck tumours: a 10-year experience with topical fluoride and a randomized trial between two fluoridation methods. *Radiother Oncol* 1983; **1**: 77–82.
15. Ekstrand J, Koch G, Lindgren LE, Petersson LG. Pharmacokinetics of fluoride gels in children and adults. *Caries Res* 1981; **15**: 213–220.
16. Reynolds EC, Riley PF, Storey E. Phosphoprotein inhibition of hydroxyapatite dissolution. *Calcif Tissue Int* 1982; **34**: 552–556.
17. Gaengler P and Hoyer I. *In vivo* remineralization of etched human and rat enamel. *Caries Res* 1984; **18**: 336–343.
18. Wucherpfennig G, Hoyer I and Gaengler P. Mikromorphologie der superfiziellen Remineralisation des Zahnschmelzes. *Zahn-Mund-Kieferheilk* 1990; **78**: 223–229.
19. Heiferman A, Gedalia I, Brayer L, Rajstein J. Absorption of milk fat on tooth enamel and the effect on debris accumulation. *Israel J Dent Med* 1981; **29**: 61–63.
20. Jenkins GN, Hargreaves JA. Effect of eating cheese on Ca and P concentrations of whole mouth saliva and plaque. *Caries Res* 1989; **23**: 159–164.
21. Thomson ME, Dever JG, Pearce EI. Intraoral testing of flavoured sweetened milk. *NZ Dent J* 1984; **80**: 44–46.