FLUORIDE AND MAGNESIUM CONTENT IN SUPERFICIAL ENAMEL LAYERS OF TEETH WITH EROSIONS

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SUMMARY: In the absence of any previous research on the content of fluoride and magnesium in superficial enamel layers of eroded teeth in relation to acid resistance of the enamel, a study was undertaken to determine the concentration of both elements in eroded teeth using the acid biopsy technique. Biopsies were obtained from the labial surface below (upper dentition) or above (lower dentition) the erosion from the following teeth: one upper central incisor, five upper canines, three lower canines, two upper premolars, and three lower premolars. Corresponding teeth without erosions from age- and gender-matched patients living in the same area served as controls. Microsamples of enamel were obtained using 3 μL of 0.5 M perchloric acid. Fluoride concentrations were measured chromatographically, and magnesium and calcium were determined by atomic absorption spectrometry. Teeth with enamel erosions had significantly lower concentrations of both fluoride and magnesium as well as lower resistance to penetration by acid. Significantly lower concentrations of fluoride and magnesium were found in upper as well as in deeper enamel layers of eroded teeth compared to controls. These findings confirm an association between the content of fluoride and magnesium and acid resistance in dental enamel, which is reduced in teeth with erosions. In areas of low exposure to fluoride, topical fluoride may be protective against enamel erosion by acids, especially in carbonated beverages.

Keywords: Enamel acid biopsy; Dental enamel erosion; Enamel fluoride; Enamel magnesium.

INTRODUCTION

Enamel erosion results from the action of acids on hard dental tissues. Numerous predisposing factors interacting with individually varying structural features and chemical composition of enamel play an important role in the etiology of these lesions.[1-4] Enamel is essentially composed of hydroxyapatite and an organic matrix fraction containing proteins, lipids, and water.

Hydroxyapatite contains calcium ions replaceable by sodium, magnesium, zinc, strontium, or lead ions, as well as hydroxyl and phosphate groups which can be substituted by chloride, bicarbonate, and fluoride ions giving rise to apatites with reduced calcium content (carboxyapatites, chloroapatites, fluoroapatites, etc.).[5-7] Enamel and other hard dental tissues are eroded by acids from food or produced by bacteria in the dental plaque. Since the resistance of enamel to acids depends largely on its chemical composition, it is of interest to determine the composition that supports or prevents the process of erosion. Accordingly, this work was undertaken to determine the content of fluoride and magnesium in the superficial layers of enamel of teeth with erosions using the acid biopsy technique.

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MATERIALS AND METHODS

Fourteen teeth with enamel erosions were studied in six males and eight females, aged 18-30 years, living in the city of Białystok, a poorly industrialized north-eastern region of Poland. Corresponding teeth without erosions from age and gender-matched patients living in the same area served as controls. All examined individuals completed a questionnaire concerning nutritional preferences, oral hygiene, and tooth brushing habits. Patients with enamel erosions consumed citrus fruits, apples, fruit juices, carbonated drinks, and herbal teas more frequently than those without erosions. No significant differences in oral hygiene and tooth brushing practices were found between the two groups.

Biopsies were obtained from the labial surface below (upper dentition) or above (lower dentition) the erosion from the following teeth: one upper central incisor, five upper canines, two upper premolars, three lower canines, and three lower premolars. Enamel samples were collected from recumbent patients. The dental surface was mechanically cleaned with a brush, thoroughly rinsed with distilled water, dried, and defatted with ether. Contamination with saliva was prevented and an adhesive tape with a central hole measuring 1.75 mm in diameter and 2.4 mm^2 in area was placed over the tooth. Microsamples of enamel were obtained using 3 μL of 0.5 M perchloric acid ($HClO_4$) and an Eppendorf micropipette (Varipette 4710) with a plastic disposable tip. The acid droplet was dispensed with the tip positioned vertically to the dental surface under the hole, left for 3 s, and aspirated. The biopsied surface was rinsed with distilled water, dried, and a second sample was collected in the same manner. Fast remineralization of the biopsy site was ensured with Duraphat sealant.

The enamel biopsy was placed in 1.5 mL Safe-Lock tube (Eppendorf, Germany) which was then sealed and stored at 4 $^{\circ}C$. Tubes were transported in dry ice for determination of fluoride, calcium, and magnesium content at the laboratory of the Department of Biochemistry and Chemistry, Pomeranian Medical University in Szczecin.

Fluoride concentrations were measured with a Chrom 5 gas chromatograph (Laboratorni Pristroje).[8,9] For this purpose, 50 μL of a solution containing 10 μL trimethylchlorosilane (TMCS) and 0.1 μL 2-methylbutane in 20 mL of benzene was added to each tube. Tubes were shaken for 20 min to transform TMCS into trifluoromethylsilane, which passed from the aqueous solution into the benzene layer. Phases were separated by centrifugation for 1 min, and 2 μL of the benzene phase was injected into the chromatograph.

Prior to determination of magnesium and calcium content with atomic absorption spectrometry,[10] tubes were evaporated to dryness, and 2 mL of 0.1% lanthanum solution was added. Measurements were performed with a PU 9100X instrument (Philips) in acetylene-oxygen flame and 1% solution of $La(NO_3)_3$ at a wavelength of 285.2 nm for magnesium and 422.7 nm for calcium.

The mass of enamel samples was calculated on the base of calcium measurements, taking 37.4% as the content of calcium.[11] The thickness of each biopsied layer was estimated using 2.95 g/cm^3 as the enamel density and assuming cylindrical digestion of the enamel by perchloric acid. The following equation was used:[8]

$$d = \frac{\text{enamel mass}}{2.95} \times \pi r^2$$

where d = depth of penetration (biopsied layer thickness); 2.95 = density of enamel (g/cm^3); r = radius of digestion (0.0875 cm).

The results were submitted to a statistical comparison with the Student's t test (Statgraphic 5.0 software) and tested for a significance level of $p< 0.05$ or less.

RESULTS AND DISCUSSION

Mean mass and thickness of the enamel layers are given in Table 1. In teeth with erosions, both the enamel mass and calculated depth of acid penetration (layer thickness) were significantly greater than in the control group ($p<0.02$ and $p<0.05$, respectively)

Table 1. Mean mass and thickness of enamel layers of teeth with and without erosions biopsied with 0.5 M perchloric acid

Group	Layer	Enamel mass (µg)	Layer thickness (µm)
Study	a	7.82 ± 1.21*	1.10 ± 0.40†
	b	9.18 ± 1.34*	1.29 ± 0.43†
Control	a	6.61 ± 1.09	0.93 ± 0.22
	b	7.19 ± 1.19	1.01 ±0.25

Compared with the control group: *$p<0.02$; †$p<0.05$.

Table 2 shows the mean concentrations of total fluoride and magnesium in teeth with and without erosions. Significantly higher F and Mg concentrations were found in the upper layers in comparison with the deeper layers. Teeth with erosions had significantly lower concentrations of both elements.

Table 2. Mean content of magnesium and total fluoride in enamel of teeth with and without erosions biopsied with 0.5 M perchloric acid

Group	Layer	Fluoride (mmol/kg)	Magnesium (mmol/kg)
Study	a	33.49 ± 6.12*	32.63 ± 8.30‡
	b	16.76 ± 4.88†	19.89 ± 6.21§
Control	a	55.77 ± 8.21	97.93 ± 6.51
	b	43.50 ± 7.98	85.60 ±6.03

Compared with the control group: *$p<0.05$; †$p<0.02$; ‡$p<0.04$; §$p<0.004$.

The relationship between the chemical composition of hard dental tissues, resistance to acids, and dental erosions is well known. Although the chemical structure of enamel is determined during mineralization of growing teeth, hydroxyapatite crystals remain chemically active and capable of exchanging ions with the external environment. In this way, the composition of enamel is subject to modification.[12-14]

It is generally acknowledged that greater resistance of enamel to acids is chiefly dependent on its fluoride content. The structure of enamel supports the diffusion of fluoride ions into deeper layers where hydroxyapatites are transformed into fluorohydroxyapatites and fluoroapatites. Upon contact of acids with the dental surface, minerals are displaced from apatite in a process called demineralization. When the contact is transient, minerals are deposited again during remineralization. Thus, demineralization and remineralization result in loss and recovery of minerals in enamel. The rates of both processes depend on conditions in the oral cavity[5,7,15] and are significantly modified by fluoride which can be incorporated in the apatite structures producing fluoroapatites with a lower calcium content and greater chemical stability.[6] It is worth noting the remineralized enamel is more resistant to acids as compared with normal enamel.[16]

The highest enamel content of fluoride in the form of fluorohydroxyapatites and fluoroapatites is observed in the external layer. Enamel is enriched with fluoride by uptake from food, water, air, and toothpastes and incorporation into apatite structures of part of fluoride ions from calcium fluoride.[17-19] Total fluoride is the sum of fluoride in hard dental tissues chemically bound in the form of fluoroapatites and fluorohydroxyapatites, along with calcium fluoride deposited on the enamel surface and inside enamel pores. Removal of calcium fluoride from the enamel surface reduces total fluoride content by approximately 50%.[17] However, this is not accompanied by a significant increase in acid solubility of superficial enamel layers. In this work the total fluoride content in the first layer obtained from teeth with erosions was markedly higher than in the second layer where the fluoride content was reduced by about 50%. Teeth without erosions also contained significantly more fluoride in the first than in the second enamel layer.

Thus our results reveal decreased total fluoride concentrations in superficial enamel layers of teeth with erosions as compared with normal teeth. Low fluoride content may therefore contribute to reduced acid resistance of enamel, and hence this condition may support the formation of erosions.[20] In a recent *in vitro* study, Hughes and co-workers advocated the addition of fluoride to drinks or pre-treatment of enamel with fluoride-containing oral hygiene products to protect against erosion by citric acid and carbonated beverages (soft drinks).[21] This finding is supported by Santaella *et al*., who regard topical fluoride treatment as more effective than diode laser treatment for enhancing demineralization resistance of sound enamel in primary teeth.[22]

Magnesium is yet another element that affects the quality and structure of hard dental tissues. However, the metabolism of this element is unclear, and there is much controversy regarding the influence of magnesium on biological processes and their clinical relevance.[23-27] Undoubtedly, magnesium modifies the activity of alkaline phosphatase which participates in the formation of physiological hydroxyapatite crystals. According to some authors, magnesium ions are capable of suppressing precipitation and growth of calcium apatite crystals and of stabilizing the amorphous form of calcium phosphate, thereby preventing its transformation to a crystalline form.[23,24,27]

Further research is needed to elucidate the mechanism of incorporation of magnesium into the enamel structure. Terpstra and Driessens[12] have suggested that magnesium is bound inside enamel. Like calcium, magnesium reacts with phosphate groups but its content in enamel is negligible in comparison to calcium. Weatherell[28] believes that magnesium is one of those rare elements that are absorbed but not incorporated into the hydroxyapatite structure.

The influence of magnesium on acid resistance of hard dental tissues remains unclear. Sorvari reports no definite improvement from the addition of magnesium to sports drinks that induced marked erosion in rat teeth.[29] On the other hand, Parry *et al*. found that the greatest amount of reduction in hydroxyapatite dissolution was caused by calcium ions alone and was decreased in the presence of other ions such as magnesium and sulfate.[30]

In the present study we have demonstrated a relationship between the content of magnesium and depth of enamel etching by perchloric acid. The higher magnesium content in the first enamel layer than in the second layer (with or without erosions) is probably due to accumulation of this element through exchange of ions between saliva and enamel, and enamel and dental plaque. We also found an almost two-fold higher content of magnesium in the first than second enamel layer and a significantly lower content in teeth with than without erosions.

Our results confirm that fluoride and magnesium concentrations in superficial enamel layer are inversely related to the resistance of the enamel to acid attack.

Teeth with erosions showed a reduced concentration of both elements, and lower acid resistance of enamel was reflected by deeper penetration of perchloric acid. Since the manner in which magnesium influences acid resistance remains unclear,[29,30] the interpretation of the magnitude of the Mg differences is also uncertain. On the other hand, it appears likely that in areas with low exposure to fluorine, topical fluoride, which enhances acid resistance of dental enamel, may be protective against erosion by acids present in soft drinks. It is also possible that the lower fluoride and magnesium concentrations found in eroded teeth may reflect inadequate calcium intake which are insufficient to maintain and restore dental integrity. Clearly, further investigations concerning dietary and serum/salivary levels of calcium, magnesium, and fluoride in subjects with enamel erosions are needed.

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