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Effect of Acidic Challenge Preceded by Food Consumption on Enamel Erosion

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ABSTRACT

Objectives: This *in vitro* study aimed to evaluate the effect of food consumption followed by acidic challenge on enamel erosion.

Methods: Seventy-five enamel blocks obtained from bovine teeth were divided randomly into five groups (n=15 per group): GI – erosion with previous immersion in milk; GII – erosion with previous immersion in cheese extract; GIII – erosion with previous immersion in liver extract; GIV – erosion with previous immersion in broccoli extract; and GV – erosive effect of cola drink (control). Over 24 h, the slabs were submitted to 3 pH-cycles, each consisting of immersion in the studied food (GI to GIV) for 5 min followed by immersion in a cola drink for 5 min, and subsequently, the slabs were stored in artificial saliva (110 min). At the end of the pH-cycles, the slabs were stored in artificial saliva for 18 h. Enamel alterations were assessed by profilometry (μm). Data were tested using ANOVA and Scott-Knott's tests (P<.05).

Results: Mean erosion depths for enamel (μm) were 0.46 in GI, 0.55 in GII, 0.64 in GIII, 0.54 in GIV, and 1.18 in GVI. Enamel loss by acidic challenge alone (GV) was significantly higher than when the acidic challenges were preceded by food extract immersion.

Conclusions: The data suggest that all studied foods could minimize the erosive effect on enamel. (Eur J Dent 2010;4:412-417)

Key words: Erosion; Dental wear; Enamel; *in vitro*.

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INTRODUCTION

The aetiology and prevalence of tooth wear have received increasing interest in the literature.[1-4] Tooth wear has a multifactorial character; however, erosion has been found to be the main contributory factor,[5,6] and there is a strong belief that attrition and abrasion are of lesser importance.[7,8] Epidemiological studies have established that the prevalence of dental erosion is high in young people and adolescents.[9-11] One main goal in the management of dental erosion is the development of preventive strategies, which should be introduced at an early age.

Dental erosion is the surface loss of tooth structure due to the action of acids without involvement of microorganisms.[5,12,13] Enamel exposed to acid loses minerals from a layer extending a few micrometres below the surface, a process known as softening.[14] With time, as softening progresses further into the enamel, dissolution in the most superficial enamel will reach the point where this layer of enamel is lost completely.[14] *In vivo*, erosion could, therefore, involve two types of enamel wear: the direct removal of hard tissue by complete dissolution and the creation of a thin softened layer, which is vulnerable to subsequent mechanical wear.[14] Although a multitude of factors seem to be involved in this process, the most important factors are dietary acids[15,16] and intrinsic acids from the stomach.[17,18] Currently, the increased consumption of acidic foods and soft drinks is becoming an important factor in the development of erosive wear.[15,16] On the other hand, the potential of dairy foods and drinks to protect teeth against dental erosion has been documented.[19,20] Gedalia et al[19] and Lewinstein et al[20] showed the rehardening effects of cow's milk and cheese *in situ* following the softening of human enamel with an acidic soft drink (cola drink), and other studies have shown that milk and cheese rich in calcium.[21,22] On the other hand, Kato, Sales-Peres and Buzalaf,[23] and Kato et al[24] have studied the role of iron in dental erosion, showing that this element may play an important role in preventing this alteration. Since liver is an important source of iron[25] and broccoli is a vegetable very rich in this chemical element,[26] these foods would also have an effect on dental erosion. The rehardening reported by the studies mentioned above refers to a reparative effect of the treatments after the erosion had been promoted.[19,20] However, few studies have investigated the preventive effect of foods and drinks used before the erosive challenge.

Taking these considerations into account and considering that liver/broccoli and milk/cheese are foods rich in iron and calcium respectively, this *in vitro* study aimed to evaluate the effect of acidic challenge preceded by food consumption on enamel erosion.

MATERIAL AND METHODS

Enamel slab preparation

Seventy-five enamel slabs (4x4x3 mm) were prepared from extracted sound bovine incisors. One slab was cut from each crown using a low-speed saw cutting machine (Isomet – Buehler Ltda., Lake Bluff, IL, USA) and two diamond disks (Extec Corp., Enfield, CT, USA) separated by a 4-mm diameter spacer. The enamel surface was ground flat with water-cooled carborundum discs (320, 600, and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet by diamond spray (1 µm; Buehler). This procedure resulted in the removal of about a 100 µm depth of enamel.

After the slab preparation, the surface microhardness was determined by making five indentations (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan) for selection and randomized distribution. Enamel slabs with a microhardness ranging from 333 to 359 KHN were distributed randomly into 5 groups (n=15, mean 344±7.6 KHN): GI – erosive effect of cola drink with previous immersion in milk; GII – erosive effect of cola drink with previous immersion in cheese extract; GIII – erosive effect of cola drink with previous immersion in liver extract; GIV – erosive effect of cola drink with previous immersion in broccoli extract, and GV – erosive effect of cola drink (control). Two layers of nail varnish were applied on half of the surface of the enamel in order to maintain reference surfaces for lesion depth determination.

Preparation of food extracts and erosive pH-cycling

Prior to the experiment, 250 g of each food (cheese, broccolis or liver) was triturated and diluted by 300 ml of deionised water, resulting in a food extract. The cow milk was not diluted.

During 24 h, the slabs from groups GI, GII, GIII and GIV were subjected to 3 pH-cycles (37°C). In each cycle, the slabs were immersed in the studied food extract (GI to GIV) for 5 minutes (32 mL per slab) followed by immersion in a cola drink (Coca-Cola®, Coca-Cola Company, Spal, Porto Real, RJ, Brazil) for 5 minutes (pH 2.6). The GV specimens (control group) in each cycle were immersed in artificial saliva for 5 minutes (pH 7.0) followed by immersion in a cola drink for 5 minutes. Between the erosive challenges, the slabs were immersed in artificial saliva [1.5 mM $Ca(NO_3)_2.4H_2O$, 0.9 mM $NaH_2PO_4.2H_2O$, 150 mM KCl, 0.1 M Tris buffer, 0.03

ppm F, pH 7.0] for 110 minutes (32 mL per slab) at room temperature. At the end of the pH-cycles, all slabs were stored in artificial saliva for 18 h.

Wear assessment

After pH cycling, the nail varnish over the surfaces was cleaned carefully with acetone-soaked cotton.[27] The enamel wear was determined profilometrically in relation to the reference surface (Hommel Tester T 1000, Hommelwerke, Schwenningen, Germany).[28-30] The tracing parameters were established at Lt: 1.5 mm and Lc: 0.25 mm and the profilometry accuracy is 0.4 µm. Five readings were performed on each slab, and the average amount of wear was calculated. These profilometric traces were taken by moving the stylus from the reference surface to the exposed surface.

Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked. Since the assumptions were satisfied, ANOVA and Scott-Knott's tests were carried out for statistical comparisons, and the significance limit was set at 5%.

RESULTS

Table 1 shows the mean wear (µm±SD) of the groups (GI-GV). Enamel loss provoked by the cola drink (GV) was nearly 2-fold higher when compared to that observed when the acidic challenges were preceded by food extract immersion.

DISCUSSION

The present study was conducted in order to evaluate the dental enamel after an erosive challenge preceded by the consumption of daily used foods, which could cause the loss of dental structure.

For convenience, bovine enamel was used. Although a direct relationship between human and bovine enamel for %SMHC and wear was observed in a previous study, morphological differences such as higher porosity exist when compared to human enamel, which increases the formation of erosive lesions.[28]

Three pH cycles were chosen to simulate the consumption of erosive beverages and foods in the main meals (breakfast, lunch, and dinner). The artificial saliva used allows for rehardening of the slabs between the erosive challenges for simulation of the *in vivo* situation.[31] However, under clinical conditions, human saliva is also responsible for the formation of the acquired pellicle, which is a physical barrier that protects the tooth against erosive attacks.[32] This selective barrier prevents direct contact between acids and the tooth surface, thus reducing the dissolution of hydroxyapatite. Protection of the tooth surface by the acquired pellicle is well-established in the literature and has been demonstrated by several studies.[33,34] In this *in vitro* study, there was no acquired pellicle formation, and the absence of this natural protection may have increased the erosive attack on the enamel slabs. In addition, under clinical conditions, the presence of a salivary pellicle might affect the adhesion of proteins on the enamel surface, increasing the protective effect of the studied foods.

In this study, enamel wear was used as response variable since it is able to measure the complete dental loss induced by the pH-cycles, thus reflecting the cumulative effect of the erosive challenges. It has to be taken into consideration that in the contact profilometry, as done in the present study, the stylus might be able to scratch the acid-softened surface.[35] However, even when the stylus might damage the surface to a small extent, it is assumed that this phenomenon can be observed in all groups and might not affect possible differences among the groups. The resolution of the profilometer is 0.4 µm, allowing highly precise wear measurement because the mean erosion depths of the studied groups were higher than the error limit of the equipment.

The results of the present study showed that the food contact previously to the erosive challenge minimized the enamel erosion. This data could be explained by the fat and/or protein content of the tested foods, which could have acted as a physical barrier, thus limiting the action of

Table 1. The mean wear profile (µm) and standard deviation for the study groups.

Groups	N	Wear (±Sd)*
GI – Milk	15	0.46 (±0.17)[a]
GII – cheese	15	0.55 (±0.22)[a]
GIII – liver	15	0.64 (±0.19)[a]
GIV – broccoli	15	0.54 (±0.19)[a]
GV – control	15	1.18 (±0.40)[b]

*: Means followed by distinct letters are significantly different (P<.05).

the acidic drink.[36] Lewinstein et al[20] hypothesized several mechanisms for caries inhibition by cheese: protection derived from bufferfat, buffering of dietary acids through metabolism of protein breakdown products, and prevention of demineralisation and/or promotion of remineralisation by casein, calcium lactate, ionisable calcium, and phosphate present in dairy foods. Taking these aspects into account, another hypothesis could be related to the calcium (Ca) present in the studied foods. In agreement with the present study, Weiss and Bibby[37] showed that bovine enamel exposed previously to cow's milk was 20% less soluble in acetic acid than the control enamel.

Another ion that could enhance the protective effect of the foods (liver and broccoli) is iron (Fe). The mechanism involved in this protection of iron against mineral dissolution is not completely understood. It is possible that the formation of a thin acid-resistant coating of hydrous iron oxide on the enamel mineral surface may be a possible factor.[38] It has been shown that when the enamel is incubated with solutions of ferric salts, acid-resistant enamel surfaces are established due to the precipitation of ferric phosphate on the surface of the enamel.[38] The formation of this ferric phosphate barrier was also suggested in recent *in situ* studies, simulating situations of high cariogenic[39] and erosive/abrasive challenges.[40] The formation of such a of ferric phosphate barrier would reduce the contact of the soft drink with the enamel in subsequent acid challenges, which would, in turn, diminish the wear.

Prior studies have aimed to investigate the preventive effect of calcium and iron on dental erosion. However, these ions were added to commercial soft drinks or to pure acids.[41,42] The present study more closely resembles the clinical situation, not only because of the presence of the ions in daily consumed foods or drinks but also because of the use of these ions in non-toxicological concentrations.

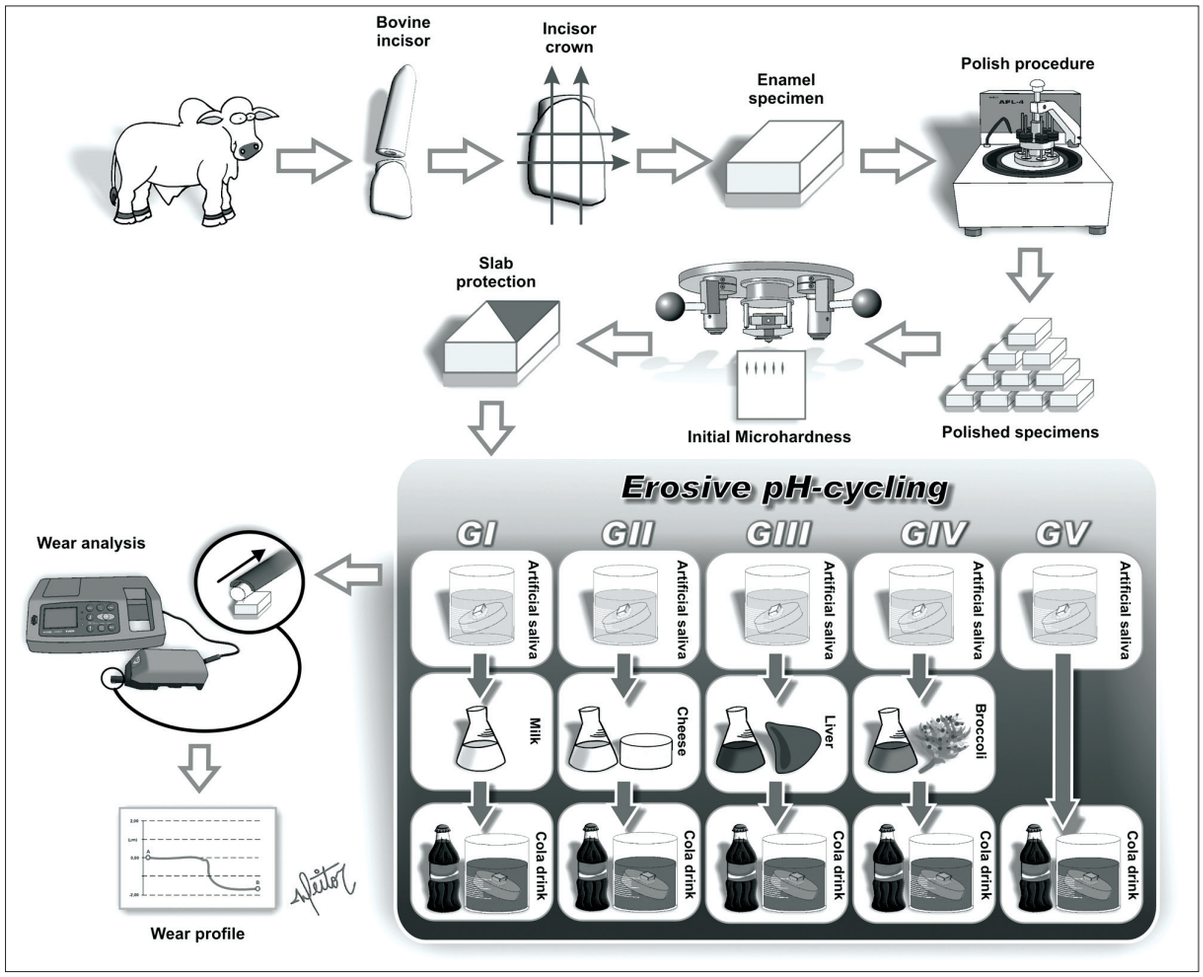

Figure 1. Schematic drawing of the experimental design.

CONCLUSIONS

The results of the present *in vitro* study suggest that the tested foods, when consumed before erosive challenges, can significantly reduce the wear. The consumption of these foods before or with acidic beverages could be a good strategy to diminish the deleterious effect of the beverage, especially for patients at high risk to dental erosion due to excessive use of these beverages. Due to the limitations of this *in vitro* protocol, however, before this preventive measure can be widely recommended, histochemical analysis of the enamel is necessary, and the data should be confirmed by *in situ* and clinical studies.

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