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In vitro Quantitative Assessment of Enamel Microhardness after Exposure to Eroding Immersion in a Cola Drink

Gerardo Maupomé [a]
Javier Díez-de-Bonilla [b]
Gabriel Torres-Villaseñor [c]
Luz del Carmen Andrade-Delgado [b]
Victor Manuel Castaño [d]

[a] Faculty of Dentistry, University of British Columbia, Vancouver, Canada,
[b] Facultad de Odontología,
[c] Instituto de Materiales,
[d] Instituto de Física, Universidad Nacional Autónoma de México, Ciudad Universitaria Coyoacán, México, Mexico

Abstract

Studies assessing the erosive potential of soft drinks have employed long time intervals of immersion that may not accurately depict the impact of frequent soft drink consumption on enamel. This in vitro study assessed the effect of a cola drink on enamel, replicating an actual drinking pattern. Six groups of 4 human enamel slabs were immersed (5 min each bath) in fresh cola drink, with immersions taking place with or without agitation, and under 3 regimes of frequency intake (low intake, 1 immersion/day; medium, 5/day; high, 10/day). Quantitative assessments of surface erosion were done over an 8-day interval using surface microhardness testing (Vickers). Results showed a sharp decrease from baseline (mean value 352.1 Vickers Hardness Number, SD 32.5) to day 1 (269.3, SD 41.0) and then continued decreasing throughout the assay, although less markedly, to reach 204.5, SD 45.4 on day 8. Microhardness decreased regardless of frequency regime, except on day 8, on which slabs from the low intake group were harder (233.2, SD 25.0) than slabs from the high intake group (169.8, SD 49.5; $p<0.05$). Results from the ANOVA on the factorial experiment indicated that the role of agitation was statistically significant (d.f. = 1, F = 7.2, p = 0.020) while the level of intake was of borderline significance (d.f. = 2, F = 3.2, p = 0.075). The main effect resulting from the joint roles of agitation and intake indicated that there was an important interaction between the two variables (d.f. = 3, F = 4.5, p = 0.023).

Key Words
Acid
Enamel
Erosion
Hardness
Microhardness
Soft drinks
Surface properties, human
Tooth demineralization

The erosive capabilities of soft drinks have been reported previously, both in studies in vivo and in vitro. However, many erosion reports have employed extremely long time intervals of a tooth or a tooth slab immersed in eroding solutions [Meurman and Frank, 1991a, b; Grenby et al., 1989; Gao et al. 1991]. Accordingly, a more realistic consumption pattern replicated under experimental conditions would be helpful in determining the actual impact of soft drinks by resembling real exposure [Maupomé et al., 1995], particularly if a quantitative estimate on surface changes is done. Clinically apparent erosion has been traced to dietary factors [Millward et al., 1994]. A survey carried out in 1993 in a large urban area where soft drinks are ubiquitous found self-reported consumption per day equivalent to 627.4 soft drinks/person/year (equivalent consumption per week reported to be 488 soft drinks/person/year) [Maupomé et al., 1995]. Over half of the 2,008 people interviewed outside subway stations indicated a consumption of at least 1 soft drink/day; while nearly 5% drank between 5 and 10 soft drinks/day. Thus, the aim of the present in vitro study was to

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Dr. Gerardo Maupomé
Department of Oral Health Sciences, Faculty of Dentistry
University of British Columbia
2199 Wesbrook Mall, Vancouver, BC V6T 1Z3 (Canada)
Tel. +1 604 822 2350, Fax +1 604 822 3562, E-Mail maupome@unixg.ubc.ca

quantitatively assess the erosive effect of a cola drink on human enamel under regimes depicting the drinking patterns found in that particular survey. It was hypothesized that even short intervals of exposure to the drink, resembling actual consumption, would lead to erosion. Since these beverages are drunk in a dynamic physical environment where the drink flows around several tooth structures, the decision was made to also study the effect of the drink being static or in motion.

Materials and Methods

Preparation of Enamel Blocks

Enamel blocks were cut from noncarious teeth extracted for orthodontic reasons. Teeth were cleaned from debris upon extraction, examined under a magnifying glass (×5) and good light for carious lesions and enamel defects, and stored in a low-level, neutral pH disinfectant for a week. The slabs were prepared using a method modified from Ximénez-Fyvie et al. [1996]: briefly, the root of each tooth was embedded in self-curing acrylic into a microtome tray. The specimen was mounted onto a microtome device which had the original cutting blade removed and replaced by a diamond disk (100 μm thick) (X929–7-TP Abrasive Technology, Inc., USA) and an electric motor. Using abundant cold tap water as a cooling agent, slices were cut on the buccal and lingual surfaces of each tooth to create a rectangular slab (3×4×1 mm). Each slab was serially polished flat as described by Zero et al. [1990] with 600 grit silicon carbide paper (3M, USA), and with aluminum hydroxide powder (sizes 0.3 and 0.05 μm) and stored in double-deionized water (DDW) at room temperature (20–25°C).

Test Procedure

Enamel slabs were divided into six experimental groups and immersed in a cola drink over an 8-day period. Following a factorial design, groups differed in that: (1) they were immersed with or without agitation provided by magnetic stirrers, and (2) they were immersed under three different regimes of frequency intake (low intake, i.e., immersion once a day; medium intake, i.e., immersion 5 times a day, and high intake, i.e., immersion 10 times a day). The 5 and 10 immersions took place evenly distributed over a 12-hour interval.

Slabs were protected from damage which could have been caused by stirring devices by being enclosed in nylon nets (10 × 15 × 20 mm; diagonal measurement of opening, approximately 1 mm), placed inside containers. Regardless of whether it was subject to agitation or not, every slab was immersed enclosed in a net. Four slabs were randomly allocated to each one of the six groups and each specimen individually treated by immersion (5 min each bath [Meurman et al., 1990]) in fresh cola drink (pH 2.6). Before and after immersion in the drink, slabs were copiously rinsed in 0.1 *M* phosphate-buffered saline (PBS; pH 7.2). When not exposed to the drink, specimens were stored in DDW at room temperature (20–25°C).

Quantitative Assessments

Four slabs from each group were used for the quantitative assessments. After completion of each day of immersions following the schedule for its experimental group, each slab was placed on the rotating table of a microhardness tester (Shimadzu, Kyoto) and two indentations spaced 100 μm apart were made with a Vickers diamond under a 100-gram load for 15 s. A positioning device designed to orientate slabs allowed parallel indentations. Enamel microhardness (EMH) was determined (assuming that net change in hardness is directly correlated to mineral loss [Kodaka et al., 1992]) by measuring the indentation length along the vertical and horizontal axes included in the Vickers Hardness Number formula. The mean value of the two readings was used.

Controls

A negative control using DDW water instead of the eroding agent was done over the full 8 days of the assay on three slabs, under the high intake and agitation regime.

Statistical Analyses

The mean values for the slabs allocated to each one of the levels of intake (8 slabs in each group) and agitation (12 slabs in each group) regimes were used in data analysis (24 slabs in total) following a factorial design [Altman, 1991]. Data were analyzed using Student's t test, one-way ANOVA (Scheffé test), fixed-effects two-factor randomized ANOVA, and multiple linear regression analysis.

Results

On pilot tests, it was found that after day 8 EMH testing was not feasible due to surface changes in enamel slabs. EMH indentations could no longer be safely distinguished from the eroded background. Therefore, the assays were limited to the first 8 days.

There were no statistically significant differences (SSD) in EMH readings on day 0 (baseline) between slabs allocated to different experimental groups. Thus, all the slabs had the same hardness prior to the baths.

EMH readings done in the negative control suggested that no erosion was caused by DDW (mean 339.9 Vickers Units (VU), SD 17.8). The coefficient of variation was small (0.052), suggesting that changes in experimental slabs were due to the erosive nature of cola drink when compared to the lack of effect by immersion in an inert, liquid medium such as DDW.

EMH decreased after immersions in cola. EMH readings continued to decrease as the baths took place but the sharpest drop in EMH occurred after the first day in all instances (tables 1, 2, and fig. 1 and 2). Overall mean values for EMH changes over time indicated that SSD occurred between days 0 and 1 ($t = 10.9$, $p < 0.001$); 1 and 2 ($t = 2.2$, $p = 0.03$); 4 and 5 ($t = 3.9$, $p = 0.001$); 5 and 6 ($t = 3.5$, $p = 0.003$), and 6 and 7 ($t = 2.5$, $p = 0.02$).

No SSD could be found between EMH readings performed on slabs subjected to the three regimes of cola drink exposure on days 1–7. On day 8, slabs in the high intake group had significantly lower EMH readings compared to the low intake group (d.f. 2, $F = 4.0$, $p = 0.038$) (fig. 1, ta-

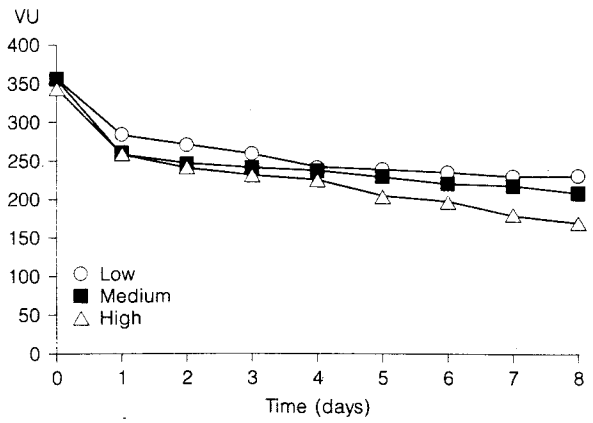

Fig. 1. Microhardness values across three levels of intake.

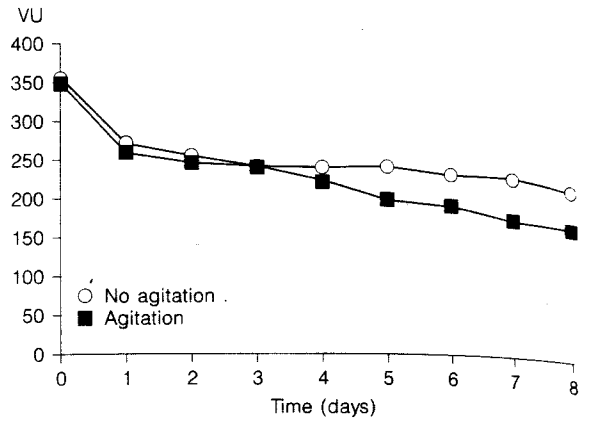

Fig. 2. Microhardness values on assays with and without agitation.

Table 1. Microhardness values (mean VU ± SD) on assays with low, medium or high intake levels

Day	Intake levels			Mean	SD
	low	medium	high		
0	356.7±32.1	356.6±24.3	343.1±41.4	352.1	32.5
1	286.3±22.9	262.3±38.1	259.2±55.2	269.3	41.0
2	271.7±31.2	246.8±29.6	241.5±39.5	253.3	34.9
3	260.2±17.2	241.8±41.1	230.2±42.4	244.0	36.2
4	241.8±25.9	237.8±44.4	224.5±34.5	234.7	35.0
5	239.7±22.7	231.0±42.0	203.5±46.1	224.8	39.3
6	236.3±20.6	221.9±39.2	196.3±45.2	218.1	38.3
7	231.3±22.8	219.2±35.9	180.5±60.6	210.3	45.9
8	233.2±25.0	210.3±38.2	169.8±49.5	204.5	45.4

Table 2. Microhardness values (mean VU ± SD) on assays with and without agitation

Day	No agitation	Agitation
0	354.8±18.8	349.5±43.0
1	271.9±33.1	266.7±49.0
2	257.4±31.1	249.3±39.3
3	245.2±38.2	242.8±35.8
4	244.5±34.6	224.9±33.9
5	246.4±20.1	203.1±42.8
6	238.9±19.2	197.3±42.2
7	237.8±19.1	182.8±49.2
8	229.7±22.4	179.3±49.5

ble 1). Since only minor statistically significant differences could be found for the intake assay, it was decided to undertake, first, separate statistical analyses for the intake and the agitation assays. Slabs eroded under agitation (all three intake levels together) had statistically significantly lower EMH values than slabs eroded in static baths (again all three intake levels together) only on days 5 ($t = 2.7$, $p = 0.019$), 6 ($t = 2.6$, $p = 0.021$), 7 ($t = 3.1$, $p = 0.010$), and 8 ($t = 2.7$, $p = 0.018$) (fig. 2, table 2). Secondly, results from the ANOVA on the factorial experiment indicated that the role of agitation was statistically significant (d.f. = 1, $F = 7.2$, $p = 0.020$) while the level of intake was of borderline significance (d.f. = 2, $F = 3.2$, $p = 0.075$). The main effect resulting from the joint roles of agitation and intake indicated that there was an interaction (d.f. = 3, $F = 4.5$, $p = 0.023$) [Daniel, 1995]. This interaction was evident when values for the 8-day time point were plotted (fig. 3).

Multiple linear regression analysis of the data showed a behavior pattern (hardness related to time) that may be summarized by the following formula:

$$\sigma = EMH = a + (b)(\ln(\text{time})) \qquad \text{In terms of time: } \text{Time} = e^{((\sigma - a)/b)}$$

Values of a and b correlated well when entered in the formula: no agitation, coefficient a, 272.5; coefficient b, –35.3; correlation coefficient 0.85. Agitation, coefficient a, 278.2; coefficient b, –27.7; correlation coefficient 0.85. Also, low intake, coefficient a, 311.0; coefficient b, –29.1; correlation coefficient 0.88. Medium intake, coefficient a,

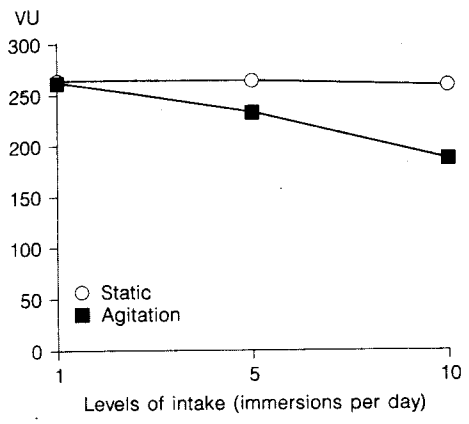

Fig. 3. Interaction between levels of intake and presence of agitation in the two-factor ANOVA.

243.6; coefficient *b*, –37.6; correlation coefficient 0.93. High intake, coefficient *a*, 245.8; coefficient *b*, –45.4; correlation coefficient 0.85.

Discussion

The epidemiology of erosion does not appear to indicate that it is a cause for serious concern as a public health problem. Prevalence varies markedly between population groups [Xhonga and Valdmanis, 1983; Xhonga-Oja and Valdmanis, 1986]. It does appear, however, that frequently drunk acidic drinks indeed cause erosion of enamel [Zero, 1996] and that this clinical concern is justified considering the increasing intake patterns and their implications for dental health [Millward et al., 1994; Touyz, 1994]. Even though a considerable number of laboratory investigations have been carried out regarding the effect of carbohydrates in relation to caries, lesser attention had been given to the effect of soft drinks which also contain fermentable sugars [Grenby et al., 1989] until relatively recently. This lack of reliable data may not be justified given the unabated upward worldwide tendency of soft drink consumption [Burt, 1993].

The erosive potential of soft drinks has been reported previously, both in vivo and in vitro. However, many erosion studies have employed extremely long time intervals of immersion in eroding solutions: e.g., 15–180 min [Meurman and Frank, 1991a]; 120 min [Meurman and Frank, 1991b]; 24 h [Grenby et al., 1989] or more [Gao et al., 1991]. There are a number of possible shortcomings in these studies if we attempt to use them as proxies of the situation in real life. For instance, it is likely that the effects of such immersion intervals do not accurately depict the actual impact of frequent, short exposures during soft drink consumption (as opposed to prolonged exposure times), given the capacity of the oral environment to heal early structural damage when the acid attack recedes with time, by deposition of minerals from saliva, and/or by avoiding it in the first place due to the protection given by salivary acquired pellicles. Another problem is that many studies have looked at the qualitative aspects of erosion but failed to devise an appropriate methodology to quantify the impact of exposure to acidic beverages. We developed a model to assess the erosiveness potential of a soft drink while mimicking actual drinking patterns. Although it would be difficult to argue that the regimes used in the present research resemble soft drink consumption patterns everywhere, there is little doubt that high levels of intake have been reported, either from interest groups, such as the reports from the American soft drink industry [Burt, 1993]; or detected within subgroups of populations under study in markedly disimilar settings, e.g., surveyed American general population [Park and Yetley, 1993]; mothers and children in Kuwait [Petersen et al., 1990]; American pregnant women [Pastore and Savitz, 1993]; adult subway users in Mexico City [Maupomé et al., 1995]; schoolchildren in a Mexico City suburb [González et al., 1993]; preschool children in Britain [Holt, 1991], and in very young children in Norway [Rossow et al., 1990] and Brazil [Dorea and Furumoto, 1992]. The distinctive feature among these (and probably most) studies is that there is always a minority within the population that has a high intake of acidic beverages of a fairly regular basis. Level of intake is clinically important [Järvinen et al., 1991]. Differences between study designs, aims of the research, and inherent features of the populations usually lead to highly variable sizes of that specific minority. It can only be said, looking at the international scene, that it would be extremely difficult to assume that the intake patterns used in the present study provide anything other than a series of yardsticks to objectively appraise the effects of differing levels of consumption (towards the higher end of the consumption continuum) under controlled conditions.

This comparison framework is important insofar as dental problems derived from excessive soft drink intake can be classified in two main groups: (1) most soft drinks are fruit-based and/or carbonated and thus are acidic enough to erode dental tissues. Canned or bottled, cariogenic or noncaloric soft drinks have a pH between 2.4 and 3.3 [Maupomé et al., 1995]. Those low pH levels appear to be common to soft drinks in many parts of the world. (2) Many of the formulations are rich in fermentable carbohydrates that can lead to

decay. The time scales of the two processes differ, however, but it is likely that both phenomena add up to potentialize the joint presence of carbohydrates and acidic beverages.

Since quantitative studies on enamel erosion were rather scarce until relatively recently [Featherstone et al., 1993] or have major differences in experimental design [Zero, 1996], it is difficult to compare the results of the present study with theirs. However, in other experimental designs [Lussi et al., 1993], it has been found that the time of immersion in acidic beverages produces a drop in hardness readings directly proportional to the exposure time. Our results substantiate the findings of Lussi et al. [1993]. It is not surprising that exposure to acidic beverages does undermine enamel integrity. It is, however, surprising how quickly this effect is evident. The greatest erosive effect in the present study occurred right after the first immersion. It is unfortunate that the effect of intake level on enamel hardness could only be detected shortly before the sensitivity technique was no longer able to identify differences. We found that the indentations made after day 8 could not be reliably distinguished from the pitted background under the present experimental conditions.

The presence of agitation could effect the availability of carbonic acid in the drink assayed and thus influence the differences found in the assay [Mistry and Grenby, 1993]. Carbonic acid may suffer some rapid changes even in freshly poured drinks. However, it is likely that agitation increased the rate of loss of ions from enamel by providing an avid, revolving environment for such an exchange, as opposed to static baths. Whatever degassing of the drink was caused by agitation appears to have little short-term effect on pH [Ireland et al., 1995]. Factorial analysis indicated that agitation appeared to potentialize the role of intake level. Whether this is or is not a factor bearing an important role in vivo is difficult to establish from the present data, in particular because host factors are likely to vary markedly [Zero, 1996]. However, it seems certain that at least some degree of agitation occurs in vivo, and that this characteristic of intake in our experimental drink increases its capabilities of causing erosion due to the high thermodynamic work of adhesion [Ireland et al., 1995].

Multiple linear regression analysis of the data suggested that the time interval physically represents an activation time or critical time in an Arrhenius-type phenomenon (i.e., a process that can only be present when a physical threshold is crossed). Different values of a and b correlated well when entered into the above formula, thereby allowing adjustment under various experimental conditions, which enables the establishment of one general behavior pattern. Such behavior permits some prediction capabilities with regard to what hardness values will be obtained after certain immersion times. These results, of course, are extremely attractive, especially if a long-term assessment of hardness is to be obtained. However, such extrapolations might be unwarranted unless hardness can be safely determined for time intervals beyond 8 days. From a broader perspective, it is striking to notice that virtually no difference could be identified between the low and the medium intake levels, which leads us to believe that a similar sharp drop in microhardness can result from 1 or 5 soft drinks a day. In a case-control study, Järvinen et al. [1991] found that more frequent soft drink consumption was strongly associated with dental erosion, except in individuals with the highest intake (more than 2 soft drinks per day), whose risk was somewhat similar to the study subjects drinking up to 2 drinks per day.

Erosion takes place by producing pitting on and roughening the corresponding surfaces. The holes and the sharp edges on them act as stress concentrators according to the Griffith criterion for brittle ceramic materials [Smith, 1996]. Gollys et al. [1993] concluded that, at the initial rate of rehardening for this type of surface deterioration, remineralization occurs in microspaces created in the slightly destroyed enamel structures. The influence of the pits could become dominating when remineralization takes place, presumably by means of the protective agents present in vivo, both through remineralizing agents naturally present in saliva and through some external factors, such as the therapeutic or household use of fluorides [Rugg-Gunn, 1993; Sorvary et al., 1994]. The protective effect of salivary pellicles in reducing surface dissolution rates of apatites has been established [Kautsky and Featherstone, 1993] but seems to vary according to the type of saliva used. Further studies incorporating salivary pellicles are necessary to explore the role of saliva in enamel protection under these particular in vitro conditions. This could prove to be a difficult endeavor in that Featherstone et al. [1993] looked at the actual fractions of saliva, aiming to establish more accurately the role of each combination, and found that no specific protein or group of proteins on their own could account for the results observed in whole and dialyzed saliva assays. It would appear that the next step towards unraveling some of the interpretation problems posed by the present research would be to introduce carefully controlled saliva environments in the model, and to compare their effects on microhardness assays.

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