ELSEVIER

Journal of Dentistry 28 (2000) 257–263

Journal of Dentistry

www.elsevier.com/locate/jdent

Erosion of deciduous and permanent dental hard tissue in the oral environment

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Received 26 April 1999; received in revised form 7 July 1999; accepted 23 November 1999

Abstract

Objectives: The objectives of this study were two-fold: (1) to determine (by surfometry) loss of deciduous and permanent enamel and dentine following consumption of a single low pH orange drink for 15 days; and (2) to determine (by surfometry) loss of deciduous and permanent enamel and dentine following consumption of the product 2 versus 4 times per day for 15 days.

Methods: Sixteen healthy volunteers participated in a single centre, single blind, 2-phase crossover study, conducted according to Good Clinical Practice, and employing the validated model described by West and co-workers (Journal of Dentistry 1998; 26:329–335).

Results: In all tissues, erosion was progressive over time, the pattern being more linear in enamel than in dentine. In general, erosion of deciduous enamel was greater than that of permanent enamel, though this difference was significant only for those specimens exposed to 4 drinks per day. Conversely, erosion of dentine was generally greater in the permanent tissue, though differences rarely reached conventional levels of statistical significance. Increasing frequency of consumption resulted in increased loss of tissue, but this difference was neither proportional nor consistently statistically significant.

Conclusions: It is concluded that statistically significant differences in susceptibility of deciduous and permanent enamel to erosion appear to emerge over time and with increasing frequency of consumption. This is of importance clinically given the reduced dimensions of the deciduous dentition and the element of 'abuse' of soft drinks by the child population. Further development of soft drinks with low erosive potential is recommended.

Keywords: Erosion; Enamel; Dentine

1. Introduction

During the last decade, in vitro experiments using acidic drinks such as cola, orange juice or sport drinks [1–3] have clearly proved the erosive potential of dietary acids. In addition, prevalence studies and case reports have shown the association of dietary habits with dental erosion [4–8]. Dietary factors known to cause erosion include all kinds of acidic foods with a low concentration of calcium or phosphate [1,9,10]. The erosive agents are characterised as being highly undersaturated with respect to both hydroxyapatite and fluoroapatite [11,12]. This ensures that enamel apatite dissolves without any formation of surface fluorohydroxyapatite [13].

Consumption of soft drinks has increased dramatically since 1950 [14]. In that year, 1000 million litres of soft drinks were sold in the United Kingdom: by 1990, this had increased sevenfold, and the trend shows no indication of levelling off. It is important to bear in mind that mean consumption figures may hide significant variations. Soft drink intake is much higher in younger age groups, providing as much as one-fifth of the added sugars in the diet of 11- to 12-year-old children [15]. It is also known that children aged between 2 and 9 years consume 42% of fruit drinks [14].

It is perhaps lamentable that marketing is often directed at younger members of the population, drinks not uncommonly being associated with peer group acceptability. Even the infant market has not been neglected, and, unfortunately, baby juices have been shown to give rise to extreme destruction of dental hard tissue with prolonged misuse [16].

A recent study [17] has shown that differences in susceptibility of deciduous and permanent tissues to erosion by a low pH drink appear to exist, though, in vitro, these were not consistently of statistical significance. In general, erosion of

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0300-5712/00/$ - see front matter
PII: S0300-5712(99)00079-2

enamel was greater in the deciduous tissue, while erosion of dentine was greater in the permanent tissue. Increasing frequency of exposure resulted in a non-proportional increase in tissue loss.

The authors recognised that in vitro models are incapable of replicating the biological variations of the oral environment. It is also well recognised that actual erosion in vivo largely depends on consumption practices. As a result, this study aimed to examine the existence of a difference in susceptibility of deciduous and permanent enamel and dentine to erosion by the same low pH drink in situ. The primary objective was to determine the amount of deciduous and permanent enamel and dentine lost by erosion following consumption of the product for 15 days. Determination of tissue loss at 5 and 10 days was considered to be a secondary objective.

The concept of 'abuse', as opposed to 'use' of soft drinks appears to be of importance in the aetiology of erosion. For example, Millward and co-workers [18] have identified a strong correlation between acidic drink consumption and the presence of the condition in children. Those presenting with severe erosion were consuming an average of 39 fruit based and carbonated drinks per week (5–6 drinks per day), whereas those with no or only mild erosion consumed 18 per week (2.5 per day). As a secondary aim, this study also sought to determine the role of frequency in the erosion of deciduous and permanent enamel and dentine in situ. This was accomplished by comparing the amount of tissue loss resulting from 2 versus 4 drinks per day.

2. Methods and materials

2.1. Clinical study

This in situ study employed the validated methodology described by West and co-workers [19], and was conducted in accordance with Good Clinical Practice. A single centre, single blind (blinded to the volunteers), 2-phase crossover design was chosen, and ethical approval obtained from the Research Ethics Committee of the United Bristol Healthcare NHS Trust. Healthy volunteers were recruited from among the staff of Bristol Dental Hospital and School.

Specimens of permanent enamel and dentine were derived from recently extracted, caries free, unerupted third permanent molars. These were collected from patients aged between 18 and 30 years of either gender. Specimens of deciduous enamel and dentine were derived from recently extracted, caries free, deciduous canines. These were collected from children of either gender who were undergoing their extraction for the relief of crowding. At the time of extraction, donors were resident in areas where the water supplies contained less than 0.3 ppm fluoride. However, details of previous residence were not available and it is also likely that fluoride-containing toothpastes were being used.

Following extraction, each tooth was soaked for at least 24 h in 50% sodium hypochlorite before being scraped of any remaining tissue with a scalpel. It was then rinsed in copious amounts of distilled water. Finally, the crown was sectioned from the root and both portions cut vertically to produce approximately equal sections of enamel and dentine respectively. In the case of the third permanent molars, 4 or 6 equal sections were cut from the crown of each tooth, depending on its size. However, due to differences in both size and morphology, it was only possible to cut two sections from the crown of each deciduous canine. Throughout this process, the necessity to produce sections from the same area of the crown in order to ensure consistency of enamel prism arrangement was constantly borne in mind.

Each section was embedded in a vacuum-formed polyurethane mould filled with epoxy resin. Twenty-four hours later, when the epoxy resin had cured, the specimen was removed and, using an automatic lapping and polishing unit, ground to fit a stainless steel jig. This had been specifically constructed to hold the specimen precisely during surfometry and ensure that a stable horizontal platform was maintained. Using abrasive discs of decreasing coarseness, a smooth, flat area of buccal or lingual/palatal enamel or dentine (as required) was exposed, care being taken to remove the minimum amount of tissue. This process was monitored by surfometry, the specimen only being accepted for use when two consecutive readings across the exposed area fell within the range -0.3 to $+0.3$ μm. Finally, each specimen was checked by eye and those in which dentine had been exposed were discarded. A total of 32 specimens of each tissue were prepared in this way.

Following acceptance, each specimen was given a unique reference number which was recorded on its reverse side in indelible ink. It was then stored in isotonic saline at room temperature in an eppendorf tube marked with the same reference number, and its two baseline surfometry measurements recorded for future reference. Immediately before use, an area of enamel or dentine was delineated by placing PVC insulating tape over the specimen, leaving a 2 mm wide zone of hard tissue exposed.

Prior to the commencement of the study, each potential volunteer was required to attend a screening appointment at which its nature and purpose were explained both verbally and in the form of a volunteer information sheet. The potential volunteer was given the opportunity to ask questions and allowed sufficient time to decide whether or not to participate. Oral and written informed consent was subsequently obtained.

In order to be eligible for inclusion in the study, volunteers (of either gender) were required to be aged between 18 and 60 years, enjoy good general health and understand and be able to comply fully with the study procedures and restrictions. Individuals suffering any condition or receiving any medication which might interfere with the interpretation of the study were specifically excluded from participating,

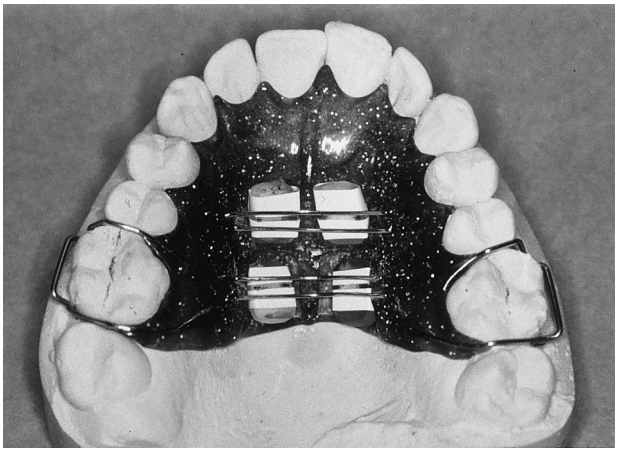

Fig. 1. Upper removable appliance containing four specimens of dental hard tissue.

as were those suffering any condition which presented undue risk from the study products or procedures.

As part of the screening interview, volunteers received an oral examination. Those with orthodontic appliances, restorations or bridgework considered likely to interfere with the study were excluded, as were sufferers of recurrent aphthous ulceration, and those exhibiting excessive gingival inflammation or tooth wear.

Sixteen volunteers, 12 females and 4 males ranging in age from 20.71 to 35.02 years (mean 25.27, SD 4.44) were enrolled in the study. Following acceptance, each volunteer had an impression of his/her maxillary arch recorded in alginate using a perforated stock tray. This was poured in dental stone within 30 min, and an upper removable appliance prepared from self-curing acrylic was subsequently constructed (Fig. 1).

On each study day, volunteers were asked to wear the intra-oral appliance and its retained specimens of dental hard tissue between 0900 and 1700 h, except for 1 h over lunch-time. In each study period, they were allocated to receive either four enamel specimens (two deciduous and two permanent) or four dentine specimens (two deciduous and two permanent) according to the randomisation schedule illustrated in Table 1.

Drinks were stored in a locked room, at room temperature, and out of direct light. On each study day, the sugar free concentrate (Asda Farm Stores, No Added Sugar Orange Squash, Asda Stores Limited, Leeds, UK) was diluted 1:4 with a bottled mineral water (Volvic, Premier Waters Ltd, London, UK). For each volunteer, 250 ml of the drink were decanted into each of four previously sterilised glass bottles.

Volunteers were asked to drink 250 ml of orange drink four times per day, consumption taking place over a 10-min period at 0900, 1100, 1300 and 1500 h. They were instructed to sip the drink at the rate of 25 ml/min, rather than consuming it all at once or through a straw. Drinks were issued by a research technician who also supervised their consumption; stopwatches were provided for monitoring purposes.

At midday on each study day, two specimens (one deciduous and one permanent) were removed from the appliance by the research technician according to a schedule which constituted part of the randomisation (Table 1). Following removal from the appliance, these specimens were stored in isotonic saline at room temperature in eppendorf tubes marked with the relevant volunteer's trial number. In order to facilitate their correct replacement for the next study day, each eppendorf tube was also marked with the required position of the specimen in the intra-oral appliance. Removal and replacement of specimens was carried out using an aseptic technique.

During the study, volunteers were asked to use a standard toothpaste and perform manual brushing twice daily with a standard toothbrush supplied at its commencement. No toothbrushing was allowed throughout the day, and volunteers were not allowed to use any mouthrinses. Plaque control was achieved by dipping the appliance and its retained specimens in 0.2% chlorhexidine gluconate mouthrinse for 3 min at the extremes of the study day (0900 and 1700 h) and on removal for lunch.

Consumption of tea, coffee and water were permitted while the appliance was in situ, but acidic drinks, including lemon or herbal teas and fruit flavoured spring were prohibited. Volunteers were not allowed to consume food products while wearing the appliance, but no restrictions were placed

Table 1
Randomisation and removal schedule (P and D stand for permanent and deciduous respectively, and 2 and 4 for the number of drinks per day)

Subject	Period 1				Period 2			
			Right	Left			Right	Left
1,5,9,13	Enamel	Posterior	P2	D2	Dentine	Posterior	D4	P4
		Anterior	D4	P4		Anterior	P2	D2
2.6.10,14	Dentine	Posterior	D2	P2	Enamel	Posterior	P4	D4
		Anterior	P4	D4		Anterior	D2	P2
3,7,11,15	Enamel	Posterior	D4	P4	Dentine	Posterior	P2	D2
		Anterior	P2	D2		Anterior	D4	P4
4,8,12,16	Dentine	Posterior	P4	D4	Enamel	Posterior	D2	P2
		Anterior	D2	P2		Anterior	P4	D4

Table 2
Progression of erosion (in μm) over time by frequency of exposure

Tissue	Exposures/day	Mean (SD)		
		Day 5	Day 10	Day 15
Deciduous enamel	2	−1.20 (1.33)	−2.79 (2.46)	−5.16 (4.61)
	4	−2.85(3.70)	−6.28 (7.51)	−7.85 (7.27)
Permanent enamel	2	−1.11 (1.88)	−2.61 (3.07)	−5.90 (5.04)
	4	−1.51 (1.58)	−2.71 (3.42)	−4.71 (6.35)
Deciduous dentine	2	−6.46 (2.28)	−8.08 (3.58)	−9.25 (4.21)
	4	−8.26 (3.09)	−9.33 (3.80)	−10.50 (5.39)
Permanent dentine	2	−7.33 (3.79)	−8.02 (3.85)	−10.04 (4.23)
	4	−9.60 (3.14)	−12.41 (4.93)	−11.68 (4.28)

on the type of food, which could be consumed when this was not in situ. Likewise, acidic medication (pH < 5.3), antacids or vitamin C preparations were prohibited while the appliance was in situ.

At completion of days 5, 10 and 15, tissue loss was determined by surfometry as described by West and co-workers [19]. Before a measurement, each sample was dipped in 0.2% chlorhexidine gluconate mouthrinse for 3 min. Overnight and at weekends, as during the lunch period, the appliance and its specimens were stored in isotonic saline at room temperature.

On the morning after days 5 and 10 (surfometry measurement days), the appliance and specimens were disinfected before wearing by dipping them in a mixture of 0.5% chlorhexidine and 70% spirit base for a period of at least 30 min.

As a safety measure, provision was made for withdrawal from the study of any volunteer who was recorded as having lost 20 μm or more from any specimen of permanent enamel.

2.2. Statistical and analytical methods

This study had a complex split-unit design in which each volunteer participated in two periods. It was therefore possible to evaluate, on a within-subjects basis, differences between deciduous and permanent teeth as well as the effect of frequency of consumption. Enamel and dentine were considered separately. The primary outcome measure was mean tissue loss on each measurement day. This was calculated by taking the mean of the two replicate readings on days 5, 10 and 15 and subtracting the mean of the two replicate readings at baseline. Summary statistics (mean, median, trimmed mean, standard deviation, standard error, quartiles and range values) produced from the raw data showed the application of parametric tests to be appropriate. Paired *t*-tests were therefore employed to examine differences between deciduous and permanent tissues and the two frequencies of consumption, as well as between erosion at anterior and posterior and left and right sites. Unpaired *t* tests were used to examine differences between erosion in Periods 1 and 2. Only the first and second of these analyses were of prime interest, the remainder arising as part of the split unit design. Further analyses were performed by analysis of variance, modelling on subject, side and frequency. Anterior/posterior analysis was not included, as no significant differences were found in the initial analyses.

Table 3
Probability values for the influence of frequency and site on erosion of deciduous and permanent enamel and dentine

Tissue	Variable	*p* value		
		Day 5	Day 10	Day 15
Deciduous enamel	2/4	0.047	0.05	0.20
	Ant./post.	0.30	0.16	0.12
	L/R	0.33	0.70	0.82
Permanent enamel	2/4	0.31	0.88	0.45
	Ant./post.	0.53	0.75	0.21
	L/R	0.78	0.31	0.22
Deciduous dentine	2/4	0.049	0.30	0.45
	Ant./post.	0.74	0.91	0.89
	L/R	0.055	0.015	0.015
Permanent dentine	2/4	0.40	0.014	0.22
	Ant./post.	0.35	0.89	0.29
	L/R	0.94	0.035	0.08

3. Results

All sixteen volunteers completed the study, thus maintaining the orthogonality of the data. This permitted analysis without adjusting for the confounding influences of any one variable upon another.

Means for erosion at days 5, 10 and 15 are presented in Table 2. This illustrates progression of erosion over time for all four tissues and for both exposure frequencies. In general, erosion in all four tissues was progressive over time, though this pattern was more linear in enamel than in dentine. In deciduous dentine, at least 70% of the total erosion had occurred by day 5; in permanent dentine, at least 60% of the total erosion had occurred by the same time point. Though not tested formally, erosion of deciduous and permanent dentine was considerably greater than that of deciduous and permanent enamel. This difference was marked at day 5; thereafter erosion progressed at a broadly similar rate for both tissues.

In all but one instance, increasing frequency of

consumption resulted in increased tissue loss. However, doubling the frequency of consumption rarely resulted in doubling the amount of erosion recorded. In contrast to the in vitro model [17], differences between frequencies in the in situ model were not consistently statistically significant (Table 3), and never so at day 15, when the degree of haphazard variation was greatest. In only two cases (days 5 and 10 for deciduous enamel) did doubling the frequency of consumption result in at least double the amount of tissue loss. In common with the findings of the in vitro study, no consistent ratio for the degree of erosion produced by 4 versus 2 drinks per day emerged.

Anterior/posterior siting made no statistically significant difference to the amount of erosion recorded (Table 3). However, in the case of permanent dentine, significantly more erosion was seen for specimens placed on the left side of the appliance (as viewed in Fig. 1). This pattern was reversed in deciduous dentine specimens, with those placed on the right showing statistically significantly more erosion.

Deciduous enamel was seen to erode more severely than permanent enamel. However, statistically significant differences were only found for the higher frequency at day 10 ($p = 0.017$) and day 15 ($p = 0.0035$). Conversely, deciduous dentine showed less erosion than permanent dentine. However, this difference only reached statistical significance at the higher frequency (day 10) when $p = 0.0064$. Erosion was always greater in Period 2 than in Period 1, but differences only reached statistical significance in two of the analyses. Analyses of variance revealed similar findings, but tended to enhance the significance of any differences. It should however be noted that variation between subjects was often highly significant, while the interaction between frequency of exposure and tissue was consistently non-significant.

4. Discussion

The main aim of this study was to determine whether deciduous and permanent enamel and dentine differ in their susceptibility to erosion by a low pH fruit drink. The study employed in situ methodology recently developed to study dental erosion by extrinsic agents. This method is highly supervised to ensure compliance and control confounding variables. Thus, all drinks were supervised by a research technician, while specimens were not exposed to extraneous influences other than those known to be minimally erosive [20], being removed at mealtimes and overnight. All specimens were prepared using a standardised method, and were derived from similar teeth. Although some biological variation would be expected, this was controlled to some degree by the random allocation of specimens to subjects. The observed inter-subject variation was also expected, and controlled for by the crossover nature of the study and the allocation of specimens to different periods and sites within the appliance.

At least with regard to enamel, this model probably creates a worst case scenario for two reasons. The use of a flattened surface facilitates surfometry since the problem of surface curvature is eliminated. In addition, when the surface layer with its larger crystallites [21] and higher carbonate and fluoride concentrations is removed, the more uniform mineral composition can be expected to produce more uniform lesions. However, it should be borne in mind that the subsurface enamel demineralises more readily than the original tooth surface. The siting of the specimens in the palate corresponds to the sip and swallow pathway of drink consumption. In studies concerned with the development of soft drinks with low erosive potential, these factors would be considered advantageous since, if anything, the results obtained would represent an exaggeration of the erosion, which might occur with natural use. In the present study, these factors are irrelevant.

Although not formally tested, the data clearly showed that deciduous and permanent enamel were considerably less susceptible to erosion than their dentine counterparts. This observation was in direct contrast to the findings of the in vitro study mentioned above [17]. This may be due, at least in part, to the role played by the acquired pellicle in the prevention of enamel erosion [20,22,23]. Of possible relevance is the finding that distinct differences in chemical composition, rate of formation, and ultrastructural appearance exist between pellicles formed on deciduous and permanent enamel [24]. Although the amino acid composition of 2-h pellicles on deciduous and permanent enamel has an overall similar pattern, the contents of serine, glycine and tyrosine are statistically significantly different. This indicates that there may be different amounts of some proteins (or even different proteins) in the acquired pellicle on deciduous enamel compared with that on permanent enamel in the same individual. By way of explanation, it should be emphasised that the adsorption of salivary proteins to enamel is dependent on the chemical composition of the surface; small changes in the latter influence the chemical composition of the acquired pellicle [25,26], probably as a result of variations in surface charge.

Sönju Clasen and co-workers [24] also observed pellicle formation to be initially slower on deciduous enamel; in addition, the adsorption process levelled out at a thickness corresponding to one-third that of the pellicle on permanent enamel. Transmission electron microscopy showed a dense, homogeneous layer after 2 h, the second globular layer, known to be present in the pellicle on permanent enamel [27], appearing not to have formed. The protein content of whole saliva has been shown to increase with age [28]. Therefore, this latter observation may relate more to the fact that the children's saliva did not contain enough protein to support the formation of the more usual globular structure than to inherent differences between deciduous and permanent enamel. This is an area, which requires further research. The upper removable appliance used in the in

situ studies reported herein could be employed, together with suitable analytical and ultrastructural technology, to study pellicle on specimens of deciduous and permanent enamel worn by adult volunteers.

Although increased frequency of consumption resulted in increased erosion of all tissues, the effect was clearly not proportional. Had this phenomenon been observed only in this in situ study, it might have been tempting to ascribe its occurrence to the chosen methodology. Those specimens subjected to two drinks per day were removed after only 3–4 h in the mouth, whereas those subjected to four drinks per day were in situ for approximately 7 h. However, the same lack of proportional effect was observed in the in vitro study [17], where a similar argument was not applicable. Nevertheless, in future studies, if only the effects of frequency are to be tested, specimens subjected to different frequency regimes should ideally be in situ for identical times.

The non-linear erosion of dentine observed in our in vitro study [17] was also observed in this study. This may be ascribed, at least in part, to the rapid initial loss of the smear layer, but further research is necessary to clarify whether this is the sole reason for the observation of this pattern of tissue loss.

Erosion of specimens according to site within the intra-oral appliance was of interest, though an explanation for the observed left/right differences is not immediately clear. Secondary to the differences between deciduous and permanent enamel and dentine, perhaps the finding of greatest interest related to inter-subject variation. Given the highly controlled nature of the study, it was apparent that factors, presumably intrinsic, influenced the amount of erosion, which occurred in each subject's mouth. Such factors most probably relate to variables such as saliva and pellicle, but unfortunately no data concerning the volunteers' salivary flow rate or buffering capacity were collected. One of the objectives of future in situ studies could be to analyse the inter-individual variation in 'treatment' effect by correlating it to various volunteer characteristics, in particular their salivary values.

5. Conclusions

This in situ study has shown that deciduous enamel appears to wear at a faster rate than permanent enamel following exposure to a low pH fruit drink. The data highlight the potential damage, which these products can inflict on teeth in a cumulative manner. However, it is necessary to exercise caution in interpretation since the difference in susceptibility only becomes of statistical significance over prolonged periods of time and with higher frequency of consumption. The observed increased loss associated with the deciduous tissue is of importance clinically given the reduced dimensions of the deciduous dentition [29,30] and the element of 'abuse' of soft drinks which has been shown to exist in the child population [18]. In certain groups, erosive fruit-based drinks tend to be introduced at a very early age, thereby increasing the time over which deciduous teeth are exposed to their damaging effects. In a recent study in South Wales, more than 50% of mothers who admitted to using a baby feeding bottle for drinks other than infant formula had used dilutable fruit concentrates or carbonated drinks [31]. Clearly, consideration must be given to prevention of erosion by product modification if major restorative problems are to be avoided.

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