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The effect on dental enamel of the frequency of consumption of fluoridated milk with a cariogenic challenge in situ

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ABSTRACT

Objectives: To evaluate the effect on enamel of varying the frequency of consumption of 1.0 mg F in milk once per day, twice per day or once every other day under cariogenic challenge in situ.
Materials and methods: In a controlled, randomised, cross-over, single-blind study, 25 subjects wore an intra-oral lower removable appliance with enamel slabs for 21 days during each study arm. Subjects used F-free toothpaste, the cariogenic challenge comprising of five 2 min dippings per day in a 12% sucrose solution. Subjects dipped the appliances in 50 ml of 5.0 ppm fluoridated milk for five minutes during the test period once per day, twice per day, and once every other day and drank 200 ml of the same milk, once per day, twice per day (100 ml each time), or once every other day (200 ml) immediately on re-inserting their appliance in order to replicate topical and systemic effects. Slabs were analysed with surface microhardness (SMH) for protection against further demineralisation and transverse microradiography (TMR) to assess changes in mineralisation.
Results: Using SMH, 200 ml of 5.0 ppm F milk once per day was significantly better than 100 ml of 5.0 ppm F twice/day ($p < 0.05$) and 200 ml once every other day, but not significantly. Using TMR there was a statistically significant difference in mineral loss of enamel between baseline and treatment for all groups, but not between groups.
Conclusions: Drinking 200 ml of 5.0 ppm milk once per day every day protected enamel against further demineralisation whereas all three frequencies were effective in promoting remineralisation.

1. Introduction

The provision of milk in schools is a public health policy in many countries. In 1988 the first community-based scheme was introduced in Bulgaria and reached 15.000 children [1]. More recently there has been further expansion particularly in Thailand and Chile and there are now over one million children participating in the international programme [2]. As milk fluoridation mostly targets the child population, milk fluoridation schemes have been established within the context of school health programmes [3] and programmes for healthy diet and nutrition. The evidence of milk fluoridation as a public health measure has been shown in a wealth of studies [4,5].

The design of studies varied from evaluation of community preventive programmes and a few randomised controlled studies. The RCTs [6,7] reported statistically significant reductions in caries compared with control groups. Some studies [1,8] showed a caries preventive effect in primary teeth and in permanent teeth [9,10]. Other studies, [11] showed no effect in either dentition. Many of the older studies were conducted in a period where the use of fluoridated toothpaste was not as wide spread as it is at the present time. It could be anticipated therefore, that the magnitude of the effect of fluoridated milk on caries reduction would most likely be less in studies conducted in populations already benefiting from the caries preventing effect of fluoridated toothpaste.

Studies have also shown that the effectiveness of fluoridated milk on prevention of dental caries with different fluoride dose: 1.5 mg/day [6], 1.0 mg/day [12] and also increased with the number of days of exposure to fluoridated milk per year [4,13]. A Cochrane review [14] concluded that there was not enough robust evidence to support fluoridated milk. However, this does not imply that fluoridated milk is ineffective in caries prevention, but that evidence from RCT's is lacking in this area. The review recommended that additional studies be performed particularly with regards to the frequency of consumption and the optimum concentration of fluoride. Such data would be useful if recommendations made to the general public are to be evidence-based and for possible modification to the existing National milk fluoridation programmes.

It has been shown in a randomised controlled double blind, cross-

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over design in situ study that concentrations of 2.5 and 5.0 ppm F in milk significantly protected dental enamel from demineralisation [15]. The role of the frequency of intake of fluoridated milk has also been investigated [16], where 200 ml of milk with 1.5 mg of F (approximately 7.5 ppm of F) in 1 daily dose was compared with 200 ml of milk containing 0.5 mg of F (approximately 2.5 ppm of F) in 3 daily doses. However, in this study as a fluoride toothpaste was also used in the situ design the authors failed to see any benefit of fluoride milk over and above that associated with the use of fluoride toothpaste.

Fluoridated milk is currently given to children under supervision during school days only and furthermore there are only 5 school days per week. In addition, there are numerous school holidays throughout each year when children do not have their daily fluoridated milk. Thus children do not ingest fluoridated milk every day. Therefore, the aim of our study was to evaluate the effect of varying the frequency of consumption of the same amount of 5.0 ppm of fluoridated milk either once per day or twice per day in two divided doses and once every other day on dental enamel under cariogenic challenge in situ.

1.1. Subject and methods

Ethical approval for this study was sought and informed consent obtained from all participants. The Principal Investigators ensured that this study was conducted according to the Declaration of Helsinki/Venice/Tokyo/Hong Kong/South Africa [17]. Following statistical advice on the number of volunteers required, thirty subjects were screened, and 25 were found to meet the inclusion criteria. Adults with normal salivary function, a minimum salivary flow rate of 0.25 ml/min, free from clinical signs of periodontal disease or any untreated carious lesions, able to comply with the protocol instructions and those who were able to provide informed consent were included in the study. Any subjects who had a medical history such as epilepsy, risk of infective endocarditis, haemophilia, or pregnant/nursing subjects were not included. The mean age of volunteers was 34.2. They were given a dental examination before the start of the study to determine their DMFT/DMFS using BASCD criteria [18].

1.2. Study groups

There was one test product: 200 ml of 5.0 ppm F semi-skimmed Milk. The difference was in the frequency and hence the dose of consumption at any given time of consumption of fluoridated milk by subjects. The groups were:

- Control group: Consumption of 200 ml of 5.0 ppm F milk once per day, equivalent to 1.0 mg F per day. This group was chosen as the control group because the amount of fluoride ingested daily is 1.0 mg.
- Test frequency group 1: Twice per day, 2 × 100 ml of 5.0 ppm F milk;
- Test frequency group 2: Once every other day 200 ml of 5.0 ppm F milk.

1.3. Preparation of fluoridated milk

Fluoridated pasteurised milk was prepared by adding an aqueous solution of sodium fluoride to milk in a fixed ratio, so as to achieve the required concentration of fluoride in the product. In order to produce this product, the aqueous stock solution of sodium fluoride (1000 ppm) was made by dissolving 2.21 g of sodium fluoride (extra pure, BP grade) per litre in distilled water. Then the stock solution was diluted with milk in order to gain the concentrations required. Therefore to obtain milk with a concentration of 5.0 ppm, 5 ml of aqueous solution of sodium fluoride was diluted to 1 l with milk.

1.4. Enamel slabs

1.4.1. Preparation of slabs for microhardness (SMH) testing

The enamel slabs used in the study were from human premolar teeth extracted for orthodontic purposes. Enamel was sectioned using a Well Diamond Wire Saw, water-cooled, cutting machine (Well® Walter EBNER, CH-2400 Le Loche) to create slabs measuring 2 × 4 × 2 mm. The buccal surface of each tooth was separated and then cut into four slabs. After cutting, the slabs were polished whilst wet using fine grit abrasive paper (P1000 Wet & Dry paper, 3 M) in combination with 5 µm and 1 µm alumina paste used to remove the outermost enamel layer (100–200 µm) and achieve a flat, plano-parallelism of the test surface to the mounting block. This was essential for the accuracy of the microhardness reading.

The enamel slabs were assessed at baseline and at the end of the 21 day test period using Microhardness, using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark). The indentations were made using a Knoop diamond under a 100 g load for 30 s [19]. The depth of indenter penetration was measured by means of an image analysis system. The average was calculated using the length of five indentations made across the surface of each enamel slab. The length of each indent was recorded three times and the mean was calculated. An average of 62–69 µm of indent length was considered as an acceptable inclusion criterion for enamel respectively in order to standardise the base line of the enamel slabs. Once the slabs had been prepared, they were kept moist in de-ionised distilled water in microcentrifuge tubes and left at room temperature.

1.4.2. Preparation of slabs for transverse microradiography (TMR) testing

The slabs that were assessed with TMR required the creation of a white spot lesion in vitro.

Human premolars extracted for orthodontic treatment were prepared and left in an acidified gel system for 7 days as described previously [20]. On removal from the gel each tooth was carefully sectioned to give the white spot lesion surrounded by a margin of sound enamel. The enamel slabs with the artificial caries-like lesions were divided into three groups: once per day, twice per day or once every other day frequency of consumption of fluoridated milk. The enamel slabs were stored damp in sealed containers and exposed to gamma irradiation (4080 Gy) for sterilisation [21]. They were then immersed in 5% sodium hypochlorite for 24 h to eliminate any possible prions [22]. After treatment the slabs were placed in de-ionised water until the date of analysis.

1.5. Experimental appliance

A mandibular removable Hawley appliance with a labial arch wire and U clasps and acrylic flanges buccal to the first permanent molars was made for each subject [23]. Two enamel slabs were secured in the buccal flanges of the appliances and covered with Dacron gauze. The gauze was used to encourage the growth of plaque on the enamel slabs.

1.6. Experimental protocol/regime

The study was randomised, single blind and crossover in design. Each subject was randomly assigned to one of the study arms, using a randomisation table. The enamel slabs were assessed blindly by the investigator who did not know the groups to which the slabs belonged to at the time of analysis with either SMH or TMR.

The subjects were required to brush their teeth with a fluoride-free toothpaste and the appliance (except the enamel slabs) twice per day throughout the test period. The appliances were worn continuously by the volunteers, except at mealtimes and whilst drinking, brushing their teeth and exposure to the investigational products. The appliances were worn for 48 h to acquire plaque before commencing one of the test regimes, and the test period was for 21 days. The cariogenic challenge