Original Paper

Caries Research

Caries Res 2007;41:108–114
DOI: 10.1159/000098043

Received: January 3, 2006
Accepted after revision: July 21, 2006

Effects of Various Forms of Calcium Added to Chewing Gum on Initial Enamel Carious Lesions in situ

J.F. Schirrmeister[a] R.K. Seger[a] M.J. Altenburger[a] A. Lussi[b] E. Hellwig[a]

[a]Department of Operative Dentistry and Periodontology, University School of Dental Hospital, Albert-Ludwigs University of Freiburg, Freiburg, Germany; [b]Department of Operative, Preventive and Paediatric Dentistry, School of Dental Medicine, University of Bern, Bern, Switzerland

Key Words

Calcium phosphates · Casein phosphopeptide-amorphous calcium phosphates · Chewing gum · Enamel · Remineralization · Transversal microradiography

Abstract

The purpose of this randomized, cross-over in situ study was to determine the effects of 4 chewing gums on artificial caries-like subsurface lesions. Two chewing gums (1 with zinc citrate and 1 without) contained dicalcium phosphate (3.9%), calcium gluconate (1.8%) and calcium lactate (0.45%), 1 chewing gum contained casein phosphopeptide-amorphous calcium phosphate nanocomplexes (0.7%), and another one contained no calcium. Fifteen subjects without current caries activity (7 male, 8 female; mean age: 27.5 ± 2.5 years) wore removable buccal appliances in the lower jaw with 4 bovine enamel slabs with subsurface lesions. The appliances were inserted immediately before gum chewing for 20 min and then retained for an additional 20 min. This was performed 4 times per day. Every subject chewed 4 different chewing gums over 4 periods of 14 days each. During a fifth period (control) the subjects only wore the appliances without chewing gum. At completion of each period the enamel slabs were embedded, sectioned and subjected to transversal microradiography. With regard to change of mineral loss and of lesion depth no significant differences could be found between chewing gums containing calcium and calcium-free chewing gums. Moreover, the chewing gum groups and the control group did not differ significantly if adjustments were made for baseline values ($p > 0.05$; ANCOVA). Under the conditions of the present study it may be concluded that the use of chewing gum offers no additional remineralizing benefit to buccal tooth surfaces, even if the chewing gum contains calcium compounds.

Chewing gums are known to be a useful adjunct to common oral hygiene. Gum chewing is a potent stimulant of salivary flow rate [Imfeld, 1999]. It is effective in raising plaque pH [Imfeld, 1999]. The chewing of sugar-free gums after meals and snacks can promote remineralization of enamel [Imfeld, 1999].

Even in the early 1960s, dietary supplements of calcium and phosphate were proposed for the prevention of dental caries. Dicalcium phosphates reduced caries by 90% in hamsters [Stralfors, 1961], and calcium lactate reduced caries in rats [McClure, 1960]. Two clinical studies performed with chewing gums containing dicalcium phosphate dihydrate showed a significant reduction in dental caries in the mineral-salt-containing gum group when compared with the control group [Finn and Jamison, 1967; Richardson et al., 1972].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0008–6568/07/0412–0108$23.50/0

Accessible online at:
www.karger.com/cre

Dr. Jörg F. Schirrmeister, Abteilung für Zahnerhaltungskunde und Parodontologie
Universitätsklinik für Zahn-, Mund- und Kieferheilkunde Freiburg
Hugstetter Strasse 55, DE–79106 Freiburg (Germany)
Tel. +49 761 2704 910 or +49 761 2704 885, Fax +49 761 2704 762
E-Mail joerg.schirrmeister@uniklinik-freiburg.de

Casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) have been demonstrated to have anticariogenic activity in laboratory, animal and human in situ experiments [Reynolds, 1998; Reynolds et al., 2003; Shen et al., 2001]. Casein phosphopeptides (CPP) containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- have the ability to stabilize amorphous calcium phosphate (ACP) in a metastable solution. Through the multiple phosphoseryl residues, the CPP binds to forming nanoclusters of ACP, preventing their growth to the critical size required for nucleation and phase transformation [Reynolds, 1998].

In a recent study the CPP-ACP-containing chewing gum produced higher levels of enamel remineralization than the chewing gum containing other forms of calcium ($CaHPO_4/CaCO_3$ or only $CaCO_3$) [Reynolds et al., 2003; Shen et al., 2001].

In the current study 2 chewing gums containing dicalcium phosphate, calcium gluconate and calcium lactate were compared to 1 chewing gum containing CPP-ACP and another without calcium regarding their effects on artificial subsurface lesions in situ.

Subjects and Methods

The independent ethics committee of the Albert-Ludwigs University of Freiburg approved the present study design. The randomized, observer-blind cross-over study was ethically conducted in accordance with the Declaration of Helsinki.

Subject Recruitment

A reduction of mineral loss of 20% (from 1,500 to 1,200 vol% × μm) for the chewing gums containing calcium was expected. It was supposed that no or little decrease of mineral loss (from 1,500 to 1,450 vol% × μm) would be detected in the control group (no chewing gum). Based on 0.9 power to detect a significant difference ($p = 0.05$), 15 participants in each group were required.

Subjects were recruited from the staff and students of the University School and Dental Hospital of the University of Freiburg, Germany. All volunteers were residents of Freiburg, Germany, and surrounding villages with negligible tap water fluoride content. Before the beginning of the study, the volunteers gave their written informed consent to the study protocol, which was reviewed and approved by the ethics committee. Demographic and ethnic data, medical history, and information on previous and current medical and dental treatment were recorded. The oral mucosa and the dentition were clinically investigated. The stimulated salivary flow rate and buffer capacity were measured by means of a commercially available test on 3 consecutive days (CRT buffer, Vivadent, Schaan, Liechtenstein). Finally, 15 volunteers who fulfilled the inclusion criteria without violating the exclusion criteria (table 1) were enrolled in the study.

Preparation of Specimens Having Enamel Subsurface Lesions

Four cylindrical specimens (3 mm diameter) were prepared from each of 75 bovine incisors and gamma sterilized. Then the enamel samples were embedded in epoxy resin (Technovit 4071, Kulzer, Wehrheim, Germany). The enamel surfaces were ground flat and polished, thereby removing the outer 100–200 μm. Then the specimens were flattened from the dentin side to a thickness of 2 mm (Exakt Mikroschleiftechnik, Grünewald, Laudenbach, Germany).

Five litres of a demineralizing solution according to Buskes et al. [1985] (pH 5.0; 3 mmol/l of calcium chloride dihydrate, 3 mmol/l of potassium dihydrogen phosphate, 50 mmol/l of lactic acid, 10 mol/l of potassium hydroxide, 6 μmol/l of hydroxymethylenediphosphonic acid, traces of thymol and distilled water) were prepared to create initial carious lesions. The prepared enamel specimens (n = 300) were placed in the solution for a period of 96 h at 37°C. The pH value of the solution was controlled daily (pH-Meter 526, Wissenschaftlich-Technische Werkstätten, Weilheim, Germany), and slight elevations were corrected with lactic acid to maintain a constant pH value between 5.0 and 5.1 during the whole demineralization period.

The specimens were removed from the resin, and for control one third of the demineralized enamel area was covered with an unfilled, light-cured resin (Heliobond, Vivadent).

Intra-Oral Appliances

A removable appliance was fabricated for each volunteer's lower jaw, with a buccal resin wing on each side [Koulourides et al., 1974]. Two specimens were mounted in each wing flush with the buccal surface.

Study Protocols

The composition of the chewing gums is shown in table 2. According to cyclic permutation, during each of 5 periods each type of gum was chewed by 3 volunteers, and 3 subjects chewed no gum for control. For each period, all subjects crossed over to each assigned treatment, with at least 1week between treatments. Random numbers were assigned to participants. E.g., volunteers 1–3 chewed gum 1 in the first period, gum 2 in the second, gum 3 in the third, gum 4 in the fourth and no gum in the fifth period; volunteers 4–6 started with gum 2 in the first period, followed by gum 3 in the second period, etc. The chewing gums in their original wrappers and the intra-oral appliances for each period were given to the participants at the beginning of the respective period. The volunteers started with the first period after a 1-week washout phase.

For each of the 5 periods, all subjects chewed the gums at their natural chewing frequency for 20 min 4 times a day for 14 days. They chewed the gum at the following times: 10:00 a.m., 2:00 p.m., 6:00 p.m. and 9:00 p.m. The appliances were worn for 20 min of gum chewing and for 20 min following. In case of the no-treatment control, the appliances were worn for 40 min. At all other times, the appliances were enveloped in a moist tissue handkerchief and stored in a plastic box at room temperature. The subjects were instructed not to eat, drink or smoke while wearing the appliance and to rinse and clean it with an extra-soft toothbrush (Meridol toothbrush, GABA, Lörrach, Germany) without using a dentifrice. They were instructed not to brush the area containing the enamel blocks. Except for a standardized fluoride-containing dentifrice (Meridol dentifrice, GABA), no al-

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Male or female, 18–50 years old • Classified as healthy • ≥20 teeth, no current caries activity and average oral hygiene (toothbrushing ≥twice a day) • Mean stimulated saliva flow rate measured on 3 days ≥0.7 ml/min each time • Salivary buffer capacity measured on 3 days medium or high (CRT buffer test) • Willing and able to give written informed consent prior to study participation • Willing to abstain from oral hygiene products except those provided and dental floss without fluoride	• Ongoing dental treatment or any other therapy of the oral cavity • Current periodontitis or non-physiological tooth mobility • Any pathological change in the oral mucosa or gingiva • Any known allergy to previously used oral hygiene products or oral therapeutic agents or dental materials used in the oral cavity or in the throat • Any known allergy to any of the ingredients of the study products or the standard toothpaste which was used during the study and the washout phase • Use of any chewing gum, fluoridated toothpaste (except for the standard toothpaste which is used during the study and the washout phase) or mouthrinse in the days prior to insertion of the appliances • Use of any of the following remineralizing or fluoridated products in the 30 days prior to insertion of the appliances: gels, pills, varnishes, fillings, and any erosive expectorants or antiasthmatic drugs • Pregnancy or breastfeeding • Participation in another clinical study in the 30 days prior to insertion of the appliances

terations were made to the subjects' oral hygiene procedures and diet for the duration of the study. They were informed not to use any remineralizing products (e.g. mouthrinse containing fluoride). After completion of each treatment period, the appliances were refilled with a new set of specimens during a 1-week washout phase.

Microradiographic Evaluation of Mineral Content

After each period the specimens were cut perpendicular to the surface of the specimens using a band saw (Exakt Trennschleifsystem) with continuous water cooling. One half of the specimen was embedded in epoxy resin without covering the demineralized area. The cut surface was polished (up to 4,000 grit).

The specimens were mounted on a transparent Plexiglas, and sections of 250 μm thickness were cut (Exakt Trennschleifsystem). The slabs were ground (2,400 grit; Exakt Mikroschleiftechnik) on the reverse side to a uniform thickness of 100 μm. Contact microradiographs of the enamel specimens were obtained as described previously [Kielbassa et al., 2001] using a nickel-filtered copper (CuKα) X-ray source (PW 1830/40; Philips, Kassel, Germany), operating at 20 kV and 20 mA. The radiation source-to-film distance was 34 cm. The exposure time of the high-speed holographic film (Kodak SO-253; Kodak, Stuttgart, Germany) was 12 s.

Microradiographs were studied with a digital image analyzing system (CCD Videocamera Modul XC77E; Sony, Japan) interfaced to a universal microscope (Axioplan; Zeiss, Oberkochen, Germany) and a personal computer. The mineral loss (ΔZ) was calculated by integrating the difference between the mineral (vol.%) in sound and demineralized enamel over the depth of the lesion. Lesion depth was defined as the distance from the surface (0%) to the location in the lesion where the mineral content was larger than 95% of the mineral content in sound enamel. Mineral content of sound enamel was set at 87 vol%. Dedicated software (TMR for Windows, release 1.24; Inspektor Research Systems BV, Amsterdam, Netherlands) was used to calculate the mineral content depth profiles from the image scans and the reference step wedge data. Mineral content (ΔZ, vol% × μm) and lesion depth (ld, μm) were determined.

Mineral change (ΔΔZ) was calculated as the difference in mineral loss (ΔZ) between the remineralized and the protected demineralized (control) area:

$$\Delta\Delta Z = \Delta Z_{remineralized} - \Delta Z_{control}$$

Table 2. Gum type and compounds

Group 1	Dicalcium phosphate 3.9% Calcium gluconate 1.8% Calcium lactate 0.45% Sodium bicarbonate 0.45%	Sugar substitutes (maltitol, mannitol, xylitol, aspartame, acesulfame-K), gum base (including antioxidant E320), flavours, E422, hydrolyzed milk protein, water, phenylalanine
Group 2	Dicalcium phosphate 3.9% Calcium gluconate 1.8% Calcium lactate 0.45% Sodium bicarbonate 0.45%	Sugar substitutes (maltitol, mannitol, xylitol, aspartame, acesulfame-K), gum base (including antioxidant E320), flavours, E422, hydrolyzed milk protein, water, phenylalanine, zinc citrate
Group 3	CPP-ACP 0.7% Calcium carbonate	Sugar substitutes (maltitol, sorbitol, mannitol, xylitol, aspartame, acesulfame-K), gum base (including antioxidant E321), flavours, E414, E472a, sodium stearate, E171, E903, phenylalanine
Group 4	No calcium	Sugar substitutes (maltitol, sorbitol, mannitol, xylitol, aspartame, acesulfame-K), gum base (including antioxidant E321), flavours, E414, E422, E171, E903, water, phenylalanine
Group 5 control	No chewing gum	

Lesion depth reduction was calculated accordingly:

$$\Delta ld = ld_{remineralized} - ld_{control}$$

As in 2 previous studies, percentage enamel remineralization (%R) was calculated as follows [Reynolds et al., 2003; Shen et al., 2001]:

$$\%R = (1 - \Delta Z_{remineralized}/\Delta Z_{control}) \times 100$$

Statistical Evaluation

Analysis of covariance (ANCOVA), $\alpha = 0.05$, was used to compare the means of mineral change and lesion depth reduction among the experimental groups, considering the baseline measurement as a covariate. All statistics were performed using SAS 8.2 (SAS Institute Inc., Cary, N.C., USA).

Results

Every subject attended all examinations before and after each test period. There were no adverse events. The study was performed between November 2003 and March 2004. At baseline, the mean age of the 15 volunteers (7 male, 8 female) was 27.5 ± 2.5 years. The mean salivary flow rate was 1.7 ± 0.5 ml/min, and the salivary buffer capacity was high for all volunteers.

Microradiographs showed that no erosion took place, and no surface mineral deposits were acquired. All artificially demineralized specimens showed homogeneous subsurface lesions. The demineralized control lesions had an average mineral loss of 1,229.7 ± 206.2 vol% × μm [mean ± standard deviation (SD)] and an average lesion depth of 52.0 ± 7.3 μm.

Mineral Change

The highest mean mineral gain was found in group 1 (–114.1 ± 157.6 vol% × μm; mean ± SD). In the group with the chewing gum containing zinc citrate (group 2), the mineral loss was striking (14.3 ± 101.6 vol% × μm). In group 3 (containing CPP-ACP) the mean mineral gain was –55.7 ± 129.7 vol% × μm and in group 4 (chewing gum without calcium) –29.4 ± 137.9 vol% × μm, whereas in group 5 (control) it was –84.3 ± 190.0 vol% × μm (table 3; fig. 1).

There were no statistical differences among the 5 groups concerning mineral change ($\Delta\Delta Z$; $p = 0.36$; ANCOVA).

Lesion Depth Reduction

The highest mean lesion depth reduction was found again in group 1 (–4.1 ± 5.7 μm; mean ± SD). Group 2 showed the lowest mean lesion depth reduction (0.1 ± 3.8 μm), followed by group 3 (–2.3 ± 5.4 μm), group 5 (–2.8 ± 5.0 μm) and group 4 (–3.1 ± 3.4 μm; table 3; fig. 2).

Table 3. Mean values (± SD) of mineral loss (vol% × μm) and lesion depth (μm) before and after the treatment period

	Mineral loss	Lesion depth
Group 1		
Before	1,321.0 ± 216.7	53.4 ± 5.3
After	1,207.0 ± 274.5	49.3 ± 7.5
Change (after-before)	–114.1 ± 157.6	–4.1 ± 5.7
Mean change, %	8.9 ± 12.7	7.6 ± 10.8
Group 2		
Before	1,187.0 ± 205.0	49.7 ± 7.7
After	1,201.3 ± 240.1	49.8 ± 7.5
Change	14.3 ± 101.6	0.1 ± 3.8
Mean change, %	–1.2 ± 8.0	–0.6 ± 7.8
Group 3		
Before	1,258.4 ± 155.8	53.1 ± 5.8
After	1,202.6 ± 196.6	50.8 ± 8.2
Change	–55.7 ± 129.7	–2.3 ± 5.4
Mean change, %	4.4 ± 10.6	4.4 ± 10.8
Group 4		
Before	1,139.5 ± 135.1	51.5 ± 4.2
After	1,110.1 ± 159.4	48.4 ± 5.3
Change	–29.4 ± 137.9	–3.1 ± 3.4
Mean change, %	2.2 ± 12.8	6.1 ± 6.8
Group 5		
Before	1,239.7 ± 249.1	52.3 ± 10.9
After	1,155.4 ± 233.5	49.5 ± 10.7
Change	–84.3 ± 190.0	–2.8 ± 5.0
Mean change, %	5.4 ± 15.9	5.2 ± 9.7

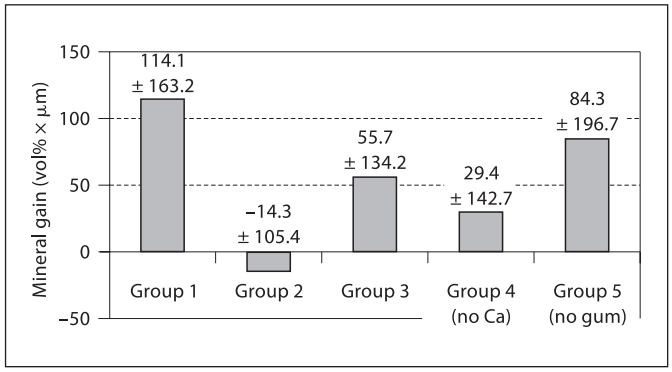

Fig. 1. Mineral gain.

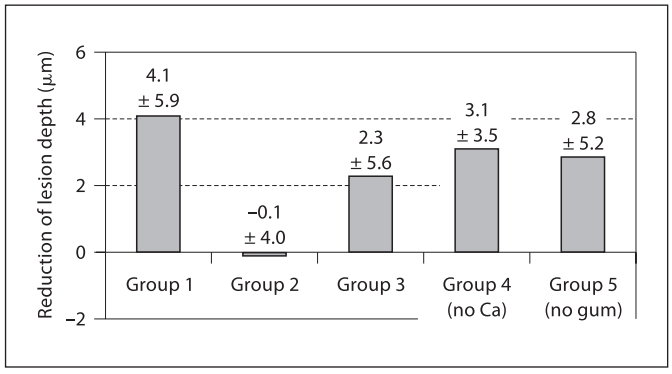

Fig. 2. Reduction of lesion depth.

Lesion depth reductions did not differ significantly either among the 5 groups (p = 0.31; ANCOVA).

Discussion

Although the volunteers chewed for the same period of time as in 2 previous studies [Reynolds et al., 2003; Shen et al., 2001], the results of those studies could not be confirmed. In comparison to the present study showing enamel remineralization of 4.4% for the chewing gum containing 0.7% CPP-ACP, in 1 of the previous studies about 18% enamel remineralization was found for an equivalent chewing gum [Shen et al., 2001]. The study design differed in 1 main fact: in the previous studies the enamel slabs were placed at the palate of mid-palatal appliances; in the present study the enamel slabs were mounted in buccal resin wings of mandibular appliances as in other remineralization studies [Buchalla et al., 2002; Koulourides et al., 1974]. The position of the enamel slabs of the present study imitated buccal lesions of posterior teeth of the lower jaw, whereas the position of specimens of the 2 previous studies imitated palatal lesions of maxillary premolars and molars. It may be argued that the specimens were less in direct contact with chewing gums in the present study than in both previous studies. It has been demonstrated that the degree of remineralization using chewing gums varied within intra-oral sites [Amaechi and Higham, 2001]. Other authors found lower salivary film velocity at the facial surface of lower molars compared to the lingual surface of upper molars during gum chewing [Dawes and MacPherson, 1993]. This fact may explain the enhanced remineralization using mid-palatal appliances. Looking at the minor differences among the groups, it seems doubtful that a larger group of subjects – for example 30 as in the 2 previous studies [Reynolds et al., 2003; Shen et al., 2001] – would have led to significant differences. It should be mentioned that in

the no-gum control group only the placement of the appliance into the mouth may have transiently stimulated salivary flow and thereby enhanced remineralization.

Further differences between the present study design and the above-mentioned previous study designs may explain this controversial outcome. In the present study enamel lesions were induced using the demineralizing solution according to Buskes et al. [1985] that resulted in a lesion depth of 52.0 ± 7.3 μm and a mineral loss of 1229.7 ± 206.2 vol% × μm. In the study of Shen et al. [2001] for example, demineralization was conducted according to a modified technique of White [1987], and lesion depth and mineral loss were about twice as large (110 ± 9 μm and 3,222 ± 545 vol% × μm, respectively), possibly making it easier to detect changes during remineralization. Moreover, the SD of lesion depth before treatment was higher in the present study. The high SDs after the treatment may be explained by individual chewing habits and individual biological variation among the volunteers, e.g. composition of saliva. In contrast, the SDs in the studies of Reynolds et al. [2003] and Shen et al. [2001] were extremely low.

According to these studies, the same group of authors [Iijima et al., 2004] showed again that sugar-free gum containing CPP-ACP was superior to an equivalent gum not containing CPP-ACP in remineralization of enamel subsurface lesions in situ. Furthermore, they found that enamel was more resistant to subsequent acid challenge after remineralization with CPP-ACP.

Since preliminary studies of Shen et al. [2001] indicated that no CPP-ACP could be detected in the gum bolus after 10 min of chewing, the subjects in the present study were instructed to chew the test gums for 20 min and then to retain the intra-oral appliance for a further 20-min period according to the studies of Shen et al. [2001] and Reynolds et al. [2003].

In a recent study, 12 volunteers wore intra-oral appliances all day long with specimens mounted lingually in the lower jaw and chewed 3 chewing gums containing urea [Itthagarun et al., 2005]. One of the chewing gums contained no calcium phosphate, one dicalcium phosphate and another one CPP-ACP. Using the chewing gum without calcium phosphate, lesion depth decreased significantly less, and mineral gain was lower compared with the 2 other gums. It may be argued again that the lingual position of the specimens led to this significant difference that is in contrast to the present study. However, these authors did not find any significant difference between the chewing gums containing dicalcium phosphate and the CPP-ACP gums either. This finding is in contrast to the results of the studies by Shen et al. [2001] and Reynolds et al. [2003], where subjects were told to wear the appliances only 4 times a day during chewing and the following 20 min. On the one hand wearing appliances all day long simulates the in vivo situation better than wearing the appliances only 20 min during chewing and the following 20 min, on the other hand variables unrelated to gum chewing may have an impact on the outcome of the study.

In a similar in situ model, remineralizing effects of a chewing gum containing sorbitol and a no-gum control were compared [Creanor et al., 1992]. Enamel specimens containing subsurface lesions were positioned lingually to the mandibular molars by means of a removable appliance. In contrast to the present study and the above-mentioned 2 previous studies [Reynolds et al., 2003; Shen et al., 2001], the subjects chewed 5 sticks of gum daily, each for 20 min, immediately after meals and snacks, and wore the appliances for a 7-week period except during oral hygiene procedures. The sorbitol gum treatment resulted in 18.2% enamel remineralization compared with 12.1% remineralization for the no-gum control. As in the present study, even after 7 weeks of gum chewing, the difference between the chewing gum (without calcium) and the no-gum control was not significant ($p > 0.05$). Thus, it seems questionable whether longer experimental periods or longer wearing of the intra-oral appliances might lead to significant differences in the present study or not.

In another study this problem was circumvented by mounting the enamel slabs in cast silver bands cemented to lower first molars [Leach et al., 1989]. After 3 weeks of gum chewing 5 times a day for 20 min each, the remineralization values were 16.8% for the no-gum control and 28.9% for a gum containing sorbitol ($p < 0.01$).

Under the conditions of the present study it may be concluded that chewing gums with and without calcium have no significant effect on the remineralization of buccal initial caries lesions in the mandibular posterior teeth. It may be argued that they do not enhance remineralization of initial lesions that are not in direct contact with the chewing gum. Extrapolation of these results to any other gum usage protocol is not justified, and further work is required for the investigation of the effects of chewing gums containing various forms of calcium.

Acknowledgement

We gratefully acknowledge the enthusiastic participation and compliance of all the subjects involved in the study.

References

Amaechi BT, Higham SM: Eroded enamel lesion remineralization by saliva as a possible factor in the site-specificity of human dental erosion. Arch Oral Biol 2001;46:697–703.

Buchalla W, Attin T, Schulte Mönting J, Hellwig E: Fluoride uptake, retention, and remineralization efficacy of a highly concentrated fluoride solution on enamel lesions in situ. J Dent Res 2002;81:329–333.

Buskes JA, Christoffersen J, Arends J: Lesion formation and lesion remineralization in enamel under constant composition conditions: a new technique with applications. Caries Res 1985;19:490–496.

Creanor SL, Strang R, Gilmour WH, Foye RH, Brown J, Geddes DA, Hall AF: The effect of chewing gum use on in situ enamel lesion remineralization. J Dent Res 1992;71:1895–1900.

Dawes C, MacPherson LM: The distribution of saliva and sucrose around the mouth during the use of chewing gum and the implications for the site-specificity of caries and calculus deposition. J Dent Res 1993;72:852–857.

Finn SB, Jamison HC: The effect of a dicalcium phosphate chewing gum on caries incidence in children: 30-month results. J Am Dent Assoc 1967;74:987–995.

Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC: Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004;38:551–556.

Imfeld T: Chewing gum – facts and fiction: a review of gum-chewing and oral health. Crit Rev Oral Biol Med 1999;10:405–419.

Itthagarun A, King NM, Yiu C, Dawes C: The effect of chewing gums containing calcium phosphates on the remineralization of artificial caries-like lesions in situ. Caries Res 2005;39:251–254.

Kielbassa AM, Shohadai SP, Schulte-Monting J: Effect of saliva substitutes on mineral content of demineralized and sound dental enamel. Support Care Cancer 2001;9:40–47.

Koulourides T, Phantumvanit P, Munksgaard EC, Housch T: An intraoral model used for studies of fluoride incorporation in enamel. J Oral Pathol 1974;3:185–196.

Leach SA, Lee GT, Edgar WM: Remineralization of artificial caries-like lesions in human enamel in situ by chewing sorbitol gum. J Dent Res 1989;68:1064–1068.

McClure FJ: The cariostatic effect in white rats of phosphorus and calcium supplements added to the flour of bread formulas and to bread diets. J Nutr 1960;72:131–136.

Reynolds EC: Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist 1998;18:8–16.

Reynolds EC, Cai F, Shen P, Walker GD: Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res 2003;82:206–211.

Richardson AS, Hole LW, McCombie F, Kolthammer J: Anticariogenic effect of dicalcium phosphate dihydrate chewing gum: results after two years. J Can Dent Assoc 1972;38:213–218.

Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC: Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80:2066–2070.

Stralfors A: Inhibition of dental caries in hamsters. V. The effect of dibasic and monobasic calcium phosphate. Odontol Revy 1961;12:236–256.

White DJ: Use of synthetic polymer gels for artificial carious lesion preparation. Caries Res 1987;21:228–242.