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Reversal of primary root caries lesions after daily intake of milk supplemented with fluoride and probiotic lactobacilli in older adults

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Abstract

Objective. To evaluate the effect of milk supplemented with fluoride and/or probiotic bacteria on primary root caries lesions (PRCL) in older adults. ***Materials and methods.*** After informed consent, 160 healthy subjects, 58–84 years of age, with at least two PRCL were recruited and randomly assigned to one of four parallel study groups drinking 200 ml milk once daily for 15 months. Group A consumed standard milk (placebo); Group B ingested milk supplemented with 5 ppm F and probiotic bacteria (*Lactobacillus rhamnosus* LB21, 10^7 CFU/mL); Group C drank milk with only probiotic bacteria and group D milk contained only fluoride. Primary endpoints were Root Caries Index (RCI) and electric resistance measurements (ECM) carried out by one blinded single examiner. Secondary endpoints were mutans streptococci and lactobacilli counts in saliva and plaque estimated with chair-side tests. Data were compared within and between groups with non-parametric tests. ***Results.*** The drop out rate was 38%. At baseline there were no statistical differences between the groups. Significantly higher numbers of RCI reversals were found in groups B, C and D compared with group A ($p < 0.05$). The mean ECM values increased significantly ($p < 0.05$) in all groups except for the placebo group A, indicating that remineralization occurred. The effect was most beneficial in the two groups that contained fluoride. No significant alterations were displayed regarding the microbial counts. No severe adverse effects were reported during intervention. ***Conclusion.*** Daily intake of milk supplemented with fluoride and/or probiotic bacteria may reverse soft and leathery PRCL in older adults.

Key Words: *ECM, fluoride, milk, remineralization, root caries*

Introduction

National surveys and repeated cross-sectional studies from Scandinavia and other industrialized countries have unveiled great improvements in the dental health of adults and elderly over the last decades [1,2]. The prevalence of edentulous subjects has decreased and the number of remaining teeth has increased significantly. Factors explaining this development are improved oral hygiene combined with an increased awareness of oral health, daily use of fluoride toothpaste as well as access to regular dental care [3–5]. However, paralleled with the increasing number of remaining teeth, a corresponding increase in the prevalence and incidence of dental caries is reported [6,7]. Physical and mental dysfunctions may increase the risk for root caries in older people [1–8]. In fact, Fure [9] has concluded that coronal caries decreases with age while root caries increases although the risk factors are similar; salivary insufficiency, accumulation of caries-associated micro-organisms and frequent carbohydrate consumption [10]. Several measures have been proposed to prevent and promote remineralization of primary root caries lesions and recent reviews have highlighted the unique role of fluoride [11,12]. Especially the use of highly fluoridated toothpaste has provided promising results indicating a certain dose–response relationship in root caries remineralization [5,13]. To our knowledge, no study has previously investigated the effects of milk as a vehicle of fluoride administration on adult root caries management *in vivo*. The rationale to use

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milk as a carrier for fluoride is basically that it is a convenient and cost-effective way of targeted fluoride supplementation [14].

Probiotics are living non-virulent micro-organisms which, when administrated in adequate amounts, confer health-promoting effects on the host [15]. The most commonly investigated probiotic micro-organisms are *Lactobacillus* and *Bifodobacterium* spp. and the mechanisms of action are likely both local and systemic through a regulation of the immune response [16]. In dental medicine, the use of probiotic bacteria is relatively new, but evidence of a beneficial impact on oral ecology is currently emerging [17]. Recently, a significant caries reduction in pre-school children was reported following a daily intake of milk supplemented with fluoride and probiotic lactobacilli over a 21-month period [18]. The design of that trial did not allow for a separation of the two supplements from each other which would be of interest to further elucidate. Moreover, the probiotic concept within dentistry is, with few exceptions, still rarely investigated in older adults. The aim of this investigation was therefore to evaluate the effects of daily intake of milk supplemented with fluoride and/or probiotic lactobacilli on primary root caries lesions in older adults using clinical and microbiological endpoints. The null hypothesis was that no differences would be displayed at follow-up examinations in comparison with baseline or when compared with the intake of normal standard milk without supplements (placebo).

Materials and methods

Subjects

The material consisted of older adult patients, 58–84 years of age, regularly attending a Specialist Dental Clinic in Ljungby, Sweden, a small town located in the southern part of Sweden with ~ 35,000 inhabitants. The patients were invited to participate in the trial according to the following inclusion criteria: (i) presence of at least two primary root caries lesions (PRCL) of grade 2 or 3 (see definition later), (ii) no acute or immune compromised medical condition, (iii) no subjectively reported xerostomia and (iv) tolerance to dairy products. Subjects with obvious physical and mental disorders such as dementia or Alzheimers's disease were excluded. One hundred and sixty eligible subjects with a total number of 408 PRCL were recruited and gave their consent after verbal and written information. Medical and dental background data from the subjects were recorded and continuously upgraded during the course of the trial. A power calculation based on findings from a previous study of a similar age group [19] indicated that ~ 28 patients would be needed in each group to detect a 40% difference in the electrical recordings with $\alpha = 0.05$ and $\beta = 0.20$. It was therefore decided to enrol 40 participants in each group in order to compensate for a 25–30% drop-out rate.

Study design

The study was designed as a double-blind randomized placebo-controlled trial with four parallel groups. The subjects were recruited consecutively and assigned randomly to one of the study groups by using computer-generated group allocation, which was placed within an envelope that was opened after the subject's inclusion. The groups were coded by colour in order to conceal the patient's allocation throughout the study. The code was kept by an independent monitor at Umeå University and not unveiled until all data were computerized. The duration of the intervention was 15 months. The primary endpoints were root caries lesion severity assessed with a clinical root caries index and electric caries monitor measurements as an estimate of remineralization. Secondary endpoints were microbial counts of mutans streptococci and lactobacilli in saliva and supragingival dental plaque. The study design was approved by the Research Ethics Committee at Umeå University (§562/03, dnr 03-475) and a clinical exemption certificate (CTX) was obtained from the County Hospital, Halmstad, Sweden.

Intervention

All subjects were instructed to drink 200 mL of normal to medium-fat milk (1.5–3%) once daily after the addition of a solution (1.0 mL) from colour-marked screw-capped capsules. In Group A, the capsules contained skim milk powder dissolved in water (placebo), Group B received capsules with the same solution but supplemented with fluoride and probiotic bacteria to give a final concentration of 5.0 mg F/L and 10^7 CFU/mL *Lactobacillus rhamnosus* LB21 in the prepared glass of milk; Group C contained skim milk powder and *L. rhamnosus* LB21 to a final concentration of 10^7 CFU/mL, while in Group D the capsules contained skim milk powder and fluoride to end up with 5.0 mg/L in the prepared milk. The capsules were prepared and delivered to the Specialist Dental Clinic by a joint venture of the biochemical laboratory at Umeå University, Norrmejerier Ekonomisk Förening (a local dairy) and Probac AB (Umeå, Sweden). After allocation, the capsules were distributed to the subjects in frozen packages sufficient for 3 months use. The subjects were instructed to thaw and add the capsules immediately before the milk consumption. Around 70% of the participants used medium fat milk and the taste of the milk was not affected by the additions. All capsules were identical in appearance except for the colour code and a logbook was used to fill in the supply for

each subject. The subjects were instructed to return all unused capsules as a control of their individual compliance. They were also asked to immediately report any perceived positive or negative side-effect possibly associated with the intervention. All subjects were strongly advocated to use fluoridated toothpaste twice daily and they were supplied with free fluoride toothpaste (1450 ppm F) and toothbrushes during the study period. The use of any other additional fluoride was discouraged.

Clinical examinations and ECM (Electric Caries Monitor) measurements

The clinical examinations and the ECM measurements were performed at baseline and after a median follow-up period of 15 months (range 14–17 months). The examinations were performed in a dental chair with optimal light and after thorough drying with air spray. The texture of the primary root caries lesions were graded after visual and tactile examinations according to Petersson et al. [19] and Baysan et al. [20]: Grade 3 = soft 'active' lesion with dull, rough surface; Grade 2 = a leathery lesion and Grade 1 = a hard lesion with a highly polished surface, judged as 'inactive' lesion. The lesions were also assessed by measuring the electric resistance with the aid of the Electric Caries Monitor (ECM, Lode Diagnostics, Groningen, The Netherlands) as previously described [19]. The rationale behind the ECM-measurements was that a demineralized soft tooth structure, as found in grades 2 and 3, had a relatively lower electric resistance compared with harder grade 1 surfaces [21]. From each site and at each occasion, 10 probe measurements were performed. Thereafter, a mean value of each patient, expressed as k-Ohm, was calculated.

All recordings were performed by a blinded single experienced senior dentist (LGP) and data were transferred to computer files with help of a dental assistant for subsequent processing. Before the start of the trial, 22 subjects with 44 PCRL were examined and scored with a 1 week interval in order to determine the intra-examiner reproducibility of the clinical registrations.

Microbiological procedures

Samples of stimulated whole saliva and supragingival plaque from the selected PRCL were collected at baseline and after 6 and 15 months. The subjects were instructed not to brush their teeth on the same day and the samplings were conducted at least 2 h after a meal. Saliva was collected after paraffin stimulation and the plaque samples were collected with the aid of a micro-brush and pooled from the surfaces of the selected PRCL. The counts of mutans streptococci and lactobacilli were estimated with the aid of selective chair-side tests (Dentocult SM® *Strip Mutans* and Dentocult LB®, Orion Diagnostica, Helsinki Finland) according to the manufacturer's instructions. After cultivation in 37°C for 48 h and 96 h, respectively, the bacteria were identified thorough their typical morphology and the number of colony-forming units (CFU) was estimated with the aid a stereo-microscope. The following scores were used: Score 0 = no detectable growth; Score 1 = 1–25 CFU (low counts); Score 2 = 26–200 CFU (moderate counts); Score 3 = > 200 CFU (high counts).

Statistical methods

All data were processed with the SPSS software (version 17.0, SPSS, Inc., Chicago, IL). The clinical scores were analysed by chi-square tests using the Linear-by-Linear Association model for comparison between the study groups. The ECM conductance data were analysed with the non-parametric Mann-Whitney Test with the patient as unit and the baseline data were compared with follow-up within the study groups as well as between them. The microbial data were processed with chi-square tests. The level of significance was set at 5%. The reproducibility and accuracy of the root caries index was estimated by Cohen Kappa analysis. A reliability coefficient (r) was calculated for the subject's mean ECM score.

Results

Final study groups and dropouts

A total of 100 subjects with 291 PRCL completed the 15-month trial, giving a total dropout rate of 38%. The dropouts were equally distributed in all four study groups (Figure 1). The mean age of the remaining subjects was 67.6 years (SD = 7.1) with a mean number of 22.2 (SD = 7.6) retained teeth. The majority (65%) were females and 35% were males. There were no significant differences concerning the baseline characteristics among the drop-outs and those that completed the protocol. Only a few side-effects were registered among the participants. A small number (4%) reported slight gastrointestinal problems at the beginning of the protocol but there were also some positive reports of a subjectively perceived improved well-being, irrespective of group allocation.

Effect on primary root caries lesions (PRCL)

Clinical score. The clinical grading of the PRCL at baseline and after 15 months is shown in Table I. There were no statistically significance differences between the groups at baseline. At the follow-up, it was obvious that the number of PRCL graded with

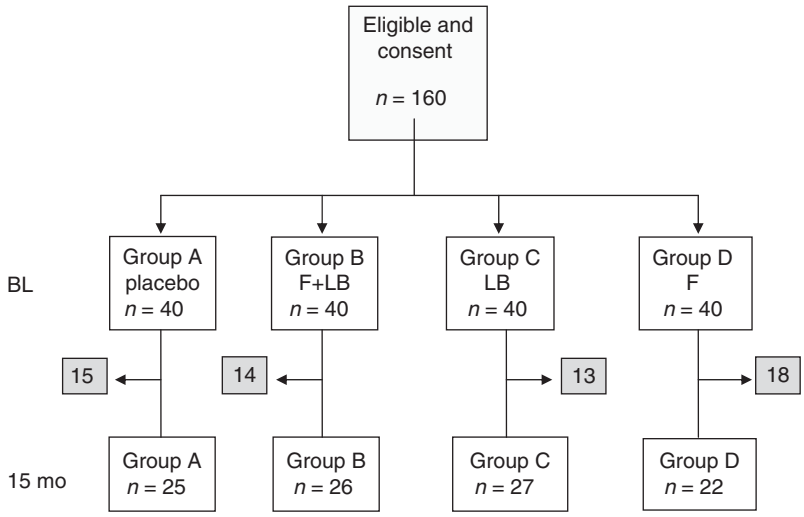

Figure 1. Flow chart of randomization at baseline (BL) and attrition of participants throughout the 15 month study period. F and LB indicate that the milk was supplemented by 5 mg fluoride/L and/or the probiotic bacterium L. rhamnosus LB21.

score 1 increased in all groups and that grade 3 decreased. The improvements were significantly greater in Groups B, C and D vs the placebo group A ($p < 0.05$). The subjects of Group B with daily intakes of milk supplemented with both fluoride and probiotic lactobacilli displayed the most frequent reversals and exhibited a significant difference compared with group C who consumed milk with probiotic bacteria only. There were no differences in the PRCL scores at follow-up between the groups B and D that both ingested milk with fluoride supplement. The reproducibility of the clinical registrations was found to be good with a Kappa value of 0.79.

ECM measurements. The ECM data are presented in Table II. At baseline, there were no statistical differences between the study groups. At the follow-up, the mean values were significantly increased ($p < 0.05$) in all intervention groups except for the placebo group. There was no significant difference between the groups with fluoride supplement (B and D), but both these groups displayed more beneficial results than group C ($p < 0.05$). The reliability coefficient between two measurements in the pre-study reproducibility test was rated as acceptable with $r = 0.69$.

Microbial findings

Sixty per cent of the subjects displayed detectable levels of salivary mutans streptococci and lactobacilli at baseline and the proportion of high counts was low, 9% and 1%, respectively. The bacterial scores in saliva at baseline and after 6 and 15 months are detailed in Table III. Although the levels varied slightly among the different study groups, no statistically significant alterations between baseline and the follow-ups were found. A generally lower prevalence of mutans streptococci and lactobacilli was detected in the supragingival plaque samples (Table IV) but, again, no significant changes in the bacterial counts were recorded throughout the study period.

Table I. Percentage distribution of primary root caries lesion grades in the four study groups at baseline (BL) and at the termination of the study after 15 months (15 mo).

	n	Grade 1 BL/15 mo	Grade 2 BL/15 mo	Grade 3 BL/15 mo	*p*
Group A (placebo)	69	0/16	71/77	29/7	—
Group B (F+LB)	87	0/59	72/38	28/3	a, b
Group C (LB)	70	0/37	74/54	26/9	a
Group D (F)	65	0/41	80/53	20/6	a

a = Statistically significantly different distribution after 15 months compared with group A ($p < 0.05$; chi-Square Linear-by-Linear association test).
b = Statistically significantly different distribution after 15 months compared with group C ($p < 0.05$; chi-Square Linear-by-Linear association test).
F and LB indicate that the milk was supplemented with 5 mg fluoride/L and/or the probiotic bacterium *L. rhamnosus* LB21. *n* denotes the number of recorded root caries lesions.

Table II. Mean values and standard deviations (SD) of the Electric Caries Monitor measurements (k-Ohm) at baseline (BL) and after a median follow-up period of 15 months in the four study groups with a daily milk intake.

	n	baseline mean (SD)	15 months mean (SD)	*p*⋆
Group A (placebo)	25	277 (172)	364 (97)	NS
Group B (F +LB)	26	256 (138)	1127 (683)	a, b, c
Group C (LB)	27	306 (226)	581 (292)	a, b
Group D (F)	22	317 (221)	883 (453)	a, b, c

⋆ Differences were calculated with the 2-tailed Mann-Whitney test.
NS = not significant.
a = Statistically significant difference compared with baseline ($p < 0.05$).
b = Statistically significant difference compared with group A at 15 months ($p < 0.05$).
c = Statistically significant difference compared with Group C at 15 months ($p < 0.05$).
F and LB indicate that the milk was supplemented with 5 mg fluoride/L and/or the probiotic bacterium *L. rhamnosus* LB21. *n* denotes the number of subjects.

Discussion

This double-blind placebo-controlled clinical trial was planned and performed to test the hypothesis that milk supplemented with either fluoride and/or probiotic bacteria could prevent further progression or even reverse primary root caries lesions of different grades in older adults. As the results clearly were in favour of the fluoride and lactobacilli-derived milk supplements, the null hypothesis was rejected. Both primary endpoints indicated strongly that remineralization occurred. A secondary aim was to investigate whether a change of caries-associated bacteria would take place but, with the chair-side techniques used, this did not seem to be the case. However, the conclusions must be drawn with some caution considering the limitations of the present study as discussed below.

Table III. Salivary mutans streptococci and lactobacilli at baseline and after 6 and 15 months in the four study groups.

Time	Group A placebo (*n* = 25)	Group B LB + F (*n* = 26)	Group C LB (*n* = 27)	Group D F (*n* = 22)
Mutans streptococci				
baseline	77/26	39/22	72/24	66/39
6 months	64/18	30/22	48/24	43/39
15 months	53/5	54/23	56/30	47/32
Lactobacilli				
baseline	64/18	61/22	64/24	67/19
6 months	54/14	70/22	72/16	71/12
15 months	79/29	56/17	79/26	77/15

No statistically significant differences were disclosed between baseline and the follow-ups in any of the study groups.
F and LB indicate that the milk was supplemented with 5 mg fluoride/L and/or the probiotic bacterium *L. rhamnosus* LB21. *n* represents the number of subjects and the values in the table denote prevalence (scores 1–3 vs moderate and high counts (scores 2–3) expressed as a percentage.

Table IV. Mutans streptococci and lactobacilli in dental plaque at baseline and after 6 and 15 months in the four study groups.

Time	Group A Placebo (*n* = 69)	Group B LB + F (*n* = 87)	Group C LB (*n* = 70)	Group D F (*n* = 65)
Mutans streptococci				
baseline	63/11	52/15	60/10	49/11
6 months	67/6	55/4	46/8	52/12
15 months	55/6	49/8	58/3	51/8
Lactobacilli				
baseline	33/5	36/1	50/4	49/3
6 months	30/2	36/1	48/2	44/2
15 months	45/2	48/2	50/2	50/3

No statistically significant differences were disclosed between baseline and the follow-ups in any of the study groups.
F and LB indicate that the milk was supplemented with 5 mg fluoride/L and/or the probiotic bacterium *L. rhamnosus* LB21. *n* represents the number of sampled sites. The values in the table denote the prevalence (scores 1–3) vs moderate and high counts (scores 2–3) expressed as a percentage.

Attrition rate was rather high and appeared most frequently shortly after the start of the study. The main reasons for dropping out were a general tiredness (40%) as well as poor remembrance (25%) and logistic difficulties in adding the allocated capsules to the milk (25%). There were also some non-regular milk consumers that accepted to participate but felt uncomfortable after a while and aborted the study. There were, however, no significant differences in the baseline caries data between those that dropped out and those that remained to the final follow-up and the dropouts were equally distributed in the four study groups. According to the check-up procedure of unused returned capsules, the compliance was considered as acceptable for those that fulfilled the study protocol. Although the power of study was somewhat impaired by the attrition, it was still considered as acceptable in the light of the clear-cut results.

The clinical and ECM intra-examiner reproducibility tests showed acceptable-to-good agreement. This was in harmony with previous findings *in vitro* and *in vivo* [19,22], but it must be stressed that especially the ECM recordings could be somewhat uncertain. There were large variations in the k-Ohm-values and the calculated mean value from the multiple measurements per lesion was made to partly overcome this problem. The variation depended probably on an uneven distribution of porosities on the diseased root surface, the grade of hydration with presence of fluid in the root apatite pores and other unknown factors [21]. It should, however, be underlined that the data from the clinical scores and the

ECM measurements were mainly in concert with each other.

The clinical findings were novel and highly interesting since this was the first study to suggest that daily intakes of fluoridated milk may have a reversing effect on root caries lesions in older adults. The mineral enhancing effect of fluoride is generally acknowledged [23] and a recent meta-analysis has concluded that any self- or professionally-applied fluoride regime can avert carious root surfaces [12]. Preventive and beneficial effects on root caries development have been reported from the use of topical fluoride solutions [24], fluoride varnish [25] and high-fluoride toothpastes [5,13]. Thus, it was highly likely that the clinical improvements displayed in the present study relied on the presence of fluoride in the oral environment. Some reversals were also obtained in the placebo group which probably was due to the fact that all subjects were supplied and strongly encouraged to use fluoride toothpaste and that milk as such is considered as a caries-protective agent [26]. A recent review of non-clinical data has suggested that the anti-caries effect from a concentration of 1 mg F/L in piped water would be similar to ~ 5 mg F/L in milk [27]. According to the ecological plaque hypothesis, demineralization of the dental structures is a result of an adaption of the oral biofilm to low pH-conditions leading to a diminished microbial diversity and an overgrowth of acid-tolerating species [28]. It is well known that fluoride can inhibit the enzyme enolase in the glycolytic pathways and thereby exert a hampering effect on the biofilm acid production [29–31]. This could contribute to a neutralization of the pH-drop that is the driving force of the biofilm stress. The key to reverse a primary root caries lesion is to manage and control the microbial balance (homeostasis) in the biofilm and the present data suggest that fluoride may contribute to this event.

Frequent sugar and starch consumption are common among elderly people and, together with an impaired salivary function, this contributes to increased caries risk. The rational to supplement the milk with lactobacilli was based on previous findings that probiotic bacteria added to dairy products can suppress the levels of mutans streptococci in saliva and reduce caries development in pre-school children [18,32–34]. There is also one report indicating that probiotic supplements can reduce the risk of hyposalivation among frail elderly [35]. We did not quantify the salivary secretion rate, but the present findings indicated a certain beneficial effect on root caries lesions compared with the placebo intervention. The milk solely supplemented with probiotic lactobacilli seemed, however, to be less effective than those with fluoride. Another important observation was that the effect of a combination of fluoride and probiotic bacteria was equal to that of fluoride alone. In agreement with the recent findings of Stecksén-Blicks et al. [18], we were unable to demonstrate any long-term effects on the levels of mutans streptococci and lactobacilli so the probiotic action did not seem to be a result of bacterial replacement or oral colonization. The findings of unchanged lactobacilli counts were, however, important since concerns that have been raised against the concept of adding a potentially cariogenic bacteria to food, especially in cases with open lesions [36]. Lactobacilli are frequently isolated in carious dentin rather than from the supragingival biofilm [37]. Thus, it is possible that the probiotic strain used here was sub-optimal. A conservative conclusion from the present study was, however, that the sole probiotic supplement seemed to do more good than harm on root caries lesion texture and this merits to be further addressed in extended studies.

In conclusion, within the limitations of this double-blind placebo-controlled trial, the results indicated that daily intake of milk supplemented with 5 ppm fluoride and/or lactobacilli-derived probiotic bacteria could reverse the soft and leathery texture of primary root caries lesions in older adults better than milk alone. There was a tendency that the beneficial effect was strongest when both fluoride and lactobacilli was added to the milk, but further studies are needed to elucidate that matter.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the performance and evaluation of the study protocol as well as for the content and writing of the paper.

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