

Milk and Oral Health

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Abstract

Oral health includes freedom from disease in the gums, the mucosa and the teeth. There has been a striking reduction in dental caries and periodontitis in industrialized countries, although the proportion with severe disease has remained at 10–15%, and the prevalence increases in less developed countries. If left untreated, these diseases may lead to pain, and impaired quality of life and nutritional status. Prevention and treatment need, besides traditional implementation of proper oral hygiene, sugar restriction and use of fluoride, newer cost-effective strategies. Non-sweetened dairy products, which are proven non-cariogenic, or specific bioactive components from alike sources might prove to be part of such strategies. Thus, milk proteins, such as bovine and human caseins and lactoferrin, inhibit initial attachment of cariogenic mutans streptococci to hydroxyapatite coated with saliva or purified saliva host ligands. In contrast, both bovine and human milk coated on hydroxyapatite promotes attachment of commensal *Actinomyces naeslundii* and other streptococci in vitro, and phosphorylated milk-derived peptides promote maintenance of tooth minerals, as shown for the β -casein-derived caseino-phosphate peptide. Observational studies are promising, but randomized clinical trials are needed to reveal if dairy products could be a complementary treatment for oral health.

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Oral Health in a Global Perspective

Dental caries and periodontitis are the most common infectious diseases worldwide. Though not life threatening, they may seriously affect quality of life and well-being due to pain, limitations in mastication and food selection, and reduced self-esteem. Other less prevalent oral diseases such as cancer and noma, may lead to untimely death. The global pattern of oral diseases has undergone a significant change in recent decades. There has been a striking reduction in dental caries in industrialized countries and a parallel increase in less developed countries. However, the proportion of

patients with severe caries has remained virtually unchanged at 10–15% in the industrialized countries, and 90% of caries in low-income countries has remained untreated. A similar pattern is seen for periodontal disease. In spite of the widespread use of effective preventive measures, such as fluoridated toothpaste and improved oral hygiene, newer strategies are needed to improve oral health. The present paper describes the potential role of milk and dairy components in oral health promotion with special focus on dental caries.

Determinants for Dental Caries

Dental caries is a chronic infectious disease with a slow progress of tooth tissue destruction in most individuals. A permanent tooth crystal demineralization results from alternating periods with low pH and neutral pH at the tooth surface, during which demineralization and remineralization, respectively, occur. Progression may be arrested if the local conditions are beneficial, such as maintenance of neutral pH and supersaturation of calcium, phosphate and/or fluoride in the fluid phase surrounding the hydroxyapatite crystals.

Teeth, and other surfaces in the mouth, are covered with a biofilm of microorganisms that are attached to host receptors or to each other [1]. In recent years, the ecosystem in the biofilm, rather than the presence of any specific bacterial species, has been recognized as a determinant for development of caries. Thus, enrichment of one or more bacterial species tolerant to low pH and qualified acid producers from sugar metabolism are held responsible for disease development [2]. Among such species are the mutans streptococci, which are frequently isolated from caries sites and induce caries development in sugar-fed animals. The mutans streptococci also have the ability to produce water-insoluble, extracellular glucans that increase bacteria adhesion capacity in the biofilm. Other bacteria, such as *Streptococcus sanguinis* and species of *Actinomyces*, are considered 'protective' against caries development. Several studies have confirmed that low pH (not sugar per se) is a driving force for plaque ecology and caries [3–5].

Bacterial colonization starts by the selective adhesion of a bacterium to a suitable host receptor. In the mouth, these receptor sites are mainly present on salivary proteins that adhere to the tooth tissues or epithelial cells (fig. 1).

Earlier research on the role of saliva for caries development focused on its role as a buffer and on the salivary proteins that limit bacterial growth and metabolism, such as lactoferrin, and lysozyme. Today, research focuses on biofilm ecology. Ligand-bacterial interaction sites are being mapped, and proteolytically released peptides with biological activities that are several-fold

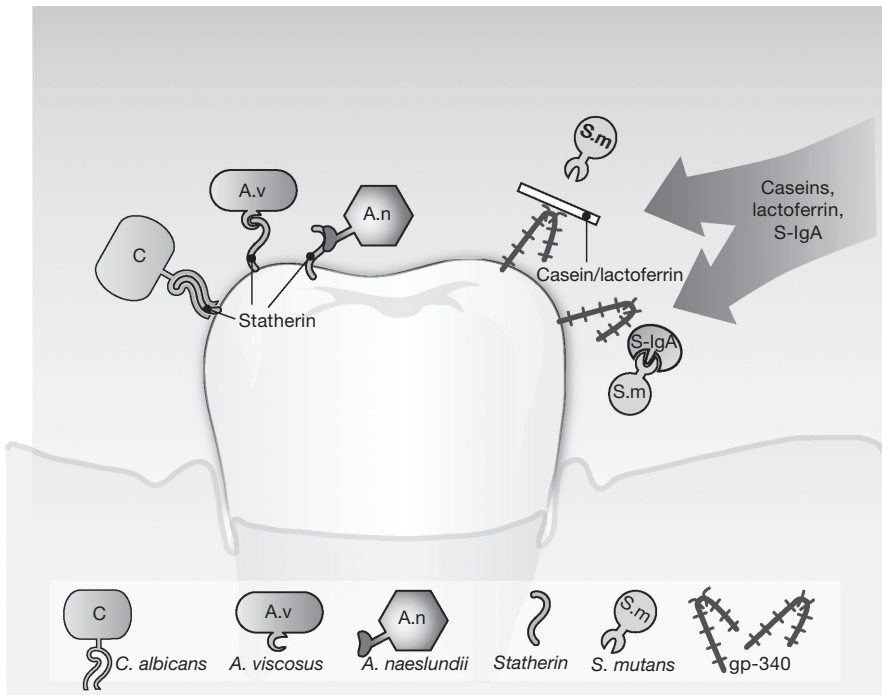


Fig. 1. Oral biofilm formation. Components from both saliva and diet form a protein/peptide pellicle on oral tissues, and display adhesion sites for bacteria attachment. Such components may also bind to free-floating bacteria and clear them from the oral cavity. Initially colonizing bacteria, such as *Actinomyces* and *Streptococcus* spp., adhere to the pellicle or co-adhere to other bacteria.

greater than the mother proteins are being studied. Externally derived proteins/peptides, such as from milk, may provide receptors for bacterial adhesion and may induce bacterial aggregation (clearance). Note that milk and saliva are exocrine secretions that contain an array of peptides/proteins and minerals that are the same and that confer similar biological functions. Other components are tissue specific, such as the lactose, fat, caseins and lactalbumins (only in milk), and the innate immunity protein gp340 (the major host receptor for mutans streptococci; only in saliva) [6]. These components confer different biological functions to the secretions. Note that some of the bacteria-binding/antimicrobial peptides with high affinity for the tooth tissues (statherin and histatins) are suggested to have evolved from an ancestral casein gene [7]. In addition, the family of saliva proline-rich proteins, which constitute >50% of the saliva protein content, share several structural similarities with the caseins.

Potential Anticariogenic Effects of Milk

Studies in rodents have shown that milk is non-cariogenic [8, 9], and have suggested that milk may have a protective effect against sugar when consumed together [9]. In vitro studies have found bioactive components in dairy products that block adhesion of cariogenic mutans streptococci, support adhesion of commensal *Actinomyces* and streptococci, reduce production of extracellular glucans, support hydroxyapatite remineralization, reduce acid production and buffer at low pH [10–13]. Several different milk components are involved in these actions, but in recent years, caseins and peptides thereof, such as caseino-phosphate peptide (CPP), have attracted special interest [for a review on the β -casein-derived CPP, see Azarpazhooh and Limeback 14].

Milk and Dental Health in Children

No randomized clinical trial on milk intake and caries development has been identified. However, several observational, cross-sectional studies find either lower caries frequency in children with milk consumption compared to those who do not drink milk, or significantly lower milk consumption in children with caries as compared to children without caries [15–19]. However, two prospective cohort studies failed to show those correlations [20, 21].

These contradictory results may be due to the fact that the studies were performed in different settings. Thus, Petti et al. [17], who studied 6- to 11-year-old non-fluoride users with poor oral hygiene, found that increasing milk consumption (0–650 ml per day) reduced caries, but only in children with high sucrose intake. Similarly, Leake et al. [19] studied small children with high caries development, i.e. 46% had severe early childhood tooth decay. The children who drank milk had fewer caries lesions after they began to walk (odds ratio 0.44, 90% CI 0.24–0.81). In contrast, the study by Öhlund et al. [20], which found no association with milk intake, was done in a low-caries prevalence area. However, Öhlund et al. [20] as well as Petridou et al. [22] found that intake of hard cheese was associated with less caries in a dose-response manner. The effect of hard cheese has been confirmed in an intervention study [23]. In a 2-year study in schoolchildren, an intervention group chewed one 5-gram piece of hard cheese daily, whereas no cheese was provided to controls. All children drank water with 0.3 ppm fluoride, brushed their teeth with fluoridated preparations and received fluoride drops. At the end of the intervention period, caries increment measured as decayed, missing and filled tooth surfaces (DMFS) was significantly lower in the cheese-eating group (0.65 DMFS) than in the control group (2.4 DMFS). Still, one prospective cohort study has found no association between reported cheese consumption and caries development [21].

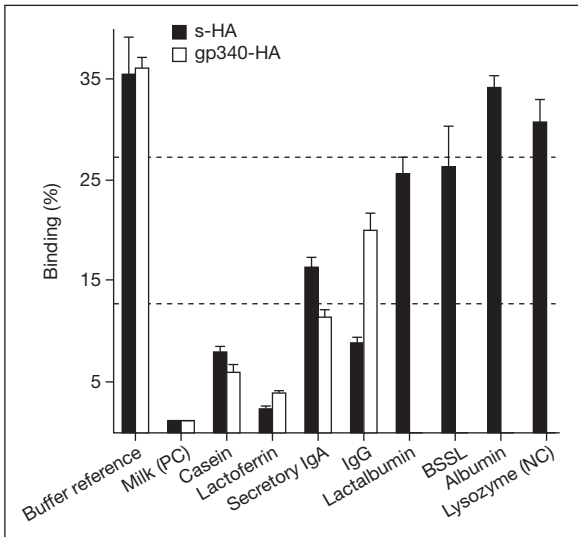


Fig. 2. Binding inhibition by human milk proteins. Binding of *S. mutans* strain Ingbritt to saliva- or gp340 (major human *S. mutans* host receptor)-coated hydroxyapatite. Bacteria were preincubated with different milk proteins. Percent *S. mutans* binding was compared with control binding of untreated bacteria. PC = Positive control; NC = negative control; BSSL = bile salt-stimulated lipase.

Milk proteins, such as bovine and human caseins and lactoferrin have been shown to inhibit attachment (the first step in colonization) of cariogenic mutans streptococci to saliva host receptors coated on synthetic hydroxyapatite (a model for tooth tissue; fig. 2).

In contrast, milk, and specifically caseins, bound to hydroxyapatite do not mediate attachment of mutans streptococci, but bind commensal and other streptococci. The delineation of human milk components mediating attachment inhibition of strain Ingbritt to parotid saliva or purified host receptor (gp340) is illustrated in figure 3.

Dairy Products and Oral Health in Adults

Epidemiologic studies in adults and elderly people confirm that higher intake of dairy products is associated with less caries. Thus, elderly who ate cheese several times per week [24] or milk products [25] had significantly less root surface caries. A possible protective role of cheese is also supported by in situ studies of remineralization of enamel slabs worn in the mouth of dry mouth patients [26].

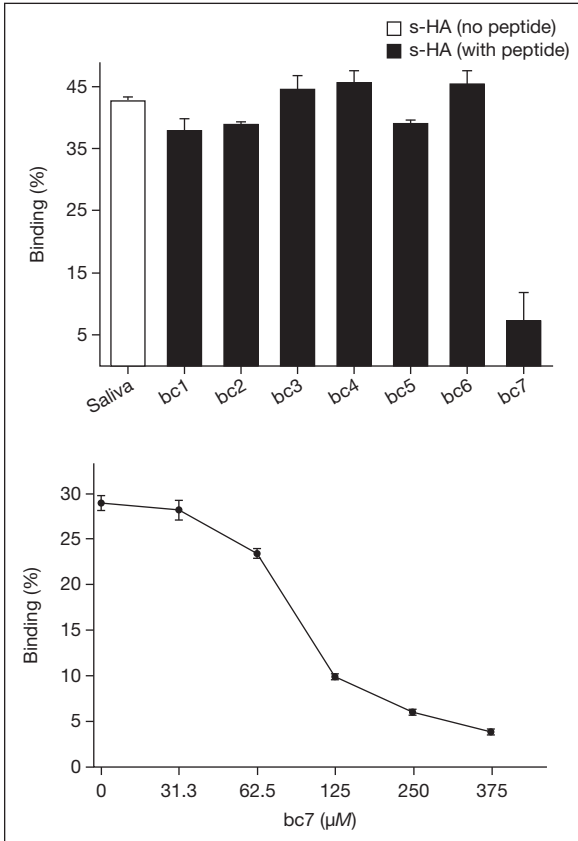


Fig. 3. Binding inhibition by β -casein-derived peptides. Binding of *S. mutans* strain Ingbritt to saliva-coated hydroxyapatite. Bacterial cells were preincubated with 30 aa synthetic peptides (bc1–bc7) covering the entire sequence of β -casein from the N to the C terminal.

The association between periodontal conditions and intake of dairy products, such as milk, cheese, and lactic acid foods (yogurt and lactic acid drinks) has also been studied in adults [27, 28]. The former authors found that subjects who ate ≥ 55 g lactic acid foods per day had less periodontitis (odds ratio after adjustment for confounders, 0.40, 95% CI 0.23–0.70) than subjects not eating those foods. Similarly, Al-Zahrani [28] found periodontitis to be 41% less prevalent in individuals in the highest quintile of intake of dairy products compared with those in the lowest quintile. After standardization for known and suspected periodontitis risk factors (age, gender, race/ethnicity, cigarette smoking, education, diabetes, poverty index, vitamin use, body mass index, physical activity, time since the last dental visit, dental cal-

culus, and gingival bleeding), it declined to 20%, but the trend remained statistically significant.

Milk as a Carrier of Therapeutic Agents

Combining dairy products and therapeutic components could theoretically lead to synergistic health effects. Such approaches have been used for prevention/treatment of dental caries using milk supplemented with fluoride or/and probiotic bacteria. Thereby, both tooth mineralization (calcium-fluoride) and biofilm ecology (buffer effect, adhesion modification, and anti-metabolic effects) are influenced. The caries-protective effect of fluoridated milk has been evaluated in several Cochrane reviews, which all came to the conclusion that fluoridated milk is beneficial, but better quality clinical studies are needed to reach stronger conclusions on its efficacy [29].

Recently, increasing numbers of publications have studied the effect of probiotic bacteria in dairy products (mainly milk) on caries-associated factors. Several short-term studies support that probiotic lactobacilli both reduce caries risk markers and improve gingival conditions, at least on short term basis [30–33]. The mechanisms are not well understood, but possibly involve blocking of host receptors, such as saliva gp340 for mutans streptococci [34] and affecting bacteria metabolism [35]. The few published long-term studies support a caries-protective effect in preschool children. Thus, in one study a group of children who consumed milk with *Lactobacillus rhamnosus* for 7 months reduced caries development compared to a group of children who drank control milk [36], and a recent 21-month randomized clinical trial showed a 75% caries reduction by milk supplemented with *L. rhamnosus* and fluoride in a low caries population with organized dental care compared to a control group [37]. The latter study design did not allow an evaluation of the separate effects of fluoride and probiotic bacteria. These studies are promising, and future long-term studies will reveal if dairy products with natural or added probiotic bacteria could be a complementary treatment for oral health.

Conclusion

Oral health inequality is a serious reality both within industrialized countries and between industrialized and less developed countries. The relative importance of different disease-associated factors, and thus the efficacy of preventive strategies, will vary between cohorts. To overcome this, a multidimensional approach must be taken. The basis will be the traditional preventive measures that have proven successful, but newer cost-effective prevention/treatment strategies must be applied. Non-sweetened dairy

products or specific bioactive components from such sources might prove to be part of such strategies. However, properly designed clinical studies in target groups are needed. Such studies must include the effects of confounders and involve caries in various risk groups and types of disease, such as children and underprivileged groups in general, orthodontic patients, dry mouth groups, and coronary and root caries conditions to overcome the shortcomings of the present studies.

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Discussion

Dr. Sankaranarayanan: Does dental caries mostly occur in milk teeth or in permanent teeth?

Dr. Johansson: Both, and these general aspects refer to both deciduous and permanent teeth.

Dr. Sankaranarayanan: We believe that multiple dental caries is likely to be due to very severe extra-epithelial manifestations of gastric refluxes. People who suffer from gastric reflux do very well particularly with anti-reflex measures and lifestyle modification, and multiple dental caries is commonly seen in neurologically impaired and mentally retarded children. Dental caries in milk tooth is a point of discussion at the differential diagnosis, and I would like to bring it to this forum, the milk-rich

feed may be a factor for constipation. I would like to have your comments about this because we think about one side of the coin, but I would like to see the other side of the coin as well.

Dr. Johansson: I assume that what you find in your reflux patients is dental erosion, and, as you describe, treatment involves antacids, modifications of toothbrushing behavior and intake of acidic products, and possibly covering of the damaged teeth. Many diseases and medications, including neurological impairment and mental retardation, as you mention, are associated with increased risk for caries. This may be due to impaired saliva secretion or muscle movements or improper oral hygiene and feeding. We find that appropriate prevention, involving oral hygiene with fluoridated toothpaste, restricted sugar intake and topical fluoride treatment, is effective. Your third question was on the risk of constipation by milk feed. The epidemiological studies supporting protective effects have not linked very high intakes of cheese or milk to caries protection, so from that point of view I do not think it would eliminate the possibility of a fiber-rich diet. I wanted to balance the not uncommon information on a risk of 'breastfeeding caries' given by some dental personnel, at least in European countries, to all parents. This is very unfortunate since human milk is not cariogenic per se. One might have to consider a risk in cases with ad libitum breastfeeding throughout the night after tooth eruption, and then 'bottle feeding caries' must be distinguished as this is a different issue.

Dr. Prentice: I wanted to thank you very much, you have introduced me to an area that I have not heard about before. My question is about the development of saliva. Many years ago, I was interested in the appearance of secretory IgA in saliva of the newborn and was interested just how long it took for concentrations to develop over a period of months. Given, as we heard today, that breast milk composition also changes, I wondered if you have any information on how long it takes the breastfed child to produce the adhesins for the bacteria – is it once the child's tooth has erupted and the child's own saliva produces those materials, or does breast milk contain some of the adhesins as well?

Dr. Johansson: Besides IgA, very few saliva components have been followed longitudinally from birth on, so I cannot give a specific description of changes in the concentrations. However, most components are detected at significant levels very soon after birth, including proteins with bacterial adhesion capacity and innate immunity functions. The low-volume saliva secreted in small babies also leads to concentrations that sometimes are higher than in the saliva of adults.

Dr. Savaiano: Could you comment on whether or not there is any evidence to believe that cheese vs. milk has differential effects?

Dr. Johansson: There are a couple of notable differences. First, there is no lactose in cheese, so there is no risk of microbial adaptation to lactose in frequent exposure. Then, there are higher concentrations of all components in cheese, and cheese contains an array of proteolytically cleaved peptides, some of which are biologically active, at least in vitro.

Dr. Garg: Until today, I had the impression that milk is cariogenic. In bottle baby syndrome, dental erosion of the front teeth occurs in children who are bottle fed. We have always thought that because milk remains in touch with the front teeth during the night, and that's what causes dental erosion. Has there been a change in the understanding of bottle baby syndrome?

Dr. Mouane: Just to join Dr. Garg, pediatricians often advise parents to avoid bottle feeding during the night.

Dr. Johansson: Bottle-feeding is one of well-documented risk factors for early childhood caries, and the advice not to use a bottle at night is correct. Often, night bottles contain sweet drinks but also the carbohydrates used in formulas are fermentable

by tooth-colonizing bacteria, and therefore should not be used ad libitum after tooth eruption, and especially not at night when there is very low saliva production. Finally, what I think you see in the bottle-fed babies you describe is dental caries and not erosion, and prevention would consist in limiting exposure frequency/duration and introducing gentle tooth cleaning as soon as the first teeth have erupted.

Dr. Haschke: Together with the University Dental Clinic in Zurich, the Nestlé Research Centre did a lot of rat studies with glycomacropeptides because they are a casein fraction with a strong anticariogenic effect. Our intention was to make a follow-up formula that is anticariogenic. The problem was that so much of glycomacropeptides was needed to show an anticariogenic effect that the cost of such a formula would have gone through the roof, and we sold the patent to Colgate. Are you aware of this?

Dr. Johansson: Yes, I know these studies from what has been published. Glycomacropeptides and casein phosphopeptides are peptides that have documented anti-caries effects in animals and the latter also in humans. The effects involve prevention of adhesion of cariogenic mutans streptococci and enrichment of calcium and phosphate in the tooth biofilm. Together with the studies by Bowen and coworkers, these studies were the foundation for a concept that might be developed into a valuable complement to fluoride.

Dr. Martin: Thank you very much for your very interesting talk. You call for high-quality studies looking at breastfeeding vs. formula feeding. Do you have any comment on the PROBIT results that were published in *Caries Research*? This was a trial with 17,000 mother-infant pairs, 8,000 randomized to the breastfeeding promotion intervention, 8,000 to the control arm, with the intervention substantially increasing exclusive and prolonged breastfeeding. In an analysis by intention to treat, removing any effect of confounding, there was no difference in caries prevalence or caries experience in 6-year-old children between the two groups [1].

Dr. Johansson: The PROBIT study in Belarus is a powerful study that demonstrates that in a population with a very high level of caries development no harmful or beneficial effects are seen from breastfeeding in a long-term perspective. However, information is also needed from other types of populations, for instance populations with poor nutritional status and populations with the dual disease distribution that has emerged in many countries today.

Dr. Martin: Just to follow up on Dr. Haschke's question about the GMP. You said that you needed so much in the formula that it was not cost effective. How much cheese do you need to have a protective effect?

Dr. Johansson: In the one intervention study that has been done, the children were given 5 g a day.

Dr. Martin: Do you need to take it daily?

Dr. Johansson: Our results support that a daily intake is needed. Thus, we found that children with one intake or more a day were caries free.

Dr. Haralappa: You said that to avoid caries one should use fluoride toothpaste. Most of the small children swallow it. My question is, how much toothpaste can they swallow? How much would be harmful and cause fluorosis?

Dr. Johansson: The main risk of developing fluorosis comes with the water that children drink during tooth formation. A fluoride water content of 1 mg/l (1 ppm) is considered optimal under most conditions. Anything above that level is associated with an increasing risk for disturbance of tooth tissue formation. Under conditions with high content of fluoride in the drinking water, one does not need fluoridated toothpaste since the teeth are rinsed with fluoride at every intake. Tea is another source that might be considered. In areas with a 'suboptimal' fluoride level, even small children can be given fluoridated toothpaste in small amounts bearing in mind that

they swallow. In Scandinavia, we recommend that parents use regular toothpaste (1,000–1,500 ppm), but limit the portion to that of half a pea. At that level, there are no indications of harmful side effects.

Dr. Bodenstab: You mentioned cheese, but how about yogurt? And does it make a difference to have live bacteria in it or not?

Dr. Johansson: There is limited epidemiology for yogurt and caries, but my assumption is that the effect is closer to cheese than milk due to bacterial fermentation and lactose reduction and peptide production. Another positive thing with yogurt is that it has naturally or often even added probiotic bacteria. A positive effect of probiotic bacteria has been indicated by several short-term studies. It must, however, be observed that many yogurts on the market have added sugar, which changes a non-cariogenic into a caries-promoting product.

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