Evaluation of plaque pH changes following oral rinse with eight infant formulas

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Abstract

Inappropriate feeding habits have been identified as major factors associated with the development of baby bottle tooth decay or nursing caries. An in vivo/in vitro combination technique was developed to investigated the plaque pH changes associated with rinsing with eight different infant formulas. These eight formulas represented four categories: 1) formulas with iron, 2) formulas with low iron, 3) soy formulas 4) and protein hydrolyzate formulas (from the manufacturers Mead Johnson Nutritionals and Ross Laboratories). All formulas had the ability to reduce the pH significantly below the pre-rinse plaque pH. Furthermore, the average minimum pH for formulas from the two manufacturers did not differ within each formula category except for the soy-based formulas, where, rinsing with Isomil™ produced a significantly lower plaque pH than ProSobee.™ These results suggest that infant formulas are acidogenic and therefore may play a significant role in the development of baby bottle tooth decay. (Pediatr Dent 18:200–4, 1996)

Infant formulas in the nursing bottle have been implicated in the development of nursing caries. Formulas are a complex synthetic combination of nutrients, including fermentable carbohydrates. There are many different formulas with different carbohydrate sources such as: lactose, corn syrup solids, sucrose, and glucose polymers. At present, there are no available data comparing the cariogenicity of different formulas. Furthermore, controversy exists concerning the cariogenicity of milk used to manufacture these formulas.

Jenkins and Ferguson,[1] after studying the effects of bovine milk, concluded that milk did not promote caries. Their results were based upon an in vivo study on plaque pH measurements with an antimony electrode and an in vitro enamel solubility study. They showed that while milk carbohydrate can be utilized by salivary bacteria for acid production, the pH values reached after 4 and 24 hr of incubation were higher than with a 4% lactose control. Furthermore, their in vitro solubility study showed that, in spite of acid production, the amount of calcium and phosphate dissolving from enamel was much less in the presence of milk than in the presence of sucrose. Therefore, they concluded that milk might provide a protective effect against cariogenic foods. In another in vitro study of bovine enamel, cow's milk was shown to reduce the solubility of enamel an average of 21.2% (5.5) when exposed to an acid solution.[2]

On the other hand, the negative features of bovine milk also have been described. Birkhed et al.[3] showed that, in humans, acid production in dental plaque increased after frequent ingestion of either lactose or milk. Other laboratory studies also have shown that, in animals, lactose will enhance oral implantation of bacteria, produce dental caries, and demineralize tooth enamel.[4–8]

These previous studies have shown that the issue of milk being linked to nursing caries is complex because milk itself is a complex fluid, and in addition to its potentially cariogenic lactose content, it contains ingredients that may protect against caries development.

Although the clinical experience — that nursing caries is linked to milk — and the experimental evidence — that under usual dietary conditions milk is not very cariogenic — appear to be contradictory we should realize that:

> "...with nursing caries condition, usual dietary conditions do not prevail. Exposure to milk is frequent and prolonged, resulting in pooling and stagnation around the necks of the teeth, especially the maxillary incisors."[9]

A further complication in the evaluation of the cariogenicity of milk has been the recent development of a variety of infant formulas. Most formulas manufactured are similar to human milk, including the lactose content. Soy-based formulas and protein hydrolyzate formulas are lactose free, but do contain different sugars, such as sucrose, glucose polymers, and corn syrup.

The objective of this study was to evaluate the plaque pH changes following rinsing with eight different commercially available infant formulas.

Materials and methods

The sample size consisted of nine adult volunteers for nine trials each. These volunteers consisted of faculty and residents from the division of pediatric dentistry at the University of Minnesota. While previous dental restorations were present in these volunteers, none of the subjects had active caries. The criteria used to accept subjects into this study were:

Inclusion criteria: Normal adult volunteers in good general and oral health.

Exclusion criteria: Subjects on antibiotic therapy; subjects with xerostomia; or subjects with lactose intolerance or general allergy to milk.

Adult subjects were asked to abstain from oral hygiene for 24 hr and to fast from all foods except water for a minimum of two hours prior to sampling. This provided us with at least 24-hr-old plaque. Supragingival plaque was sampled from maxillary buccal surfaces before and after a 1-min rinse with 5 mL of formula or sucrose control. This provided us with one prerinse and one postrinse plaque sample, which were dispersed in 50 µL of deionized water. After stabilizing for 20–30 sec, the pH of each sample was recorded every 5 min for 1hr. The positive control in this research was plaque collected after a 10% sucrose rinse. The negative control was prerinse plaque.

Eight different infant formulas were donated for this study by WALMART Co (Eagan, MN). These formulas were representative of the most commonly used infant formulas manufactured by two companies: Mead Johnson Nutritionals (MJN) and Ross Laboratories (RL).

The eight formulas were also representative of four categories (Table 1).

All formulas were premixed solutions, except Nutramigen™ which was diluted with sterile water according to manufacturer's directions. Each formula was dispensed aseptically into 10-cc tubes and the excess was discarded. A control 10% sucrose solution was made by dissolving 50 g of sucrose in distilled water to a final volume of 500 mL. This solution was then sterilized by autoclaving. Each formula and the prepared 10% sucrose solution were given a random number unknown to the operator until the end of the research. The formulas and control were stored at 4°C until use.

TABLE 1. THE EIGHT FORMULAS USED IN THIS STUDY

Brand Names	*Manufacturer*	*Lot Number*
1. *Formulas with iron:*		
Similac® with iron	RL	66737RC
Enfamil® with iron	MJN	0305-MFE 43
2. *Formulas with low iron:*		
Similac®	RL	070708 RC
Enfamil®	MJN	0302-MFE43
3. *Soy formulas:*		
Isomil®	RL	69935 RAD
ProSobee®	MJN	0309-MEE 21H
4. *Protein hydrolyzate formulas:*		
Alimentum®	RL	70616 RC
Nutramigen®	MJN	0498-ADE 64

Three plaque pH measurements were evaluated in this research.

1. Minimum pH: defined as the lowest pH recorded in the 1hr period, was recorded because hydrogen ion production potential of food items has been related to the food's cariogenic potential.
2. pH at 1 hr: defined as the postrinse plaque pH recorded at 60 min past the time of initial plaque sampling, was recorded to compare formula independent of time.
3. pH drop: defined as the difference between the initial prerinse plaque pH and the minimum plaque pH obtained, was also recorded to allow for comparisons independent of resting pH.

A paired *t*-test was used to 1) compare the pH measurements with the average pre-rinse plaque pH, 2) compare the pH measurements associated with formulas from the two manufacturers 3) and compare the pH measurements associated with each formula category.

TABLE 2. LOWEST PH RECORDED IN THE 1-HR PERIOD

Formulas	*Mean*	*SD*	*P-value*
Similac® with iron	5.76	0.53	< 0.001
Enfamil® with iron	5.79	0.40	< 0.001
Similac® low iron	5.74	0.37	< 0.001
Enfamil® low iron	5.86	0.35	< 0.001
Isomil® (soy)	5.16	0.63	< 0.001
ProSobee® (soy)	5.53	0.44	< 0.001
Alimentum® (protein hydrolyzate)	5.29	0.38	< 0.001
Nutramigen® (protein hydrolyzate)	5.38	0.68	< 0.001
Sucrose	5.25	0.40	< 0.001

Comparisons to average prerinse (mean = 6.50; *SD* = 0.24) using a paired *t*-test. $N = 9$.

Results

The average minimum pH obtained varied between milk formulas from 5.86 for Enfamil™ with low iron to 5.29 for Alimentum™ (Table 2). A paired *t*-test comparison, with the average prerinse plaque pH of 6.50 ± 0.24, showed that all formulas had the ability to reduce pH significantly below prerinse pH.

pH at 1 hr was similar to minimum pH obtained and varied from 5.98 for Enfamil with low iron to 5.43 for Alimentum (Table 3). A paired *t*-test showed that all formulas had the ability to produce pH within 1 hr significantly below prerinse pH.

pH drop was similar to the other two measures ranging from the least drop of 0.77 for Enfamil with low iron

Table 3. Postrinse plaque pH recorded at 60 min past the initial sampling

Formulas	*Mean*	*SD*	*P-value*
Similac® with iron	5.67	0.57	0.001
Enfamil® with iron	5.89	0.46	0.006
Similac® low iron	5.88	0.44	< 0.001
Enfamil® low iron	5.98	0.41	0.001
Isomil® (soy)	5.35	0.65	< 0.001
ProSobee® (soy)	5.89	0.42	0.001
Alimentum® (protein hydrolyzate)	5.43	0.48	< 0.001
Nutramigen® (protein hydrolyzate)	5.56	0.74	0.002
Sucrose	5.35	0.43	< 0.001

Comparisons to average prerinse (mean = 6.50; *SD* = 0.24) using a paired *t*-test. $N = 9$.

to the greatest drop of 1.40 for Alimentum (Table 4). A paired T-test showed that all formulas have a significant pH drop.

It is important to note that prior to testing Similac™ with iron, subject C had an acidic prerinse plaque sample. This sample did not significantly alter the average minimum plaque pH. The results for Similac with iron were significantly different from the prerinse plaque pH with or without this sample. The average pH drop was affected slightly. Fortunately, the unusual behavior of this sample only reduced the significance of Similac with iron rather than improved the significance.

The average minimum pH for formulas from the two manufacturers was similar in each category except for the soy-based formulas. The pH associated with Isomil (was significantly lower than the minimum plaque pH associated with ProSobee ($P < 0.05$). Furthermore, the minimum pH following rinsing with the soy-based and protein hydrolyzate formulas was significantly lower than that for the milk-based formulas (Figure).

Discussion

Because of the irreversible nature of dental caries, a true caries test on human subjects would be unethical. While several methods have been employed to test the cariogenic nature of foods,[11, 12] we do not have an acceptable model to investigate the caries process in the unusual conditions of nursing bottle caries. Food items have not been tested in pooling, stagnating conditions as seen in this disease. Therefore, there is a strong need to provide an experimental model that can better simulate the development of nursing caries and evaluate the potential role of infant formulas.

In our study a plaque sampling method was used. Originally proposed by Frostell, plaque was to be sampled every 5 min following oral exposure to a cariogenic substance. A modification to this technique, as suggested by Jensen,[10] was used here. The original protocol was modified to include one prerinse and one postrinse plaque sample. The postrinse plaque sample was collected immediately after exposure to the infant formula and the in vitro pH was monitored for 1 hr. This in vivo/in vitro combination appealed to us because it reduced the ability of saliva to buffer the pH as we monitor the samples. Thus, our technique involved a diminished salivary effect, which was similar to that seen at nighttime feedings due to circadian rhythm.

Our results showed that all infant formula categories produced significantly lower plaque pH values than prerinse plaque. The results also showed that the mean minimal plaque pH after rinsing with soy-based or protein hydrolyzate formulas was significantly lower than the plaque pH after rinsing with a milk-based formula. Furthermore, the soy-based and protein hydrolyzate formulas produced plaque pH changes that fell below the critical enamel demineralization pH of 5.7. The concept of critical pH was formulated in 1930s and '40s. Different estimates of critical pH ranged from 5.7 to 5.5 or lower. Muhlemann and his colleagues choose a conservative 5.7 as the basis for their "safe teeth" determination. Experience and experimentation have shown that no product judged by the Swiss system as safe for teeth has been found to promote decay.[11, 12] Therefore, in this study, we used pH of 5.7 as our critical pH.

Our data show that infant formulas are acidogenic and thus agree with Brown et al.[7] who suggested, "milk is a suitable substrate for demineralization of enamel" based upon their in vitro study of bovine milk. Our results also agree with Birkhed et. al.,[3] who showed that lactose-hydrolyzed milk or 5% sucrose control produced significantly lower plaque pH values than standard bovine milk or human breast milk.

Other studies have shown different results. Frostell[13] showed only small changes in dental

Table 4. Difference between the initial prerinse plaque pH and the minimum plaque pH

Formulas	*Mean*	*SD*	*P-value*
Similac® with iron	1.14	0.43	< 0.001
Enfamil® with iron	0.86	0.49	< 0.001
Similac® low iron	0.79	0.39	< 0.001
Enfamil® low iron	0.77	0.30	< 0.001
Isomil® (soy)	1.34	0.51	< 0.001
ProSobee® (soy)	1.12	0.34	< 0.001
Alimentum® (protein hydrolyzate)	1.40	0.44	< 0.001
Nutramigen® (protein hydrolyzate)	1.04	0.48	< 0.001
Sucrose	1.47	0.48	< 0.001

Comparisons to average prerinse (Mean = 6.50; *SD* = 0.24) using a paired *t*-test. $N = 9$.

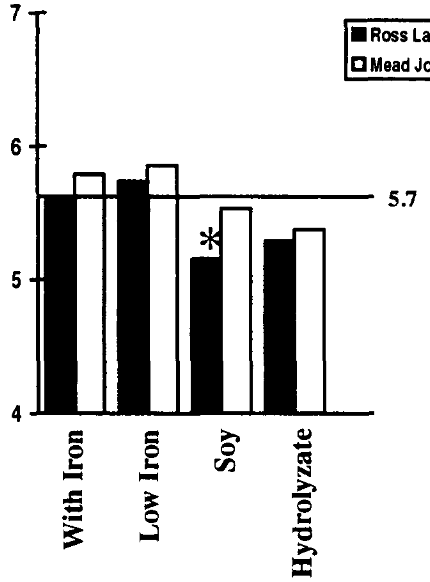

Figure. Average minimum pH following rinsing with the eight infant formulas. The minimum pH following rinsing with the soy-based and protein hydrolyzate formulas was significantly lower that that for the milk-based formulas ($P < 0.05$). The asterisk designates that the pH associated with Isomil was significantly lower than the minimum plaque pH associated with ProSobee ($P < 0.05$).

plaque pH following a rinse with cow's milk. Mor and McDougall,[14] showed milk to be the least acidogenic of the solutions they tested (5% sucrose, 5% lactose, milk, or milk plus 4 ppm fluoride). Still other studies have shown reduced enamel dissolution by milk, or milk-containing foods.[1, 2, 15]

These studies suggest that milk may provide some protection from dental caries. Several investigators have sought to identify the source of the protective action of milk.[16, 17] Dreizen et al.[16] compared rat models in which one group was given only nonfat, dry milk and the other group was fed nonfat, dry milk mixed with a caries-promoting diet (containing corn meal, wheat flour, sugar and lard). They found that the nonfat, dry milk alone was noncariogenic in the rats. They attributed the protective action to the high percentage of casein (28%) used in their study.

Casein is the major source of protein in milk. Reynolds et al.[18] later investigated the role of casein in a rat model and found that 2% casein in drinking water reduced the extent of fissure and smooth surface caries.

These studies shed light on the possibility that in protein hydrolyzate formulas, by predigestion of casein, an important anticariogenic mechanism may be removed. In our study, the soy-based formulas (with no casein) and the protein hydrolyzate formulas (with hydrolyzed casein) resulted in a pH drop significantly below the two milk-based formulas.

In addition to the differences in casein concentrations, the infant formulas we evaluated differed in the carbohydrate source and content. Therefore, our results also may be related to the cariogenicity of these sugars. Indeed, Koulourides et al.[6] studied the cariogenicity of nine sugars. They concluded that, "lactose, mannitol, melibiose, and sorbital were significantly less cariogenic than sucrose." Subsequent studies have shown that lactose-based milk was less cariogenic than sucrose-based milk.[8, 19]

Our results for formulas from the manufacturers (Mead Johnson Nutritionals and Ross Laboratories) were similar in each category except in the soy-based formulas. Although containing minor compositional differences, ProSobee and Isomil produced statistically different minimum plaque pH. The most notable difference in the manufacture of these two formulas is the carbohydrate source. While both contain corn syrup solids, Isomil also has sucrose, which may be more easily fermented by the oral bacteria. In nursing caries, it is possible that the quantity and duration of supply of the carbohydrate sources (lactose) may turn a potentially noncariogenic food (milk) into a cariogenic source.

All of the previously reported studies investigated bovine milk in an intraoral environment. None of these studies, however, was designed to investigate the conditions present in nursing caries. The removal of the saliva buffering capacity, as in our methodological approach, may play a significant role in the development of nursing caries.

Infant milk formulas and the process of their manufacture are constantly changing in an attempt to become closer to human milk characteristics. Continued studies on the cariogenicity of infant formulas are important to assess the risks associated with their consumption. Based upon this research, we recommend further evaluation of the cariogenicity of milk formulas with the understanding of a nursing child's behavior, and the formulation and evaluation of less cariogenic infant formulas.

Conclusions

From this study we conclude that:

1. Infant formulas differ significantly in their ability to alter plaque pH.
2. Rinsing with soy-based formulas or protein hydrolyzate formulas resulted in a drop in plaque pH to below the critical pH level of 5.7.

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1. Jenkins GN, Ferguson DB: Milk and dental caries. Br Dent J 120:472–77, 1966.
2. Weiss ME, Bibby BG: Effects of milk on enamel solubility.

Arch Oral Biol 11:49–57, 1966.

3. Birkhed D, Ohlsson A, Svenson C, Edwardsson S, Imfeld T: Milk and lactose acid production in human dental plaque. J Dent Res 60:1245, 1981 (Abstract 6).
4. Krasse B: The effect of the diet on the implantation of caries-inducing streptococci in hamsters. Arch Oral Biol 10:215–21, 1965.
5. Guggenheim B, Konig KG, Herzog E Muhlemann HR: The cariogenicity of different dietary carbohydrates tested on rats in relative gnotobiosis with a Streptococcus-producing extracellular polysaccharides. Helv Odont Acta 10:101–13, 1966.
6. Koulourides T, Bodden R, Keller S, Manson-Hing L, Lastra J, Housch T: Cariogenicity of nine sugars tested with an intraoral device in man. Caries Res 10:427–41, 1976.
7. Brown CR, Crawford JJ, McIver FT, Taylor DF: Effect of milk and fluoridated milk on bacterial enamel demineralization. J Dent Res 56:B210, 1977 (Abstract 632).
8. Schemmel RA, Krohn-Lutz K, Lynch P, Kabara JJ: Influence of dietary disaccharides on mouth microorganisms and experimental dental caries in rats. Arch Oral Biol 27:435–41, 1982.
9. Ripa LW: Nursing caries: a comprehensive review. Pediatr Dent 10:268–82, 1988.
10. Jensen ME: Human dental plaque pH following exposure to fermentable carbohydrate. PhD thesis at University of Minnesota, 1982.
11. Harper DS, Abelson DC, Jensen ME: Human plaque acidity models . J Dent Res 66:1503–10, 1986 (spec iss).
12. Muhlemann HR, Imfled T: Evaluation of food cariogenicity by plaque pH telemetery. In: Foods, Nutrition and dental health, Vol. 1, Hefferen JJ, Koehler HM, Eds., Chicago: Pathotox, pp 151–54, 1981.
13. Frostell G: Effects of milk, fruit juices, and sweetened beverages on the pH of dental plaques. Acta Odonto Scand 28:609–22, 1970.
14. Mor BM, McDougall WA: Effects of milk on pH of plaque and salivary sediment and the oral clearance of milk. Caries Res 11:223–30, 1977.
15. Bibby BG, Huang CT, Zero D, Mundorff SA, Little MF: Protective effect of milk against *in vitro* caries. J Dent Res 59:1565–70, 1980.
16. Dreizen S, Dreizen JG, Stone RE: Effect of cow's milk on dental caries in the rat. J D Res 40:1025–28, 1961.
17. Weiss ME, Bibby BG: Some protein effects on enamel solubility. Arch Oral Biol 11:59–63, 1966.
18. Reynolds EC, Del Rio A: Effect of casein and whey-protein solutions on caries experience and feeding patterns of the rat. Arch Oral Biol 29:927–33, 1984.
19. Tamura S, Matsmuto Y, Hattori K: A study of control of experimental dental caries development by alteration of sugar composition in milk formula. Bull Tokyo Dent Coll 21:35–40, 1980.

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