Scientific Article

Estimation of the caries-related risk associated with infant formulas

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Abstract

Purpose: *Baby bottle tooth decay (BBTD) affects 6% of children under three years of age and is associated with inappropriate bottle use. The objective of this study was to estimate the caries-related risk associated with 26 infant formulas and whole milk.*

Methods: *First, the plaque pH of adult volunteers was monitored before and after an oral rinse with infant formula to determine the minimum pH obtained in response to each formula. Second, Streptococcus sobrinus 6715 was cultured in each infant formula, and the increase in the number of colony forming units was measured. Third, each infant formula was incubated with powdered enamel and the solubility of enamel mineral was calculated in the absence of bacteria. Fourth, each formula was mixed with standardized concentrations of acid to determine the buffering capabilities. Finally, enamel windows were created on extracted permanent molars and exfoliated primary incisor crowns that were then colonized with mutans streptococci and incubated with infant formula. Caries was assessed visually and radiographically for 18 weeks. The length of time required for the development of enamel caries, dentinal caries and pulpal involvement was recorded.*

Results: *One-way or two-way ANOVA of these five assays demonstrated that*

1. *Plaque pH varied in response to oral rinsing with infant formula and most formulas did have the ability to reduce the pH significantly below the pH obtained after rinsing with water*
2. *Some infant formulas supported significant bacterial growth*
3. *Enamel mineral was dissolved by incubation with certain infant formula*
4. *The buffer capacity varied among the infant formulas tested*
5. *The length of time required for caries to reach dentin or pulp differed for the formulas, with some formulas causing dentinal caries by 3.4 weeks and pulpal involvement by 7.2 weeks. (Pediatr Dent 20:395–403, 1998)*

Baby bottle tooth decay (BBTD) is a manifestations of early childhood caries which affects about 6% of children under 3 years of age and is associated with prolonged and frequent daytime, naptime, and nighttime bottle feedings.[1-3] Infant formulas in the nursing bottle have been implicated in the development of BBTD. Carbohydrates present within the infant bottle may be utilized by oral microorganisms, especially mutans streptococci, to form a sticky plaque matrix that enables the microorganisms to adhere to the teeth. They may also serve as metabolites in the production of organic acids which with time can demineralize the teeth.

We have recently shown that infant formulas are acidogenic.[4] When adult volunteers rinsed with one of eight different infant formulas, the average plaque pH dropped to between 5.29 and 5.86, compared to a resting prerinse pH of 6.50. While variability was seen between manufacturers and type of formula, all eight formulas tested in this previous study reduced the plaque pH significantly below the resting pH, suggesting that infant formulas may play a significant role in the development of BBTD.

Controversy, however, does exists regarding the cariogenicity of infant formulas. Because most infant formulas are manufactured with bovine milk providing the base ingredient, previous research has focused on the role of bovine milk in caries development. Early on, Jenkins and Ferguson[5] found that while the carbohydrate of milk can be utilized by salivary bacteria for acid production, the pH values reached after 4 and 24 h of incubation were higher than with a 4% lactose control. Furthermore, in an in vitro solubility study, they 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. Weiss and Bibby[6,7] also showed that milk significantly reduced the solubility of blocks of bovine enamel.

In contrast, Birkhed et al.[8] demonstrated that frequent ingestion of either lactose or milk resulted in an

increase in acid production in dental plaque. Furthermore, lactose in solutions has been linked to enhanced oral implantation of bacteria and enamel demineralization.[9–13]

This study sought to determine the acidogenic and cariogenic potential of the currently available infant formulas.

Methods

Infant Formulas Tested (Table 1)

The 26 formulas tested represented six categories:

1. formulas with iron
2. formulas with low iron
3. soy formulas
4. protein hydrolyzate formula
5. special formlas, including ones for older chidren (Carnation Followup and Next Step), and for children with specific dietary restrictions (Lactofree is a lactose-free, milk-based formula supplemented with corn syrup; Lofenalac is low in phenylalanine; 3200AB is low in phenylalanine as well as tyrosine; whereas Phenyl-free has no phenylalanine; MSUD is low in leucine, isoleucine and valine)
6. experimental formulas (3200AB is low in phenylalanine and tyrosine; 3200K is soy based with no methionine; 80056 is protein free; 3232A, is a special formula which is carbohydrate free, for children with selected carbohydrate intolerance).

When available, the formulas used were premixed, ready-to-feed solutions. Otherwise, powdered formula was mixed fresh daily with sterile distilled water (also used as control solution). 10% sucrose control was made by dissolving 4 g of sucrose in water to a final volume of 40 mL, followed by autoclaving to sterilize. Bovine whole milk was purchased fresh weekly and handled utilizing sterile technique to avoid bacterial contamination.

Dental plaque pH changes after exposure to infant formula

The first part of this study included 54 child patients (18 Caucasian, 18 African-American, 18 Native American) between the ages of 12 and 24 months. Following completion of a medical-dental questionnaire for health history, parental informed consent was obtained according to the guidelines of the University of Minnesota Human Subjects Committee. The inclusion and exclusion criteria for subjects are as follows:

- Inclusion criteria: Normal, caries-free children in good general and oral health.
- Exclusion criteria: Subjects on antibiotic therapy, with xerostomia, with lactose intolerance or general allergy to milk, with soy allergy and/or intolerance.

All sampling was performed between 8:00 AM and 10:30 AM. Each parent was asked to abstain from oral hygiene for their child for 24 h and to avoid giving their child any foods, except water, for 2 h prior to sampling. Supragingival plaque (approximately 10 μg) was sampled from maxillary buccal surfaces. Alternating surfaces were sampled, prior to rinsing, to provide the pre-rinse plaque control. The remaining sites were sampled after a 1-min feeding with each of four infant formulas or control solution. No site was sampled twice. Plaque was then dispersed in 50 mL deionized water and the pH monitored for 1 h. The positive control in this research was plaque collected after a 10% sucrose rinse. The negative control was plaque collected after water rinse.

The second part of this experiment included eight adult volunteers whose plaque was assessed following oral rinse with each infant formula (Table 1). The plaque pH was monitored before and after rinsing with each sample or control solution as previously described.[4]

For all samples collected, infant or adult, four pH measurements were evaluated:

1. minimum pH, defined as the lowest pH recorded in the 1-h period, was recorded because the hydrogen ion production potential of food items has been related to the food's cariogenic potential
2. pH at 1 h, defined as the post rinse plaque pH recorded at 60 min past the time of initial plaque sampling, was recorded to allow for comparisons independent of time
3. pH drop, defined as the difference between the initial pre rinse plaque pH and the minimum plaque pH obtained, was calculated to account for the initial plaque pH of each subject
4. pH drop at 1 h, defined as the difference between the pre rinse plaque pH recorded at 60 min and the post-rinse plaque pH recorded at 60 min past the time of initial plaque sampling, was calculated to account for alterations in plaque acidogenicity caused by the sampling process.

Bacterial fermentation and growth in the presence of infant formula

Each of the formulas, control solution or Todd Hewitt broth (4 mL) were inoculated in triplicate with *S. sobrinus* 6715 (108 cells). After incubation (37°C, 5%CO_2, 3 h) the cells were dispersed by sonication and a sample (0.01 mL) of each culture was spread in triplicate onto Blood Agar Plates, incubated (37°C, 5%CO_2, 3 days), and assayed for the number of colony forming units present on each plate. The bacterial growth was assessed by comparing the number of colony forming units which grew after culture in infant formula with the optimal growth seen after culture in Todd Hewitt broth.

TABLE 1. COMPOSITION OF INFANT FORMULAS AND CONTROL SOLUTIONS

Formulas	*Carbohydrate**	*Protein**	*Fat**	*Calcium†*	*Phosphate†*
Control Solutions					
Water	0	0	0	0	0
Sucrose	100	0	0	0	0
Whole Cow's Milk	48	33	40	1250	960
Iron containing formulas					
Similac with Iron	71	14	36	487	373
Enfamil with Iron	69	14	37	520	353
Carnation Good Start	73	16	34	427	240
SMA with Iron	72	15	36	420	280
Gerber with Iron	71	15	36	500	387
Bonamil	71	15	36	460	360
Low Iron formulas					
Similac–Lo Iron	71	14	36	487	373
Enfamil–Lo Iron	69	14	37	520	353
SMA–Lo Iron	71	15	35	420	280
Gerber–Lo Iron	71	15	36	500	387
Soy-based formulas					
Isomil	69	16	36	700	500
ProSobee	67	20	35	627	493
Nursoy	69	18	36	600	420
Gerber–Soy	67	20	35	627	493
Protein hydrolyzate formulas					
Nutramigen	89	19	26	627	420
Special formulas					
Carnation Followup	88	17	27	900	600
Next Step	74	17	33	800	560
Lactofree	69	15	37	547	367
Progestimil	69	19	37	627	420
Lofenalac	87	22	26	627	467
MSUD	89	11	28	687	373
Phenyl-free	135	42	14	1062	1062
Experimental formulas					
3200AB	86	22	26	627	466
3200K	65	20	36	698	552
3232A	0	18	28	627	420
80056	83	0	26	627	347

* expressed as mg/mL † expressed as μg/mL

Enamel calcium and phosphate dissolution after incubation with infant formula

Caries-free and restoration-free, exfoliated primary incisor teeth were used for this study. The internal dentin support was removed using a high speed dental handpiece. The remaining enamel shell from 45 deciduous teeth was ground to a fine powder by use of a mortar and pestle and pooled for these experiments. 50 mg of powdered enamel (60–100 in-1 mesh) was then mixed with 1 mL of each formula or control solutions under conditions previously described.[5–7, 14] Duplicates were prepared excluding the powdered enamel. All mixtures were incubated at 37°C for 24 h with gentle agitation by constant inversion. The enamel powder was removed from each sample by centrifugation for 5 min at 1600 g. A 0.5-mL aliquot of each supernatant was removed and placed into a porcelain crucible. The samples were dried at 100°C for 2 h and then ashed at 650°C for 18 h. The residue was dissolved in 0.1 mL HCl and boiled gently to convert pyrophosphate back to orthophosphate. The dissolved ash was then made up to 1.0 mL with distilled water. Calcium was estimated in the presence of lanthanium using atomic absorption spectroscopy. Phosphorus was estimated by the ammonium-molybdate method.[15] Each sample was randomly repeated three times. The amount of Ca and P dissolved was calculated by subtracting the Ca and P concentrations in the mixtures without enamel from the Ca and P concentration in the mixtures containing the powdered enamel.

TABLE 2. IN VITRO CARIES PROGRESSION OF SELECTED INFANT FORMULAS AND CONTROL SOLUTIONS (WEEKS UNTIL DECAY REACHED DENTIN AND PULP)

Formulas	*Dentin*	*Pulp*	*Dentin*	*Pulp*
Control solutions				
Water	a	a	a	a
Sucrose	4.0 ± 0.9	10.4 ± 1.1	6.3 ± 2.4	12.3 ± 1.3
Whole milk	*11.0 ± 1.2*	*>18*	*14 ± 2.2*	*>18*
Iron-containing formulas				
Similac® with iron	NA	NA	12.0 ± 1.9	16.0 ± 2.0
Enfamil® with iron	NA	NA	11.3 ± 2.0	16.7 ± 1.6
Carnation Good Start	NA	NA	b	b
SMA with iron	NA	NA	10.7 ± 3.0	15.3 ± 2.0
Gerber with iron	NA	NA	8.6 ± 2.8	>18
Bonamil	6.0 ± 1.4	11.0 ± 1.5	7.6 ± 1.5	12.0 ± 2.5
Low-iron formulas				
Similac® low iron	NA	NA	14.6 ± 2.5	>18
Enfamil® low iron	NA	NA	9.3 ± 1.6	16.6 ± 2.0
SMA low iron	NA	NA	12.0 ± 2.0	18 ± 0
Gerber low iron	NA	NA	12.7 ± 1.6	>18
Soy-based formulas				
Isomil®	NA	NA	8.7 ± 2.0	15.3 ± 1.6
ProSobee®	3.4 ± 1.6	7.2 ± 1.8	5.0 ± 2.0	9.1 ± 2.0
Nursoy	NA	NA	b	b
Gerber soy	NA	NA	12.7 ± 2.5	>18
Protein hydrolyzate formulas				
Nutramigen®	b	b	b	b
Special formulas				
Carnation Followup	NA	NA	10.7 ± 2.0	16.0 ± 1.9
Next Step	NA	NA	12.0 ± 1.9	16.7 ± 1.9
Lactofree	NA	NA	10.0 ± 2.5	15.3 ± 1.9
Progestimil	NA	NA	b	b
Lofenalac	NA	NA	a	a
MSUD	b	b	b	b
Phenylfree	6.5 ± 1.2	14.7 ± 1.5	8.3 ± 1.1	16.7 ± 1.6
Experimental formulas				
3200AB	a	a	a	a
3200K	NA	NA	17.3 ± 1.6	>18
3232A	a	a	a	a
80056	b	b	b	b

a=No decalcification. b=Generalized surface decalcification only. NA=Not assessed.

Buffer capacity of infant formulas

Each solution was randomly tested in triplicate in two assays. Initially, 3 mL of each infant formula, water control, or sucrose control were mixed with 3 mL of 0.001M HCl for 5 min.[16] The resulting pH of each sample was measured twice using separate glass microelectrodes cross calibrated and standardized each day with standard buffer solutions of pH 7.0 and 4.0. To further test the buffer capacity, 0.01 M HCl was added to 5 mL of each solution and the number of moles required to drop the pH two pH units was recorded.

In vitro caries progression after exposure of primary incisors and permanent molars to infant formula

Extracted, caries-free, restoration-free permanent molars were used to provide enamel supported by dentin. The mesial and distal enamel surfaces were thinned to a width of 1 mm, parallel to the DEJ, using a separating disk in a slow-speed handpiece. The enamel thickness was verified radiographically and the enamel polished with medium and fine Soflex Disks (3M Corp.). In addition, exfoliated caries-free, restoration-free primary incisor crowns were also used. Each crown, molar or incisor, was embedded into a block of acrylic resin for stability.

A circular piece of masking tape (2.5 mm diameter) was fixed to the mesial and distal surface of each tooth and the remaining portion of the tooth was covered with nail varnish. After the varnish has dried, the masking tape was removed to leave two exposed enamel surfaces of 0.049 cm^2 each. The mounted coronal structures were washed, numbered and randomly assigned to each solution. Each solutions tested (see Table 2) was incubated with three mounted crowns, yielding a total of six enamel windows per infant formula.

TABLE 3. INFANT PLAQUE: LOWEST PH (MEAN ± SD) RECORDED IN THE 1-H PERIOD

Formulas	*Caucasian*	*African American*	*Native American*
Water	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Sucrose	5.3 ± 0.2*	5.3 ± 0.2*	5.3 ± 0.2*
Enfamil® with iron	5.7 ± 0.3*	5.7 ± 0.2*	5.7 ± 0.2*
Enfamil® low iron	5.8 ± 0.3*	5.9 ± 0.2*	5.8 ± 0.2*
ProSobee® (soy)	5.4 ± 0.3*	5.3 ± 0.2*	5.4 ± 0.1*
Nutramigen® (proteinhydrolyzate)	5.3 ± 0.3*	5.7 ± 0.2*	5.3 ± 0.1*

* Significantly different from water ($P < 0.001$).

In vitro enamel colonization was achieved by immersion of the mounted crowns into 2 mL of bacterial suspension (*S. sobrinus* 6715 and *S. mutans* GS5, 1 x 108 cells/mL of each strain) and incubation at 37°C for 18 h. The mounted crowns were then immersed in 2 mL of one of the infant formulas or control solution. Fresh solution was provided daily for 18 weeks. The development of enamel demineralization was assessed by clinical evaluation of the enamel. The progression of dental caries was assess radiographically utilizing Ultraspeed film (Kodak) and a standardized exposure (15mA, 75KVP, 1/5 s, 15 cm cone-film distance).

Statistical Management of the Data

Data were entered and managed by Biostatistics personnel of the University of Minnesota Comprehensive Clinical Research Center. A one-way ANOVA was used to compare pH data. A two-way ANOVA was used to compare the remaining data.

Results

pH changes of dental plaque associated with infant formula

In the study of child patients, the average minimum pH obtained in response to feeding with infant formulas varied from 5.2 to 5.9 (Table 3). These results were not statistically different from those previously obtained with adult plaque.[4] Furthermore, no statistically significant differences were present between the ethnic groups tested. Similar relationships were also obtained for pH at 1 h, pH drop, and pH drop at 1 h (data not shown). In the study of adult volunteers, the average minimum pH obtained following oral rinsing with infant formula varied from 4.4 for Carnation Followup Formula to 6.3 for MJ Experimental Formula 3232A. A One-way ANOVA showed that most solutions, except whole milk, Progestimil, Lofenalac, MSUD, Phenyl-free and #3232A, had the ability to reduce the pH significantly below the pH obtained after rinsing with water (Table 4). Similar relationships were obtained for pH at 1 h, pH drop, and pH drop at 1 h (data not shown).

Bacterial growth in the presence of infant formula

In this study we found that bacterial growth ranged from 39 to greater than 250% of optimal growth (Table 5). Nine of the infant formulas were found to be bacteriostatic, meaning that the formula did not support optimal bacterial growth, whereas eight formulas supported bacterial growth significantly higher than that recorded for Todd Hewitt broth. Interestingly, bovine whole milk was associated with one of the greatest increases in bacterial growth (>250% of optimal growth).

Mineral changes in powdered enamel after incubation with infant formula

The data presented in Table 6 demonstrates that enamel mineral was dissolved by incubation with certain infant formula. Some formulas, including Gerber soy, and the experimental formulas, did not support dissolution of mineral from tooth enamel. Similar to previous studies,[5-7,14] the sucrose control solution did dissolve calcium and phosphate from the powdered enamel, whereas the water control did not.

Buffer capacity of infant formulas

After mixing with an equal volume of 0.001M HCl, the solution pH dropped significantly for fourteen of the infant formulas, with three of these formulas allowing the pH to fall below the critical pH for enamel demineralization.[17] Furthermore, by measuring the number of moles of acid required to reduce the solution pH by two units, we were able to calculate the Buffer Value (B = dx/dpH, where dx is the number of moles of acid required to change the pH). The greater the B (i.e., more acid required to drop the pH) means a solution was more resistant to alterations in pH. Table 7 presents the Buffer Value obtained from these studies. Whole Milk showed the greatest capacity to buffer the addition of acid (B = 21.34 ± 0.59), while eight of the formulas did not buffer well with a Buffer Value below 10.

In vitro Cariogenicity of Infant Formulas

Table 2 presents the average length of time for caries to reach the dentin and pulp as expressed in weeks. For the 11 solutions tested with both modified permanent molars and with primary incisors, there was no significant difference in the caries progression. Therefore, due to their greater availability, the remaining infant formulas were only tested with modified molars. As can be seen the formulas did differ in their in vitro cariogenicity. Similar to water, Lofenalac and 3200AB

TABLE 4. ADULT PLAQUE: LOWEST PH (MEAN ± SD) RECORDED IN THE 1-H PERIOD

Formulas	*Minimal pH*
Control solutions	
Water	6.8 ± 0.2
Sucrose	5.1 ± 0.4*
Whole milk	6.5 ± 0.4
Iron-containing formulas	
Similac® with iron	5.8 ± 0.5*
Enfamil® with iron	5.8 ± 0.4*
Carnation Good Start	5.3 ± 0.2*
SMA with iron	5.8 ± 0.5*
Gerber with iron	5.9 ± 0.4*
Bonamil	5.1 ± 0.7*
Low-iron formulas	
Similac® low iron	5.4 ± 0.5*
Enfamil® low iron	5.9 ± 0.4*
SMA low iron	4.5 ± 0.4*
Gerber low iron	5.1 ± 0.4*
Soy-based formulas	
Isomil®	5.2 ± 0.6*
ProSobee®	5.4 ± 0.3*
Nursoy	6.0 ± 0.3*
Gerber soy	5.4 ± 0.9*
Protein hydrolyzate formulas	
Nutramigen®	5.4 ± 0.3*
Special formulas	
Carnation Followup	4.4 ± 0.4*
Next Step	5.5 ± 0.3*
Lactofree	5.5 ± 0.6*
Progestimil	6.2 ± 0.1
Lofenalac	6.3 ± 0.1
MSUD	6.3 ± 0.2
Phenyl-free	6.3 ± 0.2
Experimental formulas	
3200AB	4.9 ± 0.4*
3200K	5.7 ± 0.3*
3232A	6.3 ± 0.2
80056	5.0 ± 0.8*

* Significantly different from water ($P < 0.001$).

did not show any signs of dental decalcification. Carnation Good Start, Nursoy, Nutramigen, Progestimil, MSUD and 80056 only cause surface decalcification by 18 weeks, with no radiographic evidence of caries detected. In contrast, ProSobee, a soy-based formula, caused in vitro caries at a rate comparable to sucrose.

Discussion

For children to obtain adequate nutrition, nursing must continue intermittently during a 24-h period. However, this extensive exposure to fermentable carbohydrates can lead to the development of the dental decay seen in baby bottle tooth decay, a form of early childhood caries. Providing support for the pivotal nature of the frequency of carbohydrate consumption, Derkson and Ponti[18] reported that children with dental caries suckled 8.3 h/day compared to only 2.2 h/day for children without dental caries.

We have previously reported that in adult volunteers the dental plaque pH drops significantly following rinsing with eight different infant formulas.[4] We now show that while the oral microflora in children may differ from the established flora in adults, similar pH responses were recorded after children (ages 12–24 mo) were exposed to infant formulas. Furthermore, using adult volunteers, we tested most available infant formulas and demonstrate that plaque pH varied in response to oral rinsing with infant formula and most formulas did have the ability to reduce the pH significantly. We also demonstrated that certain formulas supported significant bacterial growth. Thus, these formulas may play an important role in the establishment of cariogenic organisms within the oral cavity, and, once present, may metabolize the carbohydrates available in infant formulas.

While infant formulas may support acid production in dental plaque, buffering systems present within the oral cavity, primarily salivary buffers, are important in controlling the pH of the oral fluids bathing the teeth. In the ECC condition, however, the salivary buffer effect is essentially removed due to the manner in which the infant sucks the nipple, and the reduced saliva flow during periods of sleep. Therefore, the infant formula alone may be the most important buffering system available. In this study, we demonstrate significant differences in the buffer capacity between the infant formulas. Whole milk demonstrated the greatest buffering capacity, which may explain why previous studies have failed to implicate bovine milk in the development of dental caries. In contrast, some infant formulas were unable to significantly buffer acid, which in the oral cavity, may allow the plaque pH to drop more quickly.

While the factors associated with infant formula buffering capacity are unknown, it is possible that specific proteins, such as casein may play a role. Supporting this role is the low Buffer Value calculated for two soy-based formulas, the protein hydrolyzate formula and the experimental formula #80056, all of which are either casein free or have alter casein present. Further research is now necessary to explore the possible relationship between casein and infant formula buffering capabilities.

TABLE 5. BACTERIAL GROWTH IN THE PRESENCE OF INFANT FORMULA

Formulas	*% of Optimal Growth*
Control solutions	
Todd Hewitt Broth	100
Water	53 ± 20*
Sucrose	70 ± 14*
Whole milk	>250†
Iron-containing formulas	
Similac® with iron	59 ± 15*
Enfamil® with iron	158 ± 29†
Carnation Good Start	93 ± 21
SMA with iron	139 ± 1†
Gerber with iron	119 ± 38
Bonamil	45 ± 6*
Low-iron formulas	
Similac® low iron	43 ± 14*
Enfamil® low iron	238 ± 7†
SMA low iron	147 ± 6†
Gerber low iron	128 ± 26
Soy-based formulas	
Isomil®	94 ± 6
ProSobee®	132 ± 29
Nursoy	59 ± 17*
Gerber soy	157 ± 34†
Protein hydrolyzate formulas	
Nutramigen®	73 ± 5*
Special formulas	
Carnation Followup	124 ± 22
Next Step	113 ± 1
Lactofree	68 ± 6*
Progestimil	39 ± 3*
Lofenalac	103 ± 5
MSUD	99 ± 6
Phenyl-free	104 ± 1
Experimental formulas	
3200AB	185 ± 47†
3200K	108 ± 57
3232A	>250†
80056	81 ± 44

* Significantly greater growth than in broth culture ($P < 0.001$).

† Significantly less growth than in broth culture ($P < 0.001$), i.e.: bacteriostatic.

Phosphopeptides from casein have also been suggested to protect against enamel demineralization.[6, 7, 19] Demineralization, caused by a solution or food source in the absence of micro-organisms, is another factor to consider in the development of dental caries. Previous studies.[5–7, 14] have demonstrated that sucrose can dissolve mineral from dental enamel. The results we present in this study are similar to those reported by Jenkins and Furguson[5] who demonstrated that 220 mg/mL of phosphate and 470 mg/mL of calcium dissolved from 50 mg enamel powder when incubated with 6 mL lactose solution. Their results correspond to 26 mg phosphate and 56 mg calcium dissolved/mL powdered enamel. Our results and those of Jenkins and Ferguson show a greater amount of dissolved mineral when compared to those of Rugg-Gunn, et al.,[14] who demonstrated that the incubation with a lactose/saliva combination dissolved 10 mg phosphate and 18 mg calcium/mg powdered enamel. While these results are lower than we report, it should be noted that they used a 1:2 ratio of carbohydrate to saliva in their experiments. Effectively, they incubated their samples with one-third the amount of sucrose. If we express their data on an equivalent amount of carbohydrate to enamel, the data would be similar to what we have demonstrated (e.g. 30 mg phosphate and 54 mg calcium/mg enamel).

While previous studies have suggested that bovine milk, used to manufacture infant formulas, is not cariogenic, we now demonstrate that most infant formulas do support in vitro caries development. These results are similar to those reported using a desalivated rat model.[20, 21] In these previous rat studies, a 2.5-week exposure to infant formulas caused both sulcal and smooth surface caries. However, since the animals were also exposed to sucrose containing lab chow, caution must be exercised when assessing the true cariogenic potential of infant formulas based upon these previous reports. In our in vitro model, no additional carbohydrate sources were available for bacterial fermentation. In addition, our model may better mimic the conditions that develop in the oral cavity of a sleeping child. The reduced salivary flow and the suckle-sleep-suckle cycle may lead to stagnation of the formula. This stagnation may cause an enzymatic breakdown of protective proteins, such as casein.

With the overall caries rates in children decreasing and ECC prevalence remaining stable,[22] ECC has become a major contributor to early caries in the pediatric population. Cost of care per case have been estimated at $700–1200 for the dental treatment and $200–1500 for sedation or anesthetic intervention.[23]

In contrast to chronic caries development in older children and adults, once initiated, ECC progresses rapidly until the crowns of the teeth are severely decayed. Because there are no cellular or vascular elements in the enamel of the teeth, the disease areas are incapable of healing and replacing themselves. Therefore, to reduce the morbidity associated with ECC, we need to identify patients at risk so that we can prevent the onset of disease.

TABLE 6. AMOUNT OF CALCIUM AND PHOSPHATE (MG) DISSOLVED FROM 1 MG POWDERED ENAMEL (MEAN ± SD) FOLLOWING 24 H INCUBATION WITH INFANT FORMULA.

Formulas	*Calcium*	*Phosphate*
Control solutions		
Water	0.0 ± 0.2	0.0 ± 0.1
Sucrose	34.2 ± 3.3*	18.5 ± 1.1*
Whole milk	17.4 ± 1.1*	6.9 ± 1.2*
Iron-containing formulas		
Similac® with iron	43.8 ± 3.9*	21.0 ± 1.9*
Enfamil® with iron	31.2 ± 3.0*	24.3 ± 2.1*
Carnation Good Start	27.5 ± 3.2*	6.2 ± 0.7*
SMA with iron	41.8 ± 4.2*	25.3 ± 2.5*
Gerber with iron	35.6 ± 3.1*	10.8 ± 1.3*
Bonamil	32.3 ± 3.2*	10.3 ± 1.3*
Low-iron formulas		
Similac® low iron	30.6 ± 3.2*	8.8 ± 1.0*
Enfamil® low iron	21.3 ± 2.2*	5.4 ± 0.5*
SMA low iron	23.4 ± 2.2*	10.2 ± 1.1*
Gerber low iron	13.5 ± 1.1*	12.2 ± 1.1*
Soy-based formulas		
Isomil®	48.3 ± 5.4*	33.7 ± 3.3*
ProSobee®	24.8 ± 1.9*	17.0 ± 2.0*
Nursoy	15.2 ± 0.9*	12.8 ± 1.1*
Gerber soy	0.1 ± 0.1	0.9 ± 0.1
Protein hydrolyzate formulas		
Nutramigen®	21.7 ± 1.2*	11.3 ± 1.1*
Special formulas		
Carnation Followup	18.0 ± 1.9*	11.7 ± 0.9*
Next Step	19.8 ± 1.9*	8.0 ± 0.8*
Lactofree	11.7 ± 1.0*	0.1 ± 0.1
Progestimil	21.0 ± 2.1*	6.2 ± 0.8*
Lofenalac	21.7 ± 1.8*	6.9 ± 0.7*
MSUD	18.9 ± 2.1*	8.9 ± 1.1*
Phenyl-free	6.0 ± 0.2*	0.0 ± 0.2
Experimental formulas		
3200AB	0.0 ± 0.2	0.0 ± 0.1
3200K	0.0 ± 0.1	0.0 ± 0.2
3232A	0.0 ± 0.1	0.0 ± 0.1
80056	0.0 ± 0.2	0.0 ± 0.2

* Significantly different from water ($P < 0.001$).

The goal of this research was to investigate the acidogenic and cariogenic properties of infant formulas. The results of these experiments demonstrate the need to educate parents about the association between ECC and infant formula intake. This research may therefore impact the future development of ECC. Furthermore, by reducing the cases of ECC, we may significantly decrease the caries experience in the permanent dentition.[24]

Conclusions

From this study, we conclude that:

1. Plaque pH varied in response to oral rinsing with infant formula, and most infant formulas were able to reduce the pH significantly below the pH obtained after rinsing with water.
2. Some infant formulas supported significant bacterial growth.
3. Enamel mineral was dissolved by incubation with certain infant formula even in the absence of bacterial fermentation.
4. Buffer capacity varied among the infant formulas tested, with some formulas unable to buffer the addition of acid.
5. Most infant formulas were cariogenic in an in-vitro model.
6. Further research is needed to more fully understand the relationship of infant formulas to BBTD.

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TABLE 7. RESPONSE OF INFANT FORMULAS TO ACID INCUBATION

Control solutions		
Water	2.96 ± 0.07	0.03 ± 0.00
Sucrose	2.94 ± 0.06	0.03 ± 0.00
Whole milk	6.57 ± 0.06	21.34 ± 0.59
Iron-containing formulas		
Similac® with iron	6.61 ± 0.05	11.13 ± 0.06
Enfamil® with iron	6.59 ± 0.14	9.96 ± 0.06
Carnation Good Start	6.36 ± 0.01*	12.51 ± 0.25
SMA with iron	6.83 ± 0.03	10.23 ± 0.03
Gerber with iron	6.68 ± 0.07	11.84 ± 0.12
Bonamil	6.60 ± 0.04	13.99 ± 0.10
Low-ron formulas		
Similac® low iron	6.77 ± 0.04	10.87 ± 0.06
Enfamil® low iron	6.56 ± 0.06†	9.85 ± 0.00
SMA low iron	6.88 ± 0.04	9.92 ± 0.29
Gerber low iron	6.59 ± 0.18	10.3 ± 0.00
Soy-based formulas		
Isomil®	6.27 ± 0.02*	13.77 ± 0.03
ProSobee®	6.52 ± 0.13†	8.61 ± 0.30
Nursoy	6.63 ± 0.17	13.89 ± 0.33
Gerber soy	6.76 ± 0.05	9.10 ± 0.09
Protein hydrolyzate formulas		
Nutramigen®	5.17 ± 0.12*	11.06 ± 0.07
Special formulas		
Carnation Followup	6.35 ± 0.09*	13.60 ± 0.03
Next Step	6.64 ± 0.08	13.75 ± 0.20
Lactofree	6.48 ± 0.04†	9.08 ± 0.09
Progestimil	5.95 ± 0.04*	15.92 ± 0.21
Lofenalac	5.72 ± 0.03*	18.19 ± 0.10
MSUD	4.83 ± 0.04*	15.00 ± 0.09
Phenylfree	5.34 ± 0.06*	14.41 ± 0.06
Experimental formula		
3200AB	5.74 ± 0.03*	19.6 ± 0.2
3200K	6.60 ± 0.13	6.60 ± 0.1
3232A	6.56 ± 0.05†	11.65 ± 0.0
80056	6.26 ± 0.06*	7.26 ± 0.03

* Significantly below the initial pH of the solution at the $P < 0.001$ level.
† Significantly below the initial pH of the solution at the $P < 0.01$ level.

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