



Investigation of the role of human breast milk in caries development

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Abstract

Purpose: The objective of this study was to determine the caries-related risk associated with human breast milk (HBM).

Methods: First, the plaque pH of 18 children (12–24 months) was monitored before and after a five-minute feeding with HBM to determine the pH drop and minimum pH obtained. Second, *Streptococcus sobrinus* 6715 was cultured for 3 hr in HBM, and the increase in the number of colony forming units (cfus) and the culture pH was measured. Third, HBM was incubated for 24 hr with powdered enamel to determine the solubility of mineral in the absence of bacteria. Fourth, HBM was mixed with acid to determine the buffering capabilities. Finally, enamel windows were created on extracted premolar crowns (N=33) that were colonized with *Mutans Streptococci* and incubated with HBM. Caries was assessed visually and radiographically for 12 weeks.

Results: One- and two-way ANOVAs of these five assays demonstrated that HBM did not cause a significant drop in plaque pH when compared to rinsing with water; HBM supported moderate bacterial growth; calcium and phosphate were actually deposited onto enamel powder after incubation with HBM; the buffer capacity of HBM was very poor; and HBM alone did not cause enamel decalcification even after 12 weeks exposure. However, when supplemented with 10% sucrose, HBM caused dental caries in 3.2 weeks.

Conclusion: It is concluded that human breast milk is not cariogenic. (*Pediatr Dent* 21:86–90, 1999)

Early childhood caries (ECC) is a serious oral health condition that affects about 6% of children younger than three years of age. Baby bottle tooth decay (BBTD), a manifestation of ECC, is associated with prolonged and frequent daytime, naptime, and nighttime bottle feedings.^{1,2} Recently, we have reported that most infant formulas are acidogenic^{3,4} and do support in vitro caries development.⁴

Prolonged and excessive breastfeeding also has been suspected as a causative factor in ECC.^{5–7} However, controversy exists regarding the cariogenicity of HBM. HBM has a higher carbohydrate content and lower calcium, phosphorus, and protein levels than bovine milk, thus making it potentially more cariogenic. Furthermore, the oral microflora of breast-fed children is no different from bottle-fed children.^{2,8} In contrast, studies examining the incidence of dental caries in primitive cultures—where on-demand breastfeeding, includ-

ing at will night time nursing, is common⁹ and children are not usually weaned until between 18 and 36 months—have reported extremely low rates of caries among children.^{10,11} These investigators reported caries incidences of 1.2% and 0.5% among children of Eskimo and Samoan cultures, respectively. Other studies have suggested that HBM is less cariogenic than glucose and sucrose.^{12,13} Therefore, this study sought to determine the acidogenic and cariogenic potential of HBM.

Methods

Breast milk donations

HBM donations were collected after informed consent was obtained following the guidelines of the University of Minnesota Human Subjects Committee. Mature breast milk was collected by mothers who were still nursing a child (children's ages ranged from 12 weeks to 2 years). The milk samples were collected into sterile plastic beakers after gentle manual expression of breast tissue. When necessary, the sample was then transferred to sterile tubes for further transport. All samples were stored on ice until used. Sterile distilled water and sterile 10% sucrose were used as control solutions.

Dental plaque pH changes after infant breastfeeding

Children (N=18) between the ages of 12 and 24 months who were still breast feeding were used in this study. 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 were normal, caries-free children in good general and oral health with no dental anomalies.

All sampling was performed between 10:00 AM and 10:30 AM. Parents were asked to abstain from oral hygiene for their child for 24 hr and to avoid giving their child any foods, except water, for 2 hr prior to sampling. Supragingival plaque was sampled from maxillary buccal surfaces. Alternating surfaces were sampled prior to feeding to provide the prein plaque control. The remaining sites were sampled after one min of suckling or feeding with control solution. No site was sampled twice. Plaque was then dispersed in 50 μ l deionized water and the pH monitored for one hr as previously described.³ The positive control in this study was plaque collected

Table 1. Caries-Related Variables of HBM Relative to Bovine Milk and Control Solutions

Variable	HBM*	Bovine Milk**	Sucrose*	Water*
Plaque pH changes				
Minimum pH	6.4±0.1	6.5±0.4	5.3±0.3†	6.7 ± 0.1
pH at one hour	6.5±0.1	6.7±0.1	5.8±0.2†	6.8 ± 0.1
pH drop	0.6±0.1	0.5±0.2	1.4±0.2†	0.3 ± 0.1
pH drop at 1 h	0.4±0.2	0.4±0.1	1.2±0.2†	0.3 ± 0.1
Bacterial fermentation				
% optimal growth	84±4†	>250†	70±14	53 ± 20
Culture pH	6.4±0.1	6.6±0.1†	5.7±0.2†	6.2 ± 0.1
Mineral Changes				
Calcium	↑22±3 mg/mg enamel†	↓17±1†	↓34±4 mg/mg enamel†	0
Phosphorus	↑14±2 mg/mg enamel†	↓7±1†	↓18±2 mg/mg enamel†	0
Buffer Capacity				
Initial pH	7.2±0.1	6.8±0.1	7.0±0.1	7.1 ± 0.1
B Value	0.43±0.16†	21.3±0.6†	0.03±0.00	0.03 ± 0.00

* Data presented as mean ± SD. ** Data previously reported.⁴ †Significantly different from water ($P<0.001$).

after 10% sucrose consumption. The negative control was plaque collected after water consumption.

Four pH measurements were evaluated:

1. Minimum pH, defined as the lowest pH recorded in the one-hr period, was recorded because the hydrogen ion production potential of food items has been related to the food's cariogenic potential
2. pH at one hr, defined as the postrinse 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 prerinse plaque pH and the minimum plaque pH obtained, was calculated to account for the initial plaque pH of each subject
4. pH drop at one hr, defined as the difference between the prerinse plaque pH recorded at 60 min and the postrinse 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 HBM

Samples of breast milk obtained from donors (N=7), control solution, or Todd Hewitt Broth (4 mL) were inoculated in triplicate with a highly cariogenic and extensively studied strain of *Mutans Streptococci* (*S. sobrinus* 6715, 10⁸ cells). After incubation (37°C, 5% CO₂, 3 hr) 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% CO₂, 3 days), and assayed for the number of cfus present on each plate. The bacterial growth was assessed by comparing the number of cfus, which grew after culture in HBM with the optimal growth seen after culture in Todd Hewitt broth. In addition, the pH changes associated with bacterial fermentation were then assessed by comparing the pH of each sample after 3 hr of culture with the pH prior to culturing with bacteria.

Mineral dissolution after incubation with HBM

Caries- 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 eight deciduous teeth was ground to a fine powder by use of a mortar and pestal and pooled for these experiments. Fifty milligrams of powdered enamel (60–100 in⁻¹, mesh) was then mixed with 1 mL of breast milk obtained from donors (N=12) or control solutions.^{12–15} Duplicates were prepared excluding the powdered enamel. All mixtures were incubated at 37°C for 24 hr 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 hr and then ashed at 650°C for 18 hr. 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 (Ca) was estimated in the presence of lanthanum using atomic absorption spectroscopy. Phosphorus (P) was estimated by the ammonium-molybdate method.¹⁶ 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.

Buffer capacity of HBM

To test the buffer capacity, 0.01 M HCl was added to 5 mL HBM obtained from donors (N=16), water control, or sucrose control. Each solution was assayed in duplicate. The number of moles required to drop the pH two pH units was then determined using two separate glass microelectrodes cross-calibrated and standardized each day with standard buffer solutions of pH 7.0 and 4.0.

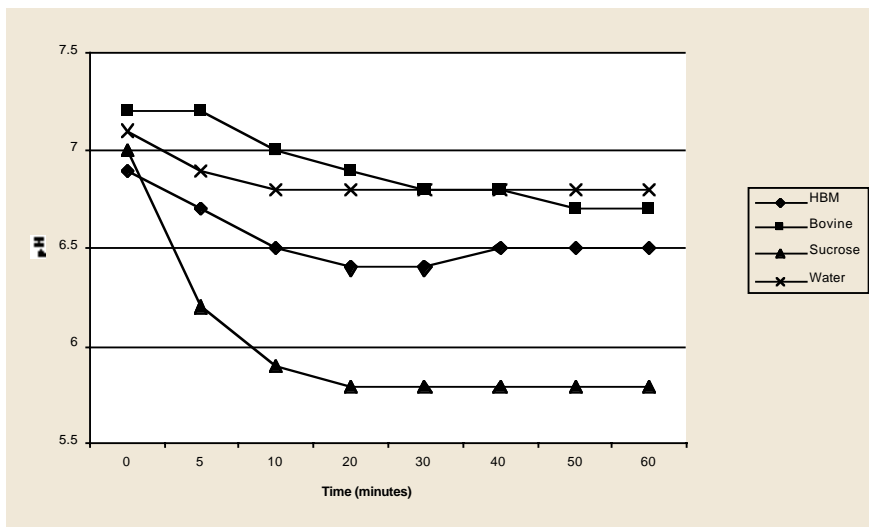


Fig 1. Acidogenic profile of human breast milk, bovine milk, sucrose, and water.

In vitro caries progression after exposure of premolars to HBM

Extracted caries- and restoration-free premolars ($N=33$) 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, St. Paul, MN). A circular piece of 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 had dried, the masking tape was removed to leave two exposed enamel surfaces of 0.049 cm² each.

In vitro enamel colonization was achieved by immersing of the mounted crowns in 1.5 mL of bacterial suspension (*S. sobrinus* 6715 and *S. mutans* GS5, 1×10^8 cells/mL of each strain), and incubating at 37°C for 18 hr and verifying weekly by bacterial culture. The mounted crowns were then immersed in 1.5 mL of test solution provided fresh daily for 12 weeks. The development of enamel demineralization was assessed by clinical evaluation of the enamel. The progression of dental caries was assessed radiographically utilizing Ultraspeed film (Eastman Kodak, Rochester, NY) and a standardized exposure (15 mA, 75 KVP, 1/5 seconds, 15 cm cone-film distance).

The 11 different samples tested were 10% sucrose, distilled water, HBM (six donors), and HBM (three donors) supplemented with 10% sucrose. Each sample tested was incubated with three mounted crowns, yielding a total of six enamel windows per test sample.

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 HBM

The average minimum pH obtained in response to feeding with HBM was 6.37 ± 0.12 (Table 1, Figure 1). The average minimum pH obtained for the control water and sucrose solutions were 6.67 and 5.29, respectively, which was not statistically different from those previously obtained in our studies of infant formulas.⁴ Similar results were also obtained for pH at one hr pH drop, and pH drop at one hr (Table 1, Figure 1).

Bacterial growth in the presence of HBM

In this study, we found that bacterial growth in the presence of HBM averaged 84% of optimal growth (Table 1).

Furthermore, the culture pH dropped from 7.11 to 6.42 after 3 hr of culturing. Supplemental studies showed that the length of time between expression of breast milk and initiation of culture had no statistically significant impact on bacterial growth (data not shown).

Mineral changes in powdered enamel after incubation with HBM

The data presented in Table 1 demonstrates that calcium and phosphorus were actually deposited onto powdered tooth enamel after incubation with HBM. Similar to previous studies,^{3, 12-15} the sucrose control solution did dissolve calcium and phosphate from the powdered enamel, whereas the water control did not. Supplemental studies showed that the length of time between expression of breast milk and incubation with powdered enamel had no statistically significant impact on dissolution of mineral (data not shown).

Buffer capacity of HBM

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.,

Table 2. In-vitro Caries Progression of HBM, HBM Supplemented with Sucrose, Bovine Milk, and Control Solutions

Sample	Weeks to Dentin*
Control solutions	
Water	ND**
Sucrose	4.0 ± 0.5
Bovine Milk†	$14.0 \pm 2.0^\ddagger$
HBM	
Unsupplemented (N=7)	ND**
Supplemented with Sucrose (N=3)	$3.2 \pm 0.4^\ddagger$

* Data presented as mean \pm SD. ** ND=No enamel decalcification present at any time during the study. † Data previously reported.³ ‡ significantly different from sucrose ($P < 0.001$).

more acid required to drop the pH) means a solution was more resistant to alterations in pH. Table 1 presents the Buffer Value obtained from these studies. We have found that HBM allows the solution pH to drop rapidly with the addition of acid. Supplemental studies showed that the length of time between expression of breast milk and acid incubation had no statistically significant impact on the measurement of buffer capacity (data not shown).

In vitro cariogenicity of HBM

Table 2 presents the average length of time for caries to reach the dentin as expressed in weeks. Similar to our previous studies with infant formula,⁴ water did not show any signs of dental decalcification. Also similar to our previous studies, sucrose caused rapid *in vitro* caries development. In contrast, HBM, when used as the only carbohydrate source, did not cause enamel decalcification, even after 12 weeks of exposure. Interestingly, when HBM was supplemented with 10% sucrose, the caries development was actually more rapid than sucrose, with dental caries identified at 3.2 weeks. Supplemental studies showed that the length of time between expression of breast milk and initiation of culture had no statistically significant impact on caries progression (data not shown).

Discussion

The buffering systems present within the oral cavity, primarily salivary buffers, are important in controlling the pH of the oral fluids bathing the teeth.¹ 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.¹ Therefore, the source of liquid nutrition during periods of nursing and sleep may be the most important buffering system available.

The pH of a buffer system is based on the equilibrium between the bound hydrogen ions and free hydrogen ions. To express the buffering capacity of solutions, VanSlyke introduced Buffer Value, or β^{17} , in 1922. Buffer Value is the reciprocal of the slope of the pH neutralization curve, and is useful to demonstrate the rapidity at which the pH of a solution will drop with the addition of acid.

When one thinks about the buffering of body fluids, the system most widely understood is the circulatory system. The buffers present within the blood are important in closely maintaining a pH of around 7.3. The main buffers within the circulation are inorganic systems such as phosphate, carbon dioxide, and bicarbonate. However, the most important control of pH within the circulation comes from the hemoglobin-oxyhemoglobin equilibrium and its effects on carbon dioxide transport.¹⁸

Other living tissues and secretions are buffered less closely, with normal fluctuations seen in the pH range of 4.0 to 7.0.¹⁹ The main buffers present in these other body secretions are phosphates, carbonates, and amino acids. The main sources of free hydrogen ions to create pH fluctuation within bodily fluids are the organic acids such as citric, ascorbic, folic, and malic acids.

An assessment of the composition of HBM is important to understand the reduced buffer capacity in comparison to bovine milk.²⁰ HBM has significantly less phosphate (15 mg/dL), especially inorganic phosphate (5 mg/dL), when com-

pared to bovine milk (100 mg/dL total phosphate, 75 mg/dL inorganic phosphate). HBM also has less protein with approximately one-fifth the amount of amino acids when compared to bovine milk. Of particular importance is the concentration of histidine with the highly buffering imidazole ring. The concentration of histidine in HBM (23 mg/dL) is significantly less than that present in bovine milk (110 mg/dL). In contrast, the concentration of organic acids is two to three times higher in HBM compared to bovine milk. It is likely that the phosphate and protein present within HBM is capable of buffering the free hydrogen ions associated with these organic acids and thereby maintaining the pH near neutral when unchallenged by other acid sources. However, when additional acid is present, the buffering capacity is exceeded.

Several studies have reported the occurrence of rampant caries in breastfed children.^{2, 21, 22} However, none of these studies has reported the composition of the remainder of the children's diet. Based upon information in this study, it is likely that nursing caries may not arise solely from breastfeeding. This research demonstrated that HBM alone does not cause enamel decalcification in our *in vitro* model. However, we also demonstrated that HBM does not buffer the addition of acid, and when HBM was supplemented with sucrose the rate of *in vitro* caries development was faster than that of sucrose alone. Therefore, we conclude that HBM alone is not a cariogenic food source. However, if a child is given a sugar-rich food and then allowed unlimited breastfeeding, HBM in combination with these other carbohydrates is highly cariogenic.

Two factors associated with nursing may explain the clinical appearance of dental caries associated with the consumption of HBM. In the nursing condition, the lips become pressed against the teeth, thus restricting the salivary flow to rinse other carbohydrates from the area. Furthermore, with the low buffering capacity of HBM, the acidogenic environment associated with bacterial fermentation of the other carbohydrates may be maintained in the oral cavity for long periods of time.

The goal of this study was to investigate the acidogenic and cariogenic properties of HBM. The results of these experiments demonstrate the need to educate parents about the association between ECC and breastfeeding so that parents can properly care for their child's teeth to prevent the development of ECC.

Conclusions

From this study, we conclude that:

1. HBM does not cause a significant pH drop in plaque.
2. HBM supports moderate bacterial growth.
3. Calcium and phosphorus are actually deposited onto enamel powder after incubation with HBM.
4. The buffer capacity of HBM is very poor.
5. HBM is not cariogenic in an *in vitro* model, unless another carbohydrate source is available for bacterial fermentation.

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ABSTRACT OF THE SCIENTIFIC LITERATURE



MEDICAL NECESSITY OF DENTAL SEDATION AND GENERAL ANESTHESIA

Who should determine the medical necessity of dental sedation and general anesthesia? A clinical commentary supported by Illinois patient and practitioner surveys.

Providing patient care under sedation and general anesthesia is common, but more third party payers are denying claims based on the lack of medical necessity. While to practitioners it may be obvious why certain services need to be provided using pain and anxiety management techniques such as conscious sedation and general anesthesia but in the managed care arena these services are seen as elective. Elective therapies are often regarded as not necessary. The purpose of this paper was to present the results of surveys polling patients, practitioners and third party payers about this issue.

The results of the survey reveal that there is very little agreement as to the definition of medically necessary. Third party payers did not have a standardized definition but generally relied on descriptions of the patient's medical condition or the procedure to be accomplished. Often the definition was what ever the medical director decided it was. The responses indicated that the third party payers were denying claims based on cost containment rather than on actual need. Practitioners surveyed agreed that the most common reason given for claim denial was that the sedation or use of general anesthesia was not medically necessary and that the problem of claim denial was increasing. Also practitioners had the perception that the third party payer's consultant or medical director did not always have the knowledge to determine when a sedation or general anesthetic was appropriate. Practitioners also agreed that one of the most important factors influencing patients seeking dental services negatively is fear or apprehension. The patient surveys showed that claims for sedation and general anesthesia were denied because these were not covered services under the patients policy; and this generally because these services are not "medically necessary." A large number of patients responded that fear and apprehension caused them to avoid necessary care. Patients agreed that sedation and general anesthesia services should be covered benefits in their health care policies.

The authors go on to state that dental sedation or general anesthesia should be considered necessary if the patient and the provider agree that it is necessary for any particular patient and that third party payers need to adopt a standard definition that reflects this. They also suggest that the dental profession adopt standard guidelines of the definition of medical necessity. They propose that a working definition include three elements: (1) the patient's needs or desires, (2) a recommendation by the patient's physician (dentist or oral surgeon) that a particular procedure or technique is necessary for the patient's condition, (3) the treatment should be scientifically proven to be safe, effective, and legitimate for the patient's condition. **MGP**

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