Scientific Article

IN VITRO

Cariogenicity of Different Commercially Available Bovine Milk Types in a Biofilm Caries Model

Rodrigo A. Giacaman, DDS, PhD[1] • Cecilia Muñoz-Sandoval, DDS[2]

Abstract: ***Purpose:*** *This study's purpose was to assess the cariogenicity of commercial bovine milk types in an experimental biofilm/caries model.* ***Methods:*** *Enamel and dentin slabs were used to grow biofilms of Streptococcus mutans UA159. Slabs/biofilms were exposed three times per day to commercial skim, semi-skim, whole, whole lactose-free, and whole with 10 percent sucrose-added bovine milk and to 10 percent sucrose and 0.9 percent sodium chloride as positive and negative caries-control, respectively. Biofilms were analyzed for bacterial counts, biomass, proteins, and polysaccharide production. Slab's demineralization was assessed by loss of surface microhardness and the biofilm acidogenicity by medium pH.* ***Results:*** *Only whole and whole lactose-free milk kept pH above the demineralization threshold, inducing the lowest demineralization in both enamel and dentin (P<.05). Skim and semi-skim milk induced similar demineralization to the sucrose control, albeit slightly lower for semi-skim milk (P<.05). Whole and whole lactose-free milk produced lower biomass and less insoluble polysaccharides than the other treatments in enamel and dentin (P<.05). Adding 10 percent sucrose to whole milk turned it as cariogenic as 10 percent sucrose solution.* ***Conclusion:*** *Bovine whole milk seemed less cariogenic than sucrose and the other commercial milk types, but not anticariogenic. Fat content in milk seemed to reduce cariogenicity of the fluid.* *(Pediatr Dent 2014;36:E1-E6) Received February 18, 2013 | Last Revision May 16, 2013 | Accepted May 16, 2013*

KEYWORDS: MILK, DENTAL CARIES, DIET, CARIOGENIC, STREPTOCOCCUS MUTANS

Bovine milk is one of the most consumed food products by humans. Hence, milk's cariogenicity results are relevant and, yet, unclear. Whole bovine milk has been typically considered as healthy and caries-protective. In fact, epidemiologic studies have stated that bovine milk consumption by children is associated with lower caries experience.[1,2] This protective effect would come from anticariogenic properties of the fluid. Thus, many components in bovine milk have been reported to have anticaries properties, including calcium and phosphates,[3] fats,[4] vitamins, iron, fluoride, iodide, and some enzymes.[5,6] Likewise, the milk proteins casein and albumin have been reported to have an antibacterial effect.[7,8] Furthermore, these proteins lead to an increase in calcium and phosphate within the oral biofilm.[9] Besides casein, lactoferrin, lysozymes, and antibodies present in milk have an antibacterial effect against *Streptococcus mutans.*[10]

Despite the putative anticariogenic effect derived from some of the milk's components, bovine milk contains the disaccharide lactose. A potentially cariogenic effect of milk, due to its lactose content, has been reported.[5,11] Results from the reported evidence are conflicting, nevertheless. While a potential role in caries onset has been shown for milk and lactose,[12] other studies have indicated that lactose is less cariogenic than sucrose.[6]

In that regard, our group recently reported an in vitro study showing that bovine milk does not possess an anticaries effect, as it had cariogenicity levels comparable to those from lactose alone.[13] Yet, when compared with sucrose, whole bovine milk had reduced cariogenicity in biofilms formed on both enamel and dentin.[13]

[1]*Dr. Giacaman is an associate professor and director of the Cariology Unit, Department of Oral Rehabilitation; and* [2]*Dr. Muñoz-Sandoval is a clinical instructor, School of Dentistry, and student of the Specialization Program in Pediatric Dentistry, University of Talca, Talca, Chile.*

Correspond with Dr. Giacaman at giacaman@utalca.cl

Given the complexity of milk's composition, it is possible to speculate that variations in some of its components could affect its cariogenicity. There are several bovine milk types commercially available, including whole, skim, semi-skim, lactose-free, and sugar-containing milk. In spite of the large variety of existing milk types, little scientific evidence is available about the caries effect of each type or how changes in milk's composition affect cariogenicity. It is reasonable to think that a lactose-free or reduced lactose formulation could be less cariogenic than whole milk, as the latter contains all the sugars from milk. Moreover, variations in the level of fat could also affect milk's cariogenicity, as an anticaries effect has been attributed to fat and fatty acids.[14,15] Likewise, an antibacterial effect has been attributed to fatty acids,[16,17] but the effect of fat at different concentrations in milk is mostly unknown.

The purpose of this study, therefore, was to compare the cariogenicity of different commercially available bovine milks in an artificial caries model and assess their effect on a biofilm of *Streptococcus mutans*. Since these products are highly consumed by the population, especially children, the results of this investigation may be of high interest to the dental profession as well as the public.

Methods

Experimental design. Biofilms of *Streptococcus mutans* UA159 were grown on bovine slabs of enamel for five days and on bovine slabs of dentin for four days, following a validated protocol.[18] Initial Knoop surface microhardness (**SH**) was assessed, and the slabs were randomly assigned to one of the treatment groups of commercial bovine milk: skim (Colún, La Unión, Chile); semi-skim (Colún); lactose-free whole (Loncoleche, Santiago, Chile); whole (Colún); and whole with 10 percent sucrose added (Colún). Total fat content for the different milk types was: skim 0.05 percent; semi-skim 1.5 percent; lactose-

free whole and whole 3.0 percent; and 10 percent sucrose-added whole 3.1 percent. A group with 10 percent sucrose only and another with 0.9 percent sodium chloride (**NaCl**) were used as positive and negative caries controls, respectively. To simulate a usual ingestion pattern of milk in children, treatments were applied three times per day (at 8:30 a.m., 12:30 p.m., and 4:30 p.m.) for five minutes on each occasion, washed with 0.9 percent NaCl, and relocated to a medium-containing well. Culture medium was changed twice a day, before the first treatment (at 8 p.m.) and after the last treatment (at 5 p.m.)

After the experimental phase, biofilms were separated from the slabs and analyzed for viable bacteria, dry weight (biomass), soluble proteins, and extra and intracellular polysaccharide production. SH on the slabs was determined again and compared with the initial assessment to obtain surface microhardness loss as percentage (**%SHL**). Acidogenicity from the biofilm was assessed by pH measurements of the culture medium twice per day. The entire experiment was performed twice, with each condition in triplicate.

Slabs preparation. Bovine incisors were stored and disinfected with sodium hypochlorite. Enamel and dentin slabs (4 x 7 x 1 mm) were obtained with diamond disks, a low-speed handpiece, and Soflex polishing disks (3M, St. Paul, Minn., USA). Initial SH was determined by three Knoop indentations 100 µm apart from each other (402 MVD, Wolpert Wilson Instruments, Norwood, Mass., USA) at 50 g for five seconds. To maintain similar initial SH across the slabs, only those with SH 361.56±11.17 kg/mm^2 (N=21) for enamel and 58.04± 6.24 kg/mm^2 (N=21) for dentin were included in the study. Slabs were sterilized with ethylene oxide,[19] covered with ultrafiltered (0.22 µm) pooled human saliva, and treated for 30 minutes to allow the formation of an acquired pellicle on the enamel for bacterial initial adherence.[20] Slabs were suspended into the wells of a 24-well plate by means of orthodontic wire.

***S mutans* biofilms and treatments.** Colonies of *S mutans* UA159 from frozen stocks were reactivated, transferred to BHI broth (Merck, Darmstadt, Germany) supplemented with one percent glucose, and incubated at 37 degrees Celsius and 10 percent carbon dioxide. Culture optical density (OD) was adjusted at 0.8 (600 nm) after 18 hours of incubation. An aliquot of 100 ml was transferred to 50 mL of one percent sucrose supplemented homogenized BHI broth, and two mL of the inoculated medium was transferred to each well of a 24-well plate (Corning Costar, Lowell, Mass., USA). Saliva-coated enamel and dentin slabs[20] were placed in the wells to form the biofilms at 37 degrees Celsius and 10 percent CO_2 for eight hours. After the initial biofilm formation, slabs were transferred to wells containing 0.1 mM glucose (basal concentration in saliva) until completion of 24 hours of growth, after which time the aforementioned treatments were applied.

Acidogenicity of the biofilms exposed to different milk types. To assess acidogenicity, medium pH was measured inside each well through a microelectrode (HI 1083B, Hanna Instruments, Woonsocket, R.I., USA) coupled to a portable digital pH-meter (HI 9126-02, Hanna instruments). Measurements were carried out twice per day before the first and the last medium change, in triplicate and for the entire duration of the experiment.

Enamel and dentin demineralization. The duration of the experimental phase was five days for enamel and four days for dentin. Dentin has a lower mineral content than enamel, making it more prone to acid dissolution. For that reason, the length of the experiment was shortened by 24 hours for dentin. A longer exposure time for dentin would make micro-hardness assessment impossible. Slabs/biofilms were washed three times with 0.9 percent NaCl and transferred to 1.5 mL Eppendorf tubes containing one mL 0.9 percent NaCl. Slabs were vortexed for 30 seconds to separate biofilms from the dental substrate. The resulting biofilm suspension was kept for further biofilm analysis. Final SH of the slabs was measured to estimate demineralization produced throughout the experimental period. Loss of SH has been extensively used as a reliable methodology to evaluate demineralization.[21] A new set of three SH readings, taken after the experiment, was obtained by three indentations separated by 100 µm from each other and from the initial measurements. Mean values from the initial and final SH were used to obtain the %SHL, calculated as:

(initial SH - final SH) x 100 ÷ initial SH

Biofilm analysis and characterization. Biofilm suspension obtained by separation from the slabs was assessed for the following characteristics:

1. Biomass (dry weight): A 200 µL aliquot of the suspension was transferred to a preweighed 1.5 mL tube. Each suspension was incubated with 100 percent ethanol at -20 degrees Celsius for 15 minutes, centrifuged (10 minutes at 5,000 g and 4 degrees Celsius), and the resulting pellet was washed with 500 µL of

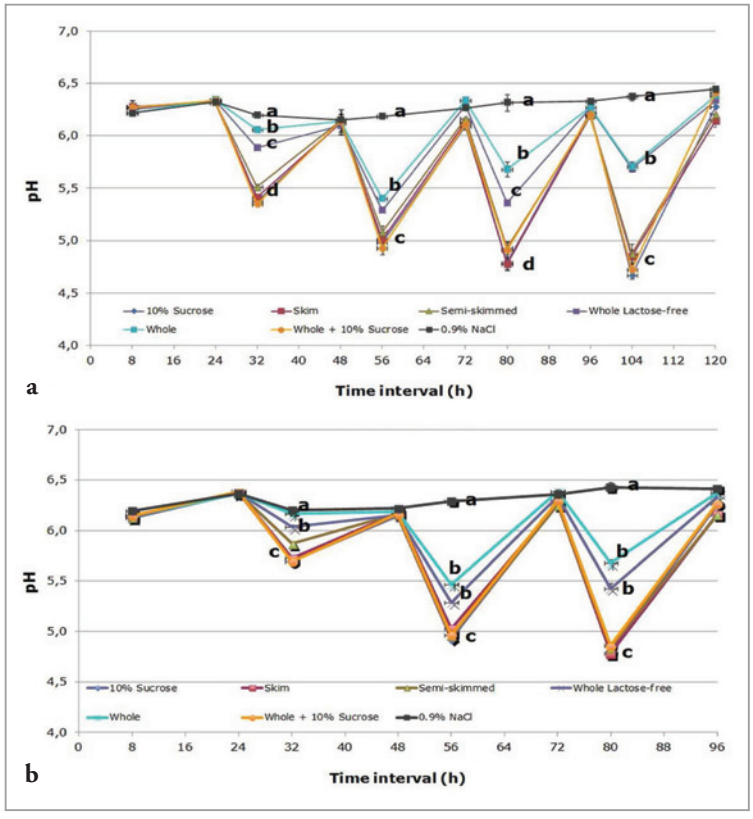

Figure 1. Medium pH of biofilms formed on (a) enamel and (b) dentin, according to treatment and time of biofilm formation. Medium pH was measured directly in the culture medium twice per day after 24 hours of biofilm growth, at indicated time points. Each point in the plot represents the mean (±SD) pH of two independent experiments in triplicate wells. Different letters represent statistically significant differences among treatments (P<.05).

75 percent ethanol. After a second centrifugation, the pellet was dried for 24 hours in a desiccator. Dry weight was calculated by subtracting the final to the initial weight of the empty tube and expressed as mg per mL of biofilm. Dry weight is used as an estimate of the biofilm biomass content.[20]

2. Bacterial counts: Serial dilutions of the biofilm suspension in 0.09 percent NaCl (v/v) were drop-plated on brain heart infusion agar plates (BHI; Merck, Darmstadt, Germany)[22] in duplicate and incubated anaerobically for 24 hours at 37 degrees Celsius; colonies were counted from the dilution that allowed better visualization of isolated colonies. Counting was corrected by the dilution factor and expressed as CFU/mg of biofilm dry weight.[23]
3. Total soluble proteins contained in the biofilm. A 50 µL aliquot of the biofilm suspension was treated with two M sodium hydroxide (NaOH) and incubated at 100 degrees Celsius for 15 minutes. The suspension was centrifuged (10,000 g for 10 minutes at four degrees Celsius), and the supernatant was used to obtain soluble proteins according to Bradford's method.[24]
4. Intra- and extracellular polysaccharides. Three different types of polysaccharides produced by the biofilm of *S mutans* were evaluated: soluble (SEPS) and insoluble (IEPS) extracellular and intracellular (**IPS**) polysaccharides, as previously described.[23] In brief: 200 µL of the biofilm suspension was centrifuged (10 kg for five minutes at four degrees Celsius) to obtain SEPS from the supernatant. For IEPS, the pellet obtained from the previous step was treated with 200 µL of one M NaOH, homogenized, centrifuged, and stored to extract the polysaccharides from the supernatant.[25] Finally, the pellet from the previous step containing the IPS was incubated with 200 µl 1M NaOH for 15 minutes at 100 degrees Celsius and centrifuged (10 kg for five minutes at four degrees Celsius). Supernatant was used to measure the concentration of IPS. The three supernatants from each extraction step were separately treated with three volumes of cold 100 percent ethanol and incubated for 30 minutes at minus 20 degrees Celsius. Samples were immediately centrifuged, and the resulting pellet was washed with cold 70 percent ethanol and centrifuged again (10 kg for five minutes at four degrees Celsius). The resulting pellet of each fraction was resuspended in 1M NaOH, and total carbohydrate concentration was estimated by the sulfuric phenol method.[26] Results were normalized by biofilm dry weight and expressed as percentage of polysaccharides by mg of biofilm biomass.

Statistical analysis. After verifying normal distribution of the data by Kolmogorov-Smirnov test, mean values for each dependent variable were compared using the analysis of variance test and a post-hoc contrast for each pair of variables (Bonferroni correction). SPSS 15.0 software (SPSS Inc, Chicago, Ill., USA) was used to analyze the data with a confidence level of 95 percent.

Results

When acidogenicity of the biofilm formed on both dental substrates was assessed by medium pH measurements, all the milk types induced a pH drop below 5.5 up to 56 hours. At 80 hours, however, only whole milk kept the pH above 5.5 ($P<.05$), but at 104 hours, whole and lactose-free showed pH values above 5.5 ($P<.05$). As expected, sucrose (caries-control) showed low pH at all time points, and 0.9 percent NaCl (caries-negative control) did not induce a pH drop throughout the experiments (Figure 1a-b).

Enamel and dentin demineralization was higher ($P<.05$) when *S mutans* biofilms were treated with sucrose control or whole milk with sucrose added (Figure 2a-b). Conversely, the lowest demineralization ($P<.05$) was observed on biofilm/slabs treated with whole and whole lactose-free milk. Interestingly, skim and semi-skim milk showed a similar demineralization pattern ($P>.05$), with skim milk inducing similar demineralization as 10 percent sucrose ($P>.05$).

Biofilms formed on enamel and dentin were recovered from the slabs to analyze their properties, which are depicted in Tables 1 and 2, respectively. Whole and whole lactose-free milk induced lower biofilm biomass and IEPS than the rest of the treatments in enamel and dentin. Both skim and semi-skim milk exposure to the biofilms induced similar amounts of biomass and IEPS as the sucrose caries-positive control, without any differences between them ($P>.05$). Regarding IPS, only whole and whole lactose-free milk showed significantly lower values than the rest of the treatment groups. When milk was supplemented with 10 percent sucrose, biomass, IEPS and IPS formation were similar to those induced by sucrose alone. For the other biofilm properties—SEPS, viable micro-organisms, and proteins—no differences were detected in either dental substrate ($P>.05$).

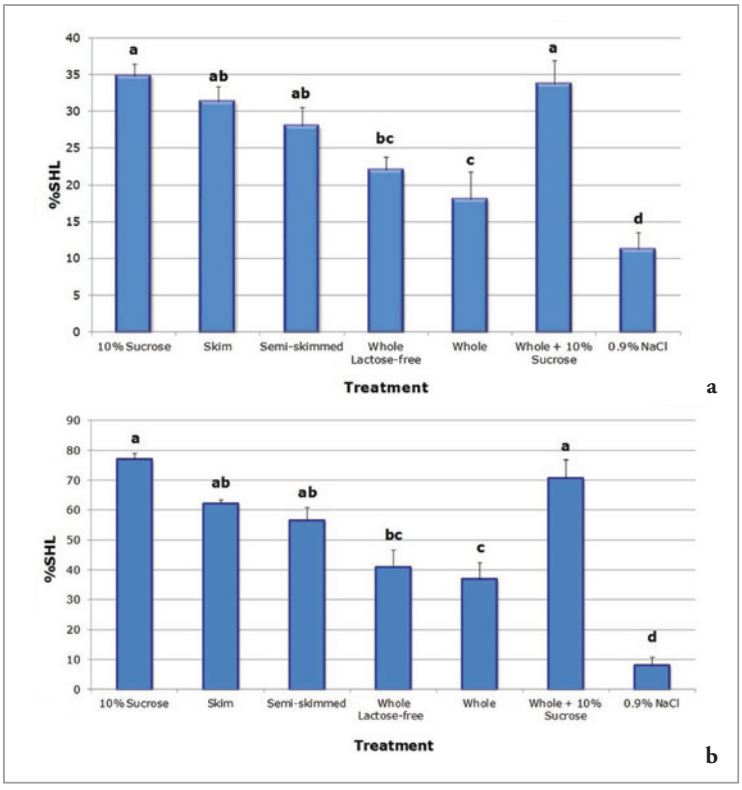

Figure 2. (a) Enamel demineralization and (b) dentin demineralization. Bars depict percentage of surface Knoop microhardness loss (%SHL), according to the various treatments, as indicated (n=6). Error bars show standard deviation. Different letters represent statistically significant differences among treatments ($P<.05$).

Table 1. PROPERTIES OF *STREPTOCOCCUS MUTANS* BIOFILMS FORMED ON ENAMEL BY TREATMENT (MEAN±(SD), N=6)*

Dependent variables	Sucrose 10%	Skim milk	Semi-skim milk	Whole lactose-free milk	Whole milk	Whole + 10% sucrose	0.9% NaCl
Biomass (mg)	2.09±0.40a	1.92±0.60ab	1.75±0.40ab	1.25±0.30bc	1.09±0.40c	2.17±0.40a	0.50±0.10d
Soluble proteins (μg/mg of biomass)	49.10±7.80a	48.70±5.40a	40.10±8.30a	38.81±6.30a	37.40±8.70a	44.60±5.70a	23.76± 8.50b
Viable bacteria, CFU/mg of biomass	3.30E+10 9.00E+09a	3.50E+10 2.00E+10a	3.30E+10 1.00E+10a	2.50E+10 6.00E+09a	2.50E+10 ±2.00E+10ab	3.10E+10 9.00E+09ab	8.50E+09 4.00E+09b
SEPS (%/mg of biomass)	1.73±0.20a	1.41±0.50a	1.26±0.60a	1.15±0.50a	1.84±1.00a	2.25±0.40a	0.60±0.10a
IEPS (%/mg of biomass)	7.05±0.80a	6.42±1.20ab	5.24±1.70ab	4.29±1.20bc	3.74±1.20c	7.00±1.20a	1.35±0.50d
IPS (%/mg of biomass)	5.34±1.40a	3.90±0.40a	3.20±1.20a	2.97±0.80a	2.61±0.90a	4.59±1.50a	0.81±0.60b

* Different letters represent statistically significant differences among treatments ($P<.05$).

Discussion

The results of this study clearly indicated that whole milk and lactose-free whole milk induced lower demineralization than the rest of the commercially available milk types. Conversely, skim and semi-skim milk provoked similar demineralization as the sucrose control. In terms of acidogenicity (Figure 1) and demineralization induced in enamel or dentin (Figure 2), whole and lactose-free whole milk showed lower values than the rest of the milk types. Since there are several types of bovine milk available from which the population can choose, we decided to explore their cariogenicity using an artificial caries model. These results are in line with our previous study showing lower demineralization of whole milk[13] than the sucrose control. In those previous studies, we also showed that 4.5 percent lactose, the disaccharide concentration found in whole milk, was as capable of demineralizing as whole milk, suggesting that milk was not anticariogenic, as had been suggested.[11,27]

If lactose is mildly cariogenic, as we showed before, it is plausible to think that a lactose-free product may be less cariogenic, based on the absence of the milk sugar, lactose. Our results, however, indicated that whole lactose-free milk induced similar levels of tissue demineralization as whole milk (Figure 2a-b). Lactose-free milk is produced by addition of lactase, an enzyme that hydrolyzes lactose from milk. Once cleaved, lactose gives rise to the monosaccharides glucose and galactose. Thus, it still contains sugars that are fermentable by *S mutans*. Consistent with previous evidence from experiments conducted in rats,[6] whole milk reduced in lactose retained the mild cariogenic potential of whole milk.

Although not cariogenic per se, bovine milk seems to be less cariogenic than sucrose.[7,13,28,29] It has been argued that this lower cariogenicity of milk derives from its peptides, mainly casein.[10] All bovine milk types tested here, however, contained approximately the same amount of protein. Yet, significant differences in their demineralizing potential and their effect on the *S mutans* biofilm were found among them. Nonetheless, commercially available milk types vary in their fat content. While whole milk contains 3.1 percent, skim and semi-skim milk contain 0.05 percent and 1.5 percent, respectively. The hypothesis behind these studies is that fat content in milk is important to decrease milk's cariogenicity.

Among the few studies available, it has been stated that fatty acids decrease the cariogenicity of sugars.[15] Likewise, in a recent investigation conducted by our laboratory,[17] we observed that free fatty acids exposed to a *S mutans* biofilm immediately after sucrose exposure decreased the cariogenicity of a caries model, similar to the one used here. Several potential mechanisms for the putative anticaries activity of fatty acids have been proposed. Fatty acids could act by inhibiting bacterial growth or directly killing the micro-organisms.[30] Yet, the exact molecular mechanism by which fatty acids act to inhibit bacterial pathogenicity is still uncertain.

Detergent properties due to fatty acids' amphipathic structure is a potential explanation for their activity. Once fatty acids make contact with the surface of bacteria, pores are created that can solubilize the cell membrane. This disrupts its structural integrity and interferes with the electron transport chain, which, in turn, interferes with oxidative phosphorylation.[31,32] Other mechanisms may also be responsible for the anticaries effect of fatty acids, including bacterial lysis, enzymatic inhibition, impairment of nutrient uptake, and toxicity by generation of byproducts.[30]

In our previous study,[17] we reported a dose-dependency for the effect of the fatty acids against cariogenicity of *S mutans*. Interestingly, a modest and dose-dependent mild effect on *S mutans* killing was observed, mainly for monounsaturated oleic acid. Milk fat contains many types of fatty acids. Approximately 70 percent of the fatty acids in milk are saturated, 25 percent are monounsaturated, and approximately three percent are polyunsaturated.[33] Although fatty acids have an antibacterial effect,[30] fat content in milk is relatively low, at 4.2 percent; hence, it was reasonable to observe no differences among the milks tested here regarding bacterial counts (Tables 1 and 2). This finding was consistent with previous results from experimental animals.[6]

Despite the lack of effect on bacterial killing, biofilms formed in the presence of the various milk types evidenced differences in other properties of the consortium. When biomass was assessed by dry weight, whole and whole lactose-free milk showed lower biomass than the other milk types (Tables 1 and 2). Biofilm comprises mainly bacterial cells and insoluble polysaccharides that form a protective scaffold for bacteria. Since IEPS were also lower in whole and whole lactose-free milks (Tables 1 and 2), it is reasonable to think that the effect on biomass reduction was due to an effect on polysaccharide production rather than on bacterial killing. Consistent with our initial hypothesis, milk types containing a higher fat content (whole and whole lactose-free milk) were the least acidogenic (Figure 1), induced lower tissue demineralization (Figure 2), and resulted in less cariogenic biofilms (Tables 1 and 2) than the other milk types. In light of our results, we think that the role of milk's proteins may be negligible and the most relevant

Table 2. PROPERTIES OF *STREPTOCOCCUS MUTANS* BIOFILMS FORMED ON DENTIN BY TREATMENT (MEAN±(SD), N=6)*

Dependent variables	Sucrose 10%	Skim milk	Semi-skin milk	Whole lactose free	Whole milk	Whole + 10% sucrose	0.9% NaCl
Biomass (mg)	2.42±0.60a	2.00±0.40ab	1.87±0.40ab	0.98±0.50bc	1.01±0.30c	2.25±0.60a	0.33±0.30d
Soluble proteins (μg/mg of biomass)	48.28±5.00a	47.10±9.00a	41.60±4.80a	39.11±9.00a	35.80±5.60a	41.70±7.80a	18.59±4.00b
Viable bacteria, CFU/mg of biomass	3.00E+10 ±7.00E+09a	3.00E+10± 3.00E+09a	3.00E+10± 7.00E+09a	2.00E+10± 1.00E+10a	2.00E+10± 1.00E+10a	2.00E+10 ±1.00E+10a	6.00E+09 ±6.00E+09b
SEPS (%/mg of biomass)	1.86±0.500a	1.10±0.30a	1.22±0.20a	1.21±0.20a	1.15±0.30a	1.77±0.60a	0.47±0.20b
IEPS (%/mg of biomass)	4.34±1.00a	3.74±1.30ab	4.30±1.50ab	2.04±0.60bc	1.75±0.40c	2.44±0.50a	0.82±0.40d
IPS (%/mg of biomass)	4.63±1.10a	4.46±1.10a	3.61±1.00a	5.20±2.40a	6.25±1.50a	4.53±1.80a	0.41±0.50b

* Different letters represent statistically significant differences among treatments ($P<.05$).

caries-modulating agent may be its fatty acid content. Indeed, those milk types with lower fat content—skim and semi-skim milk—showed demineralization values similar to 10 percent sucrose, the caries-positive control (Figure 2).

Enamel and dentin demineralization varies greatly when different milk types were exposed to the biofilms of *S mutans* (Figure 2). From the observation of the results, whole milk (whole and whole lactose-free) induced lower demineralization than skim and semi-skim milk. Although enamel demineralization was approximately half the demineralization observed for dentin (Figure 2), the pattern of %SHL for all milk types used was similar for both dental substrates. Since dentin is less mineralized than enamel, dentin showed higher microhardness values than enamel. Therefore, if both substrates were exposed to the biofilm for the same amount of time, excessive dentin microhardness would make measurements impossible. For that reason, biofilm was allowed to grow four days on dentin and five days on enamel.[18]

It is important to highlight that, even though the experimental phase for dentin slabs was shortened one day, %SHL was almost twice as high as that produced in enamel. This finding may be of relevance to people with exposed root surfaces who are more prone to root caries. The latter is of particular relevance for older adults, a growing concern in dentistry.

A very important observation was that, despite containing all the fat, sweetened whole milk with 10 percent sucrose behaves similarly to the caries-positive control. Lower in vitro cariogenicity of whole milk, as reported here, is abolished by the addition of sucrose to whole milk, which confirms evidence from experiments in rats.[6] Commercially flavored and sweetened milk typically contains 10 percent sucrose in its composition. Adding sugar to milk is a fairly common practice among children.

These results should alert clinicians and educators of the need to reinforce education to parents in this regard. Furthermore, bovine milk has been suggested as a caries-safe food, suitable to be used as replacement in case of hyposalivation.[11] From our results, however, we think that this is not an appropriate recommendation, and it should be carefully conveyed.

On the other hand, consumption of skim milk is becoming popular. Higher consumption of these products is based on concerns about gaining weight or due to cardiovascular diseases.[34] Recent evidence suggests that consumption of nonfat milk and dairy products is associated with higher rates of coronary heart disease and that milk fat may be beneficial, contrary to what was previously thought.[35] This recent evidence, in addition to the relatively low fat content of whole milk and its lower cariogenicity, suggests that the recommendation of avoiding whole milk consumption, particularly in children, should be revised. From our data, it appears clear that whole milk, with all its fat content, may be significantly less cariogenic than sucrose.

We acknowledge the fact that this is an in vitro study and, thus, implications of these results are preliminary. Yet, we used a relevant biological caries model to emulate the response of the oral biofilm to the exposure to different types of milk. We think, therefore, that these data should be contextualized as proof of principle on the cariogenicity of commercial bovine milks. Clinical trials should provide more definitive conclusions on this matter.

Conclusions

Based on this study's results, the following conclusions can be made:

1. Whole milk, despite all its fat, may be less cariogenic than sucrose but not anticariogenic.
2. Adding sugar makes whole milk as cariogenic as a sucrose solution.
3. Lower cariogenicity of whole milk is gradually reduced as fat content is removed from the commercial products.
4. Limited cariogenicity may be due to fatty acids contained in its composition, acting in a dose-dependent manner; further research appears necessary, however, to translate these findings into practice.

Acknowledgments

These investigations were funded by the Chilean government's Fondecyt Grant no. 11100005 to RAG. The authors wish to thank Dr. Manuel Zura, who was a volunteering dental student at the University of Talca, Talca, Chile, when the experiments were conducted, for helping with the experiments.

References

1. Petridou E, Athanassouli T, Panagopoulos H, Revinthi K. Sociodemographic and dietary factors in relation to dental health among Greek adolescents. Community Dent Oral Epidemiol 1996;24:307-11.
2. Petti S, Simonetti R, Simonetti D'Arca A. The effect of milk and sucrose consumption on caries in 6- to 11-year-old Italian schoolchildren. Eur J Epidemiol 1997;13: 659-64.

3. Harper DS, Osborn JC, Clayton R, Hefferren JJ. Modification of food cariogenicity in rats by mineral-rich concentrates from milk. J Dent Res 1987;66:42-5.
4. Slomiany BL, Murty VL, Zdebska E, et al. Tooth surface-pellicle lipids and their role in the protection of dental enamel against lactic-acid diffusion in man. Arch Oral Biol 1986;31:187-91.
5. Rugg-Gunn A, Roberts G, Wright W. Effect of human milk on plaque pH in situ and enamel dissolution in vitro compared with bovine milk, lactose, and sucrose. Caries Res 1985;19:327-34.
6. Bowen WH, Pearson SK. Effect of milk on cariogenesis. Caries Res 1993;27:461-6.
7. Bibby BG, Huang CT, Zero D, Mundorff SA, Little MF. Protective effect of milk against in vitro caries. J Dent Res 1980;59:1565-70.
8. Weiss ME, Bibby BG. Effects of milk on enamel solubility. Arch Oral Biol 1966;11:49-57.
9. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587-95.
10. Aimutis W. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr 2004;134: 989S-995S.
11. Bowen W, Pearson S, VanWuyckhuyse B, Tabak L. Influence of milk, lactose-reduced milk, and lactose on caries in desalivated rats. Caries Res 1991;25:283-6.
12. Mor BM, McDougall WA. Effects of milk on pH of plaque and salivary sediment and the oral clearance of milk. Caries Res 1977;11:223-30.
13. Muñoz-Sandoval C, Muñoz-Cifuentes MJ, Giacaman RA, Ccahuana-Vasquez RA, Cury JA. Effect of bovine milk on Streptococcus mutans biofilm cariogenic properties and enamel and dentin demineralization. Pediatr Dent 2012;34:197-201.
14. Bowen WH. Food components and caries. Adv Dent Res 1994;8:215-20.
15. Kabara JJ. Dietary lipids as anticariogenic agents. J Environ Pathol Toxicol Oncol 1986;6:87-113.
16. Huang CB, Alimova Y, Myers TM, Ebersole JL. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral micro-organisms. Arch Oral Biol 2011;56:650-4.
17. Giacaman RA, Muñoz-Sandoval C, Jobet P, Castro RJ. Poly and monounsaturated fatty acids reduce sucrose-induced enamel demineralization in an artificial caries model. Presented at the 59th Annual Congress of the European Organization for Caries Research (ORCA), Cabo Frio, Brazil. June 29. Submitted 2012.
18. Ccahuana-Vásquez R, Cury J. *Streptococcus mutans* biofilm model to evaluate antimicrobial substances and enamel demineralization. Braz Oral Res 2010;24:135-41.
19. Thomas RZ, Ruben JL, ten Bosch JJ, Huysmans MC. Effect of ethylene oxide sterilization on enamel and dentin demineralization in vitro. J Dent 2007;35:547-51.
20. Koo H, Hayacibara M, Schobel B, et al. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. J Antimicrob Chemother 2003;52:782-9.
21. Zero DT. In situ caries models. Adv Dent Res 1995;9: 214-30.
22. Herigstad B, Hamilton M, Heersink J. How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods 2001;44:121-9.
23. Aires CP, Del Bel Cury AA, Tenuta LM, et al. Effect of starch and sucrose on dental biofilm formation and on root dentine demineralization. Caries Res 2008;42:380-6.
24. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72: 248-54.
25. Cury J, Rebello M, Del Bel Cury A. In situ relationship between sucrose exposure and the composition of dental plaque. Caries Res 1997;31:356-60.
26. Dubois M, Gilles K, Hamilton J, Rebers P, Smith F. A colorimetric method for the determination of sugars. Nature 1951;168:167.
27. Prabhakar A, Kurthukoti A, Gupta P. Cariogenicity and acidogenicity of human milk, plain and sweetened bovine milk: an in vitro study. J Clin Pediatr Dent 2010; 34:239-47.
28. Peres RC, Coppi LC, Franco EM, et al. Cariogenicity of different types of milk: an experimental study using animal model. Braz Dent J 2002;13:27-32.
29. Jensen ME, Donly K, Wefel JS. Assessment of the effect of selected snack foods on the remineralization/demineralization of enamel and dentin. J Contemp Dent Pract 2000;1:1-17.
30. Desbois AP, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol 2010;85:1629-42.
31. Sheu CW, Freese E. Effects of fatty acids on growth and envelope proteins of Bacillus subtilis. J Bacteriol 1972; 111:516-24.
32. Wojtczak L, Wieckowski MR. The mechanisms of fatty acid-induced proton permeability of the inner mitochondrial membrane. J Bioenerg Biomembr 1999;31:447-55.
33. Månsson HL. Fatty acids in bovine milk fat. Food Nutr Res 2008;52.
34. Ha EJ, Caine-Bish N, Holloman C, Lowry-Gordon K. Evaluation of effectiveness of class-based nutrition intervention on changes in soft drink and milk consumption among young adults. Nutr J 2009;8:50.
35. Avalos EE, Barrett-Connor E, Kritz-Silverstein D, et al. Is dairy product consumption associated with the incidence of CHD? Public Health Nutr 2012:1-9.