

# In vivo dental plaque pH after consumption of dairy products

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This in vivo study assessed plaque pH in subjects following the consumption of different dairy products. After receiving parental consent to participate, subjects (12-15 years old) were asked to refrain from brushing their teeth for 48 hours prior to the study. At baseline, plaque pH was determined at 4 different sites. Each of the subjects was then assigned randomly to 1 of 4 subgroups and each subgroup was given either cheese, milk, yogurt, or paraffin (control). After baseline, all subjects were allowed to chew and/or swish their respective product for 3 minutes and pH was assessed subsequently at different time intervals. Statistical analysis was performed using unpaired t-test and 1-way ANOVA. The results showed

a statistically significant change in mean plaque pH after consuming the different dairy products, as the plaque pH after 30 minutes was higher in the cheese group than that of the milk and yogurt groups, both of which showed a pH toward baseline after 30 minutes. These results suggest that cheese has the highest anticariogenic property among the dairy products studied, and that milk and yogurt can be considered as noncariogenic.

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Dairy products are perceived to be important for one's overall and dental health.<sup>1</sup> The oral cavity shelters numerous microbial floras. Dental plaque, which develops naturally on oral tissues, is one major action of this complex ecosystem. Studies have shown a correlation between the acidogenic potential of a food and changes in the pH of human dental plaque following the ingestion of that food.<sup>2</sup> Plaque pH is reduced after carbohydrate consumption but returns rapidly to resting level by chewing paraffin wax or other substances that stimulate salivary flow.<sup>3,4</sup> Certain foods, such as cheese, produce a rapid rise in plaque pH in vivo, accelerating plaque's pH return to neutrality.<sup>5</sup> Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), which is present in cheese, also may buffer the plaque pH by adsorbing to the tooth enamel, which reduces enamel solubility and bacterial adherence.<sup>6</sup>

The increase in pH during chewing appears to be closely associated with increased salivary flow (and perhaps buffering capacity), but may involve other factors as well, including the increased supply of nitrogenous substrates.<sup>6</sup> Both in vivo and in vitro studies have shown the effect of milk on the pH of human dental plaque.<sup>7</sup> Milk contains 4%-5% disaccharide lactose, which can be fermented by oral biofilm bacteria. Normally, sucrose lowers the plaque pH to <5.0, while lactose lowers pH to approximately 6.0. Therefore, under normal conditions, the carbohydrate

content of milk confers a low cariogenic potential; however, milk or cheese preserve teeth by counteracting the acidification induced by sucrose in the dental biofilm.<sup>8</sup> Birkhed et al found that milk induced minimal pH reduction compared to fruit juice and sweetened beverages.<sup>7</sup> Milk fermentation leads to the production of lactic acid and the resulting drop in pH inhibits the growth of many pathogenic microorganisms.<sup>9</sup> The most common fermented dairy product is yogurt, which traditionally is fermented with *Lactobacillus bulgaricus*.<sup>10</sup> A 1980 study by Bibby et al found that the milk component of plain home yogurt inhibited enamel dissolution and acid production.<sup>11</sup> Ferrazzano et al also showed that the natural CPPs in yogurt prevent demineralization and promote remineralization of tooth enamel.<sup>12</sup> While milk and milk products are consumed commonly, there is remarkably little clinical research concerning their effect on oral health and diseases.<sup>13</sup> This study sought to assess the variation in plaque pH in vivo, following the consumption of different dairy products (cheese, yogurt, and milk), with paraffin used as a control.

## Materials and methods

A sample of 68 healthy subjects (12-15 years old) were selected for this study. Thirty-four of the subjects were caries-free, that is, with no decayed, missing, or filled teeth (DMFT); the remaining 34 subjects were caries-active, that is, with a DMFT >3 and at least 1 caries

lesion. Eligible participants were given an informational summary to read with their parents, and the study also was explained verbally. Participating subjects signed an informed consent form which was reviewed and approved by an ethical committee review board. Subjects with missing teeth were excluded from this study, as were those undergoing orthodontic treatment, antibiotic therapy or other chemotherapeutic procedures with a potential effect on plaque formation.

Appointments were arranged and consenting subjects were asked to abstain from all oral hygiene measures for 48 hours, so that adequate plaque accumulation could occur. Previous clinical trials have demonstrated this time interval is acceptable for plaque accumulation.<sup>14</sup> All appointments took place in the morning. Subjects were asked to refrain from eating and drinking (except water) until after that day's dental visit. Upon arrival, the subject's compliance with these parameters was confirmed.

A baseline pH was measured for all subgroups at 4 sites, (mesial surface of tooth No. 3, distal surface of tooth No. 14, mesial surface of tooth No. 19, and distal surface of tooth No. 30). Each subject was then assigned randomly to 1 of 4 subgroups: cheese (n = 8), milk (n = 8), yogurt (n = 8), and control (paraffin) (n = 10). After the baseline estimation of plaque, the subjects in each subgroup were allowed to consume their respective products: cheddar cheese (10 g), milk containing 3.5% fat (15 ml), sugarless yogurt (10 g), or paraffin (5 g) for

3 minutes. They were then asked to swish their mouths with deionized water. The pH was measured after intervals of 10, 20, and 30 minutes, using the same procedure for all subjects. This aspect of time has been shown to be adequate for significant buffering of plaque pH.<sup>14</sup>

During the entire study, *in situ* plaque pH was assessed directly using a Beetrode pH Touch Electrode (World Precision Instruments, Inc.) connected to a display unit. This miniature wire electrode was designed to measure fast pH changes in small samples. Initially, the tips of new and sterilized Beetrode pH sensors were soaked in distilled water for several hours prior to use. Once prepared, the electrodes were stored in a reference buffer (pH = 7), where calibrations were performed before assessment of each subject. Once the electrode grounding device was placed sublingually, the tip of the electrode was placed into the plaque mass and held in place until the reading on the display unit had stabilized and data was recorded. The electrode was rinsed in distilled deionized water between each reading to protect against cross-contamination. Data was analyzed (SPSS version 10.0, IBM Corporation). Difference in mean plaque pH values between the groups and at different time intervals were statistically analyzed using 1-way ANOVA, and an unpaired t-test was used for the caries-active and caries-free subjects.

## Results

Table 1 summarizes data recorded for the 4 groups analyzed at baseline, 10, 20, and 30 minutes. The results showed a statistically significant difference in mean plaque pH between baseline, 10 minutes, and 20 minutes. However, there was a statistically nonsignificant difference in mean plaque pH between baseline and 30 minutes following consumption of dairy products.

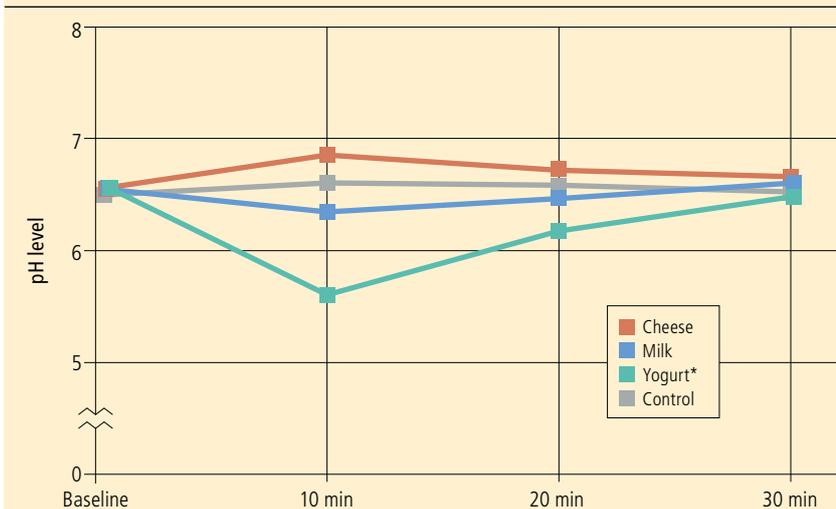
The Chart illustrates that the mean plaque pH in the cheese group rose rapidly after 10 minutes and decreased slightly after 20 and 30 minutes; however, the plaque pH at 30 minutes was slightly higher than at baseline. These variations were found to be statistically nonsignificant ( $P < 0.381$ ). Among those who drank milk, plaque pH decreased

**Table 1. Mean plaque pH of subjects ( $\pm$ SD) of different groups at different time intervals.**

Group	Baseline	10 minutes	20 minutes	30 minutes
Control	6.51 $\pm$ 0.44	6.60 $\pm$ 0.42	6.58 $\pm$ 0.36	6.53 $\pm$ 0.47
Cheese	6.54 $\pm$ 0.46	6.85 $\pm$ 0.54	6.73 $\pm$ 0.51	6.66 $\pm$ 0.52
Milk	6.59 $\pm$ 0.49	6.36 $\pm$ 0.46	6.48 $\pm$ 0.46	6.60 $\pm$ 0.47
Yogurt	6.57 $\pm$ 0.47	5.61 $\pm$ 0.05	6.18 $\pm$ 0.36	6.48 $\pm$ 0.48
ANOVA	0.114	26.914	4.166	0.437
P	0.952 <sup>c</sup>	0.000 <sup>c</sup>	0.009 <sup>b</sup>	0.727 <sup>c</sup>

*P* < 0.05, <sup>a</sup>Highly significant; <sup>b</sup>Significant; <sup>c</sup>Nonsignificant

**Chart. Mean plaque pH of subjects at different time intervals following consumption of dairy products.**



\*Highly significant at  $P < 0.00$ , Tukey post hoc significant pairs. Baseline and 10 min, baseline and 20 min, 10 and 20 min, 10 and 30 min.

after 10 minutes and increased again after 20 and 30 minutes; however, the variations at the different time intervals were also found to be nonsignificant ( $P < 0.451$ ). Among the subjects who consumed yogurt, plaque pH dropped rapidly after 10 minutes and rose after 20 and 30 minutes. In this case, the variations at different time intervals were found to be highly significant ( $P < 0.000$ ). Post hoc results showed significant differences in the yogurt group between baseline and 10 minutes, baseline and 20 minutes, 10 and 20 minutes, and 10 and 30 minutes.

Table 2 compares caries-active and caries-free subjects at different time intervals after dairy product consumption. Statistically significant differences were seen between caries-active and caries-free subjects for all the groups at each time interval, except for the yogurt group, which showed no statistically significant difference at 10 minutes. The control (paraffin) group exhibited significant differences at all time intervals.

## Discussion

Dairy products have low cariogenic potential and demonstrate anticaries activity, although additional studies are needed.<sup>15-18</sup>

Table 2. Mean plaque pH of subjects ( $\pm$ SD) at different time intervals among cheese, milk, yogurt, and control groups.

Group	Baseline		10 minutes		20 minutes		30 minutes	
	Caries	Caries-free	Caries	Caries-free	Caries	Caries-free	Caries	Caries-free
Cheese	6.10 $\pm$ 0.13 <sup>a</sup>	6.96 $\pm$ 0.15	6.33 $\pm$ 0.12 <sup>a</sup>	7.36 $\pm$ 0.13	6.25 $\pm$ 0.14 <sup>a</sup>	7.22 $\pm$ 0.09	6.18 $\pm$ 0.04 <sup>a</sup>	7.15 $\pm$ 0.13
Milk	6.14 $\pm$ 0.09 <sup>a</sup>	7.04 $\pm$ 0.23	5.94 $\pm$ 0.09 <sup>b</sup>	6.79 $\pm$ 0.17	6.05 $\pm$ 0.09 <sup>b</sup>	6.91 $\pm$ 0.19	6.18 $\pm$ 0.76 <sup>a</sup>	7.03 $\pm$ 0.22
Yogurt	6.13 $\pm$ 0.18 <sup>a</sup>	7.00 $\pm$ 0.09	5.59 $\pm$ 0.09 <sup>c</sup>	5.63 $\pm$ 0.17	5.86 $\pm$ 0.16 <sup>a</sup>	6.49 $\pm$ 0.25	6.05 $\pm$ 0.16 <sup>a</sup>	6.91 $\pm$ 0.24
Control	6.10 $\pm$ 0.17 <sup>a</sup>	6.90 $\pm$ 0.16	6.22 $\pm$ 0.19 <sup>a</sup>	6.97 $\pm$ 0.15	6.16 $\pm$ 0.18 <sup>a</sup>	6.99 $\pm$ 0.20	6.11 $\pm$ 0.18 <sup>a</sup>	6.96 $\pm$ 0.20

<sup>a</sup>Highly significant; <sup>b</sup>Significant; <sup>c</sup>Nonsignificant

The anticariogenic activity of milk and its products are attributed to the direct chemical effects of casein, phosphopeptides, calcium, and phosphate.<sup>16,17</sup> One approach for estimating the cariogenic potential of food involves evaluating the magnitude of plaque pH response following ingestion.<sup>10</sup>

The age of plaque is an important factor for plaque pH measurement. When Igarashi et al challenged plaque with a sucrose solution, the response increased with aging plaque (particularly if undisturbed for up to 2 days), producing a greater level of acid than immature plaque.<sup>19</sup> In the present study, oral prophylaxis was not performed and subjects were instructed not to brush their teeth for 48 hours prior to taking the pH measurements to ensure the presence of representative oral bacterial flora on the accessible tooth surfaces.<sup>20</sup> According to a 1986 study by Chida et al, the plaque that accumulated on enamel was colonized completely by cocci in 2 days; after 4 days, the number of rods became dominant.<sup>21</sup> In terms of pH changes, the 2-day-old plaque responded more rapidly than older, mature plaque when exposed to fermentable carbohydrates.<sup>21</sup> As a result, the plaque in the present study contained an ideal bacterial composition, since its responsiveness to challenges was optimal, which explains the lower baseline plaque pH values.

The results of the present study revealed that milk and cheese consumption led to an increase in plaque pH, but cheese showed a greater increase in plaque pH even at 30 minutes, while the plaque pH of milk and yogurt decreased at 10 and 20 minutes. After 30 minutes, the plaque pH in the milk group was similar to that of the baseline pH, while in the yogurt group, it was slightly lower.

The plaque pH values after cheese consumption were similar to those reported in previous studies.<sup>22,23</sup> The main factors for the initial rise in plaque pH values after cheese consumption seems to be the reduction of critical plaque pH. This reduction occurred due to diffusion of calcium and phosphorus into the plaque from the cheese; the buffering of the plaque pH by saliva, which was stimulated by chewing cheese (a strong sialagogue); the fact that cheese contains a significant amount of tyramine, which could be used by microorganisms to raise the pH value of plaque; and the accelerated rise in pH (similar to a salivary pH rise) due to the peptides in the cheese.<sup>7,13</sup>

Although it increased initially, the plaque pH following cheese consumption decreased after 10 minutes. The acidic constituents of cheese (lactic and sorbic acid) may have contributed directly to the subsequent reduction of plaque pH and also may have depleted the saliva's buffering power.<sup>7</sup> Minah et al reported that factors such as salivary pH rise may favor the competitiveness of less harmful oral bacteria and discourage aciduric bacterial strains such as streptococci and lactobacilli.<sup>24</sup> It can be conjectured that as the pH rises, less cariogenic bacteria may metabolize the available substrates, producing weaker acids which will help maintain an elevated pH.<sup>24</sup>

The decrease in plaque pH within 10 minutes after consuming milk was similar to a 1985 study by Rugg-Gunn et al, in which a sucrose solution caused a substantial drop in plaque pH, while milk depressed plaque pH only slightly.<sup>25</sup> By contrast, Birkhed et al reported that dental plaque microflora may adapt to

lactose in milk, increasing the ability to ferment lactose in the milk following frequent consumption.<sup>7</sup> In 2002, Johansson observed increased acid production due to bacterial enzymes involved in lactose transport and catabolism.<sup>8</sup> The pH increased slightly after 20 minutes due to the peptides and amino acids produced by the hydrolysis of casein, which upon further catabolism, can raise the plaque pH and prevent demineralization. This process of alkaline production may counteract the acid produced from lactose fermentation.<sup>8</sup>

Milk fermentation leads to the production of lactic acid and the resulting pH decrease inhibits growth of many pathogenic organisms. One of the most popular fermented foods is yogurt, which traditionally has been fermented with *L. bulgaricus*.<sup>26</sup> Yogurt consumption in this study led to a rapid drop in the plaque pH; however, the decrease did not drive it below the critical pH of 5.5 at 10 minutes, similar to the results of a 2007 study by Sonmez & Aras.<sup>10</sup> The initial fall in plaque pH was due to the acidic nature of the yogurt (4.0-4.5 pH). The increase in pH after 20 and 30 minutes may be due to the buffering capacity of stimulated saliva, and the reduced lactose content of the yogurt due to fermentation.<sup>8</sup> The increase in pH may also be due to the fact that the natural CPP content present in yogurt is higher than that of milk, due to the proteolytic activity of microorganisms contained in the yogurt, and the peptides and amino acids produced by the hydrolysis of casein. Both of these have a potential to produce a pH rise in plaque upon further catabolism, and prevent demineralization.<sup>8,10</sup>

Acid produced by plaque bacteria can be useful to the process of caries risk assessment. Previous studies have shown that the plaque pH value reflected the plaque's carious potential (that is, higher carious activity corresponds to lower plaque pH values).<sup>20</sup> There is considerable clinical evidence that cariogenic conditions are associated with increased amounts of microorganisms capable of acid production. In caries-active subjects with cariogenic diets, shifts occur in the dental plaque microflora, with increased numbers of specific organisms, such as *Streptococcus mutans*, *L. casei*, and, in the case of root surface lesions, strains of *Bifidobacterium* and *Actinomyces*.<sup>27</sup> The present study demonstrated a reverse relationship between DMFT scores and the pH values of resting dental plaque.<sup>20</sup>

Numerous clinical studies have established that the proportions of microorganisms designated as capable of acid production are significantly higher in the plaque of caries-active patients as compared to that of caries-free patients.<sup>14,19</sup> This increase in microorganisms may be due to cariogenic plaque fermenting dietary carbohydrates to produce stronger organic acids (lactate, pyruvate, and fumarate) that can readily demineralize enamel in a caries-active group. By contrast, a non-caries group produces larger amounts of weaker organic acids (such as acetate), which can efficiently buffer plaque pH changes.<sup>27</sup>

## Summary

Among the 3 dairy products consumed, cheese showed the highest plaque pH 30 minutes after consumption, followed by milk and yogurt. This suggests that cheese has the highest anticariogenic property among these dairy products. The pH levels of the milk and yogurt groups approached baseline/neutrality. None of the dairy products in this study lowered the plaque pH below the critical pH of 5.5, where enamel demineralization and dissolution are expected even at the 10-minute interval.<sup>10</sup> These findings confirm that cheese, milk, and yogurt without sugar (sucrose) are noncariogenic and to some extent cariostatic. It appears

that milk possesses many of the desired biological and physical properties for a saliva substitute.<sup>12</sup> Frequent sipping/chewing of dairy products may satisfy the need to lubricate and moisten the mouth, while at the same time providing caries protection to highly susceptible individuals.<sup>8</sup> Milk and milk-based components (without added sucrose) can be used as a substitute for carbohydrate-laden desserts and snacks, which may help reduce the incidence of dental caries.

## Author information

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