# Comparison of Salivary Calcium, Phosphate and Alkaline Phosphatase Levels in Children with Early Childhood Caries after Administration of Milk, Cheese and GC Tooth Mousse: An *in Vivo* Study

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***Background and Objectives:*** *This study compares the Salivary Calcium, Phosphate and Alkaline Phosphataselevels in children with Early Childhood Caries after administration of Milk, Cheese and GC Tooth Mousse to a control group of caries resistant children.* ***Study design:*** *90 kindergarten children both males and females aged 5 years, from the South Canara region were included in the study. Based on the dmfs score, children were divided into 3 groups: Control group, ECC group and S-ECC group. The Salivary Calcium, Phosphate and Alkaline Phosphatase levels in the sample were assessed before and after administration of Milk, Cheese and GC Tooth Mousse at three different intervals, i.e within 5 minutes, 30 minutes and 60 minutes by using Spectrophotometry.* ***Results:*** *The mean Salivary Calcium levels were higher in caries free group whereas Phosphate and Alkaline Phosphatase were lower in the caries free group which was statistically highly significant ($p<0.001$). The Tooth Mousse group showed higher bioavailability of calcium and phosphate which was statistically highly significant ($p<0.001$). Salivary Calcium and Phosphate levels within 5 minutes afterapplication of Milk, Cheese and Tooth Mousse were higher than at 30 and 60 minutes. Salivary Alkaline Phosphatase levels were lower than the baseline values at all the 3 intervals after administration ofMilk, Cheese and Tooth Mousse and was statistically not significant ($p>0.05$).* ***Conclusion:*** *Saliva should be saturated with Calcium and Phosphate to affect their bioavailability in amounts adequate for remineralizaton. Milk, Cheese and GC Tooth Mousse applicaton were equally beneficial in saturating the saliva with adequate amount of Calcium and Phosphate.*

***Key words:*** *Calcium, Phosphate, Alkaline Phosphatase, Milk, Cheese, Tooth Mousse.*

## INTRODUCTION

Saliva has many functions in protecting the integrity of the oral mucosa: it participates in the clearing of the oral cavity of food residues, debris and bacteria; it buffers the deleterious effects of strong acids and bases and provides the ions needed to remineralize the teeth like calcium ,phosphate.[1] The delicate balance between demineralization and remineralization, to which dental hard tissues are subjected to, dwells on the Salivary Calcium, Phosphate and Salivary Alkaline Phosphatase levels.[2] Saliva should be saturated to affect the bioavailability of the calcium and phosphate in amounts adequate for remineralization.[3]

Milk and Dairy products are the natural food items most commonly consumed by humans and are sources of good quality protein,carbohydrate,fats,vitamins and minerals including calcium, phosphate, magnesium, iodine and potassium.[4,5] Milk proteins are adsorbed onto the enamel surface and may impede enamel demineralization; the phosphoprotein (phosphopeptide) casein is implicated in this effect.[6] Cheese decreases caries in human subjects and may exert part of this effects by stimulating saliva which, in turn, would influence plaque ph and sugar clearance from oral cavity. However cheese also contains a number of water –soluble substances like calcium, phosphates, calcium salts of lower fatty acids, proteins that protect against dental caries.[7]

A group of peptides, known as Casein Phosphopeptides(CPP), have been shown to stabilize calcium and phosphate preserving them in an amorphous or soluble form known as Amorphous Calcium Phosphate (ACP). This CPP-ACP complex is an extract of cheese and is commercially available as GC Tooth Mousse( MI Paste in North America). This complex applied to the teeth is able to adhere to the dental biofilm and increase calcium phosphate levels which serve as a reservoir for free calcium and phosphate ions.[8]

An improvement in dental health may be expected on administration of these products, thereby reducing caries susceptibility.

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Hence, the study was done to compare the Salivary Calcium, Phosphate and Alkaline Phosphatase levels in children with Early Childhood Caries after administration of Milk, Cheese and GC Tooth Mousse to a control group

## MATERIALS AND METHOD

A total of 90 subjects both males and females aged 5 years, selected randomly from kindergartens in the South Canara region were included after taking informed consent from parents/guardians.The caries status of the children was recorded using dmfs (decayed, missing, filled surfaces) index according to criteria of WHO (World Health Organization) using a mouth mirror and probe.[9]The included visual and tactile methods were carried out under acceptable lighting conditions.

Based on the dmfs score, 90 children were selected and divided into 3 groups of 30 each.

1. Group I - Control group (dmfs 0).
2. Group II - Early Childhood Caries (ECC) group (dmfs 1-3)
3. Group III -Severe Early Childhood Caries (S-ECC) group (dmfs>3).[10]

About 5 ml of unstimulated whole saliva was collected from all the subject in a plastic tube. All subjects were instructed to refrain from eating and drinking for 1 hour prior to saliva collection. Unstimulated whole saliva samples were collected sitting in the "Coachman" position; on the edge of the chair, the patient passively drooled saliva into a funnel inserted into a graduated cylinder for 5 min.[11] Salivary Calcium, Phosphate and Alkaline Phosphatase levels were estimated.

Same quantity of unstimulated whole saliva was collected after exposure to 150ml of Double Toned Pasteurized Amul Milk (swished and swallowed in 3 minutes).Similarly 20 gms of processed Cheddar Cheese (consumed within 3 minutes) and application of GC Tooth Mousse (only pea sized amount applied to both the arch and kept for 3 minutes) was done for each group. Saliva samples were collected within 5 minutes, at 30 minutes and 60 minutes interval respectively followed by estimation of Salivary Calcium, Phosphate and Alkaline Phosphatase levels.[12]

The Salivary Calcium levels were assessed using a calcium kit (Asritha Diatech ,Kukatpally,Hyderabad,India). All glassware being used for the test was rinsed with 0.1 N HCl and then with distilled water before use to prevent contamination. Saliva sample mixed with the reagent and incubated at 37$^{o}$C for 5 min. The amount of absorbance of the standard and test sample was measured against a blank at 570nm.[13]The Salivary Phosphate levels were assessed using reagent which contains Sulfuric acid and Ammonium Molybdate (Agappe Diagnostics LTD, Ernakulam , Kerala ,India ). All glassware being used for the test was rinsed with 50% Nitric acid and then with distilled water before use to prevent contamination. Saliva samples were mixed with the reagent and then incubated at 37$^{o}$C for 1 min. The amount of absorbance of the standard and test sample was measured against the blank.[14]

The Salivary Alkaline Phosphatase levels were assessed using Alkaline Phosphatase kit (Asritha Diatech ,Kukatpally,Hyderabad,India ) in all the three groups. Saliva sample mixed with the reagent and the initial absorbance was read after 1 minute and the procedure repeated after every 1, 2 and 3 mins at 570nm and the mean value was taken.[15]

The data obtained was statistically analysed using Fischers test, TukeyHSD test and Paired't'test (p- value <0.05 were considered significant).

## RESULTS

The baseline mean Salivary Calcium level in Caries free group was 28.16mg/dl, whereas in ECC and S-ECC group it was 18.49 mg/dl and 18.12 mg/dl respectively. There was a decrease in the mean Salivary Calcium levels from Caries free group to ECC group and Caries free group to S-ECC group, which was statistically very highly significant ($p<0.001$).

The meanbaseline Salivary Phosphate level in Caries free group was 10.23mg/dl, whereas in ECC and S-ECC group it was 13.94mg/dl and 17.23 mg/dl respectively. The mean baseline Salivary Alkaline Phosphatase levels in Caries free group were 16.55 mg/dl, whereas in ECC and S-ECC group it was 18.42 mg/dl and 30.15 mg/dl respectively. There was an increase in the mean Salivary Phosphate and Alkaline Phosphatase levels from Caries free group to ECC group and Caries free group to S-ECC group, which was statistically very highly significant ($p<0.001$).

The mean Salivary Calcium levels were higher in Caries free group than ECC & S-ECC groups whereas Phosphate and Alkaline Phosphatase were lower in Caries free group as compared to ECC & S-ECC group which was statistically highly significant($p<0.001$). There was increase in Salivary Calcium and Phosphate levels in all the three groups and at all the three intervals after consumption of Cheese which was statistically very highly significant ($p<0.001$) , whereas there was minimal decrease in the mean values of Salivary Alkaline Phosphatase at all the three groups and at all the three intervals when compared to the baseline levels which was statistically not significant ($p>0.05$).

The Salivary Calcium levels increased between Tooth Mousse and Milk&Tooth Mousse and Cheese at all three intervals and in all the three groups which were statistically very highly significant ($p<0.01$). The Salivary Calcium levels between the Milk and Cheese were not significant when compared to GC Tooth Mousse ($p>0.05$).

The Salivary Phosphate levels increased between Tooth Mousse and Milk&Tooth Mousse and Cheese at all three intervals and in all the three groups which were statistically very highly significant ($p<0.01$). The Salivary Phosphate levels between the Milk and Cheese were not significant when compared to GC Tooth Mousse. ($p>0.05$) There was slight difference in the Salivary Alkaline Phosphatase levels after administration of Milk, Cheese and GC Tooth Mousse at all the three intervals and all the three groups. However they were notstatistically significant($p>0.05$).

## DISCUSSION

Saliva has the capacity to buffer strong acids and bases and provides the ions needed to remineralize the teeth like calcium, phosphate etc. The delicate balance between demineralization and remineralization,to which dental hard tissues are subjected to, dwells on the Salivary Calcium, Phosphate and Salivary Alkaline Phosphatase levels. The caries protective effects of milk, cheese and other dairy products have been postulated. Casein Phosphopeptides have been shown to stabilize calcium and phosphate preserving them in an amorphous or soluble form known as Amorphous Calcium Phosphate.[1,2]

According to the American Academy of Pediatric Dentistry, Early Childhood Caries (ECC) can be defined as the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger. In children younger than 3 years of age, any sign of smooth-surface caries is indicative of severe early childhood caries (S-ECC). From ages 3 through 5, 1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth, or a decayed, missing, or filled score of >4 (age 3), >5 (age 4), or >6 (age 5) surfaces constitutes S-ECC.[10]

The baseline Salivary Calcium Levels in our study were higher among Caries free children than the children with ECC and S-ECC. There was no significant difference seen when the baseline Salivary Calcium levels were compared between ECC and S-ECC group. This was in accordance with study done by Ashley *et al* [16] and Karshan *et al* [3]. The severity of caries in S-ECC group could be attributed to other factors responsible for dental caries than ECC group.

The Salivary Phosphate levels were lower in Caries free group when compared with ECC and S-ECC. This was in agreement with study done by Klassen et al [17] and Damle *et al* [2] and contradiction with study done by Shaw *et al* [18]. The results obtained could be due to the diffusion of solubilizing ions into and out of the lesion. The dissolution of dental hard tissues in the case of calcium and phosphate occurs in the presence of saliva. The ionic concentration of calcium and phosphate in saliva helps maintain equilibrium between dissolution and remineralization of enamel. The rate of caries formation depends on the rate of diffusion of these ions.[19]( Table 1)

In the present study, the baseline Salivary Alkaline Phosphatase levels were lower in Caries free when compared to ECC and S-ECC. This could be due to maintenance of equilibrium between demineralization and remineralization depending on the ionic concentration of calcium and phosphate in saliva, which in turn is influenced by alkaline phosphatase levels. Variation in alkaline phosphatase levels causes changes in phosphate levels which lead to initiation and progression of caries lesion.[2](Table 1)

The mean Salivary Calcium Levels after consumption of Milk at three different intervals was higher in Caries free group than the ECC and S-ECC group. This may be because of increased baseline values in Caries free group or due to uptake of calcium into the demineralized lesion, thereby reducing the Salivary Calcium levels. The Salivary Calcium levels when compared between ECC and S-ECC group there was no significant difference seen at three intervals. This could be attributed to other factors responsible for dental caries in S-ECC than ECC group. The calcium levels were not significant when compared between caries free and ECC at 30 minutes and 60 minutes intervals. This could be due to rapid clearance of milk in the mouth.

The mean Salivary Phosphate Levels were lower in Caries free group than ECC and S-ECC group at 3 different intervals but higher than the baseline values. Increase in the calcium and phosphate level after intake of milk may be due to the presence of 200 mg of calcium and 150 mg of phosphate per 150 ml of milk.[4] The Salivary Phosphate level were much higher in S-ECC group at all intervals when compared with the Caries free which could be due to the increase release of phosphates from hydroxyapatite during to demineralization. The mean Salivary Alkaline Phosphate levels were lower in Caries free group than ECC and S-ECC group at 3 different intervals but lower than the baseline values. The absence of Alkaline Phosphatase in milk indicates well pasteurized milk. So the decreased levels has the beneficial role in preventing initiation and progression of caries.[19, 20] (Table 1)

In the present study, the mean Salivary Calcium levels after consumption of Cheese at three different intervals was higher in Caries free group than the ECC and S-ECC group. The mean Salivary Phosphate and Alkaline Phosphate levels were lower in Caries Free group than ECC and S-ECC group at 3 different intervals. This may be due to increased baseline values. Increase in the Calcium and Phosphate level after intake of cheese was due to calcium and phosphate content. 20gms of cheese was equivalent to 150 ml of milk.[4] (Table 2)

There was a significant difference seen between the Caries free group and S-ECC group at all three intervals. This may be because of increased baseline values of calcium in the Caries free group or due to uptake of calcium into the demineralized lesion, thereby reducing the Salivary Calcium levels. The decreased Salivary Alkaline Phosphatase levels after administration of cheese could be due

Table 1. Salivary calcium, phosphate and alkaline phosphatase levels in different groups before and after administration of Milk.

| Variable | Group | N | Baseline | | | | MILK | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | < 5 minutes | | | | 30 minutes | | | | 60 minutes | | | |
| | | | Mean | SD | F | p | Mean | SD | F | p | Mean | SD | F | p | Mean | SD | F | p |
| Calcium | Caries | 30 | 28.16 | 12.8 | | | 56.71 | 23.5 | | | 31.71 | 15.9 | | | 28.14 | 6.15 | | |
| | free | | | | 9.45 | <0.001 | | | 8.61 | <0.001 | | | 3.06 | 0.05 | | | 6.23 | 0.002 |
| | ECC | 30 | 18.49 | 6.6 | | vhs | 41.82 | 20.2 | | vhs | 29.99 | 11.8 | | sig | 25.64 | 3.7 | | hs |
| | S-ECC | 30 | 18.12 | 6.1 | | | 34.48 | 19.3 | | | 23.86 | 10.3 | | | 20.65 | 3.0 | | |
| Inor- | Caries | 30 | 10.23 | 3.7 | | | 20.71 | 3.1 | | | 17.46 | 4.1 | | | 15.00 | 3.6 | | |
| ganic | free | | | | 19.5 | <0.001 | | | 25.5 | <0.001 | | | 13.1 | <0.001 | | | 25.7 | <0.001 |
| phos- | ECC | 30 | 13.94 | 4.0 | | vhs | 21.30 | 1.8 | | vhs | 18.23 | 3.8 | | vhs | 17.00 | 3.2 | | vhs |
| phate | S-ECC | 30 | 17.23 | 5.0 | | | 24.64 | 1.6 | | | 22.31 | 3.7 | | | 20.00 | 3.4 | | |
| Alk. | Caries | 30 | 16.55 | 4.3 | | | 15.88 | 3.9 | | | 12.53 | 4.0 | | | 11.23 | 3.5 | | |
| phos- | free | | | | 50.6 | <0.001 | | | 58.9 | <0.001 | | | 28.4 | <0.001 | | | 34.5 | <0.001 |
| phatase | ECC | 30 | 18.42 | 4.7 | | vhs | 17.75 | 4.0 | | vhs | 15.55 | 3.8 | | vhs | 13.24 | 4.4 | | vhs |
| | S-ECC | 30 | 30.15 | 7.4 | | | 29.48 | 7.1 | | | 25.42 | 6.342 | | | 22.45 | 6.4 | | |

Table 2. Salivary calcium, phosphate and alkaline phosphatase levels in different groups before and after administration of Cheese.

| Variable | Group | N | Baseline | | | | CHEESE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | F | p | < 5 minutes | | | | 30 minutes | | | | 60 minutes | | | |
| | | | | | | | Mean | SD | F | p | Mean | SD | F | p | Mean | SD | F | p |
| Calcium | Caries free | 30 | 28.16 | 12.8 | 9.45 | <0.001 vhs | 57.22 | 23.0 | 5.87 | 0.004hs | 32.37 | 15.38 | 2.71 | 0.072 | 29.03 | 5.88 | 6.41 | 0.003 hs |
| | ECC | 30 | 18.49 | 6.6 | | | 44.51 | 22.1 | | | 31.66 | 10.23 | | | 26.39 | 4.71 | | |
| | S-ECC | 30 | 18.12 | 6.1 | | | 37.99 | 20.96 | | | 25.66 | 10.41 | | | 24.72 | 3.05 | | |
| Phosphate | Caries free | 30 | 10.23 | 3.7 | 19.5 | <0.001 vhs | 23.13 | 2.82 | 5.23 | 0.008hs | 17.59 | 4.21 | 14.74 | <0.001 vhs | 15.23 | 3.12 | 18.4 | <0.001 vhs |
| | ECC | 30 | 13.94 | 4.0 | | | 24.56 | 1.91 | | | 18.54 | 3.64 | | | 17.64 | 3.25 | | |
| | S-ECC | 30 | 17.23 | 5.0 | | | 25.03 | 1.84 | | | 22.69 | 3.72 | | | 20.08 | 3.54 | | |
| Alk. phospha-tase | Caries free | 30 | 16.55 | 4.3 | 50.6 | <0.001 vhs | 15.73 | 4.11 | 59.7 | <0.001 vhs | 15.53 | 3.45 | 64.23 | <0.001 vhs | 15.35 | 3.52 | 50.4 | <0.001 vhs |
| | ECC | 30 | 18.42 | 4.7 | | | 17.55 | 3.95 | | | 17.49 | 4.72 | | | 17.46 | 5.04 | | |
| | S-ECC | 30 | 30.15 | 7.4 | | | 29.25 | 6.97 | | | 28.93 | 6.25 | | | 28.39 | 7.03 | | |

Table 3.Salivary calcium, phosphate and alkaline phosphatase levels in different groups before and after application of GC tooth mousse

| Variable | Group | N | Baseline | | | | GC TOOTH MOUSSE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | F | p | < 5 minutes | | | | 30 minutes | | | | 60 minutes | | | |
| | | | | | | | Mean | SD | F | p | Mean | SD | F | p | Mean | SD | F | p |
| Calcium | Caries free | 30 | 28.16 | 12.8 | 9.45 | <0.001 vhs | 92.60 | 36.55 | 2.46 | 0.091 | 52.67 | 22.73 | 2.93 | 0.058 | 44.50 | 8.67 | 6.49 | 0.002hs |
| | ECC | 30 | 18.49 | 6.6 | | | 87.73 | 11.15 | | | 46.73 | 21.94 | | | 34.48 | 19.3 | | |
| | S-ECC | 30 | 18.12 | 6.1 | | | 76.65 | 31.35 | | | 40.70 | 9.90 | | | 32.88 | 10.0 | | |
| Inorganic phosphate | Caries free | 30 | 10.23 | 3.7 | 19.5 | <0.001 vhs | 30.18 | 12.45 | 6.23 | 0.002 hs | 27.34 | 5.01 | 2.46 | 0.091 | 23.86 | 10.3 | 2.90 | 0.060 |
| | ECC | 30 | 13.94 | 4.0 | | | 36.25 | 12.31 | | | 31.05 | 8.13 | | | 24.65 | 1.60 | | |
| | S-ECC | 30 | 17.23 | 5.0 | | | 41.23 | 9.50 | | | 34.48 | 19.33 | | | 27.94 | 6.05 | | |
| Alk. phosphatase | Caries free | 30 | 16.55 | 4.3 | 50.6 | <0.001 vhs | 15.60 | 4.00 | 53.6 | <0.001 vhs | 15.49 | 3.59 | 68.88 | <0.001 vhs | 15.22 | 3.90 | 65.5 | <0.001 vhs |
| | ECC | 30 | 18.42 | 4.7 | | | 17.23 | 4.05 | | | 17.06 | 3.29 | | | 16.89 | 4.64 | | |
| | S-ECC | 30 | 30.15 | 7.4 | | | 28.66 | 7.24 | | | 28.49 | 6.54 | | | 28.13 | 5.56 | | |

its absence and that has a beneficial role in preventing the initiation and progression of caries.[2] (Table 2)

After the application of GC Tooth Mousse, the mean Salivary Calcium level at three different intervals was higher in the Caries free group than the ECC and S-ECC group. Whereas, the mean Salivary Phosphate levels were lower in Caries free group than ECC and S-ECC group at 3 different intervals but was higher than their baseline values. The initial Salivary Calcium and Phosphate levels did not differ much between the intergroups but at 30 minutes and 60 minutes there was significant difference seen between Caries free and ECC, S-ECC group due to the reduced local availability of calcium and phosphate.

This may be attributed to the complexes (Casein Phosphopeptides-Amorphous Calcium Phosphate ) which are found in the dental biofilm, that increase the Calcium Phosphate levels which serve as a reservoir for free calcium and phosphate ions. Thus providing a topical effect by maintaining ionic phosphate and calcium supersaturation, buffer the effects on plaque and increased remineralization. The mean Salivary Alkaline Phosphatase levels were lower in Caries free group than ECC and S-ECC group at 3 different intervals indicating its beneficial role. The Calcium levels in the saliva was higher after application of Tooth Mousse when compared with Milk and Cheese due to the more topical and stabilizing effect of calcium and phosphate by Tooth Mousse.[8](Table 3)

The Salivary Calcium levels were higher after administration of Milk and Cheese in all the three groups and at all the three intervals. But the mean value of Cheese was slightly higher than Milk. This could be due to adherence of cheese on the pits and fissures of the teeth .The increase in Salivary Phosphate levels were significant between Tooth Mousse and Milk and Cheese at all intervals in all the three groups. This could be because of the phosphate stabilizing effect by Tooth Mousse.[8] The difference was not significant between Milk and Cheese because of equal content of phosphate in them. The Salivary Alkaline Phosphatase levels were not significant after administration of Milk, Cheese and Tooth Mousse at three different intervals and at all the three groups which could be due to its absence (Tables 4, 5, 6).

Table 4. Comparison of Salivary calcium levels at different time intervals among different groups after intake of milk, cheese and application of tooth mousse.

| Group | Variable | Parameter (I) | Parameter (J) | Mean Difference (I-J) (mg/dl) | p-value |
|---|---|---|---|---|---|
| Caries free | Calcium <5 min | Milk | Tooth Mousse | -35.898 | <0.001 vhs |
| | | | Cheese | -.5133 | .997 |
| | | Tooth Mousse | Cheese | 35.385 | <0.001 vhs |
| | Calcium 30 min | Cheese | Milk | .5133 | .997 |
| | | | Tooth Mousse | -35.385 | <0.001 vhs |
| | | Milk | Tooth Mousse | -20.951 | <0.001 vhs |
| | Calcium 60 min | Tooth Mousse | Cheese | 20.293 | <0.001 vhs |
| | | Milk | Tooth Mousse | -16.363 | <0.001 vhs |
| | | | Cheese | -.888 | .876 |
| ECC | Calcium <5 min | Tooth Mousse | Cheese | 15.475 | <0.001 vhs |
| | | Milk | Tooth Mousse | -45.907 | <0.001 vhs |
| | | | Cheese | -2.688 | .840 |
| | Calcium 30 min | Tooth Mousse | Cheese | 43.219 | <0.001 vhs |
| | | Milk | Tooth Mousse | -16.741 | <0.001 vhs |
| | | | Cheese | -1.674 | .909 |
| | Calcium 60 min | Tooth Mousse | Cheese | 15.067 | <0.001 vhs |
| | | Milk | Tooth Mousse | -8.842 | 0.012 sig |
| | | | Cheese | -.743 | .967 |
| S-ECC | Calcium <5min | Tooth Mousse | Cheese | 8.099 | 0.024 sig |
| | | Milk | Tooth Mousse | -42.164 | <0.001 vhs |
| | | | Cheese | -3.510 | .844 |
| | Calcium 30 min | Tooth Mousse | Cheese | 38.654 | <0.001 vhs |
| | | Milk | Tooth Mousse | -16.839 | <0.001 vhs |
| | | | Cheese | -1.805 | .773 |
| | Calcium 60 min | Tooth Mousse | Cheese | 15.034 | <0.001 vhs |
| | | Milk | Tooth Mousse | -8.466 | <0.001 vhs |
| | | | Cheese | -.303 | .981 |

Higher levels of Calcium and Phosphate was seen in saliva of all the three groups after administration of Milk, Cheese and Tooth Mousse. Among all the three groups Tooth Mousse showed higher bioavailability of calcium and phosphate.

Saliva should be saturated with calcium and phosphate to affect their bioavailability in amounts adequate for remineralization. However, their threshold concentration in saliva that will initiate this process is yet to be established experimentally. Thus in the light of the information available at hand our study concludes that Milk, Cheese and GC Tooth Mousse are all beneficial to the dentition.

## CONCLUSIONS

Salivary Calcium levels decreased from Caries free to ECC and from ECC to S-ECC whereas Salivary Phosphate and Alkaline Phosphatase levels increased from Caries free to ECC and from ECC to S-ECC group.

Salivary Calcium and Phosphate levels were higher than the base line values after administration of Milk, Cheese and Tooth Mousse group at all 3 intervals . However the levels were statistically higher in the Tooth Mousse group followed by Cheese and Milk respectively.

Salivary Alkaline Phosphatase levels were decreased from the base line values after administration of Milk, Cheese and Tooth Mousse group at all 3 intervals. However the levels were higher in Milk group followed by Cheese and Tooth Mousse respectively.

Table 5. Comparison of Salivary Phosphate levels at different time intervals among different groups after intake of milk, cheese and application of tooth mousse

| Group | Variable | Parameter (I) | Parameter (J) | Mean Difference (I-J) (mg/dl) | p-value |
|---|---|---|---|---|---|
| Caries free | Phosphate <5 min | Milk | Tooth Mousse | -13.775 | <0.001 vhs |
| | | | Cheese | -458 | .987 |
| | | Tooth Mousse | Cheese | 13.317 | <0.001 vhs |
| | Phosphate 30 min | Cheese | Milk | -.098 | .996 |
| | | | Tooth Mousse | 9.755 | <0.001 vhs |
| | | Milk | Tooth Mousse | -9.853 | <0.001 vhs |
| | Phosphate 60 min | Tooth Mousse | Cheese | 8.639 | <0.001 vhs |
| | | Milk | Tooth Mousse | -8.865 | <0.001 vhs |
| | | | Cheese | -.230 | .988 |
| ECC | Phosphate <5 min | Tooth Mousse | Cheese | 13.185 | <0.001 vhs |
| | | Milk | Tooth Mousse | -13.185 | <0.001 vhs |
| | | | Cheese | .000 | 1.000 |
| | Phosphate 30 min | Tooth Mousse | Cheese | 12.511 | <0.001 vhs |
| | | Milk | Tooth Mousse | -12.823 | <0.001 vhs |
| | | | Cheese | -.312 | .975 |
| | Phosphate 60 min | Tooth Mousse | Cheese | 4.358 | <0.001 vhs |
| | | Milk | Tooth Mousse | -4.572 | <0.001 vhs |
| | | | Cheese | -.214 | .951 |
| S-ECC | Phosphate <5min | Tooth Mousse | Cheese | 16.574 | 0.024 sig |
| | | Milk | Tooth Mousse | -16.574 | <0.001 vhs |
| | | | Cheese | .000 | 1.000 |
| | Phosphate 30 min | Tooth Mousse | Cheese | 11.795 | <0.001 vhs |
| | | Milk | Tooth Mousse | -12.172 | <0.001 vhs |
| | | | Cheese | -.376 | .991 |
| | Phosphate 60 min | Tooth Mousse | Cheese | 9.146 | <0.001 vhs |
| | | Milk | Tooth Mousse | -9.606 | <0.001 vhs |
| | | | Cheese | -.459 | .922 |

Table 6. Comparison of Salivary alkaline Phosphatase levels at different time intervals among different groups after intake of milk, cheese and application of tooth mousse.

| Group | Dependent Variable | (I) GRP | (J) GRP | Mean Difference (I-J) | P |
|---|---|---|---|---|---|
| Caries free | <5mins | Milk | Tooth mousse | .2813 | .961 |
| | | | Cheese | .1527 | .988 |
| | | Tooth mousse | Cheese | -.1287 | .992 |
| | 30 minutes | Milk | Tooth mousse | .0410 | .999 |
| | | | Cheese | .1957 | .977 |
| | | Tooth mousse | Cheese | .1547 | .986 |
| | 60 minutes | Milk | Tooth mousse | .3300 | .935 |
| | | | Cheese | .1977 | .976 |
| | | Tooth mousse | Cheese | -.1323 | .989 |
| ECC | < 5mins | Milk | Tooth mousse | .5210 | .871 |
| | | | Cheese | .2083 | .978 |
| | | Tooth mousse | Cheese | -.3127 | .951 |
| | 30 minutes | Milk | Tooth mousse | .4900 | .885 |
| | | | Cheese | .0620 | .998 |
| | | Tooth mousse | Cheese | -.4280 | .911 |
| | 60 minutes | Milk | Tooth mousse | .5503 | .894 |
| | | | Cheese | -.0203 | 1.000 |
| | | Tooth mousse | Cheese | -.5707 | .886 |
| S-ECC | <5mins | Milk | Tooth mousse | .8193 | .896 |
| | | | Cheese | .2343 | .991 |
| | | Tooth mousse | Cheese | -.5850 | .946 |
| | 30 minutes | Milk | Tooth mousse | .5310 | .955 |
| | | | Cheese | .2877 | .987 |
| | | Tooth mousse | Cheese | -.2433 | .990 |
| | 60 minutes | Milk | Tooth mousse | 1.0693 | .823 |
| | | | Cheese | .8143 | .893 |
| | | Tooth mousse | Cheese | -.2550 | .989 |

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