Abstract

This study utilized volunteer subjects to examine caries-like lesions for remineralization and demineralization patterns in dentin and enamel when nine different snack foods were eaten between meals. Caries progression was observed in enamel and dentin when apple juice, a cola beverage, and sweetened (strawberry) yogurt were consumed as snacks. Remineralization of enamel was observed when cheddar cheese, skim milk, 2% milk, whole milk, chocolate milk, and orange juice were used as between meal snacks. Dairy products, with the exception of the sweetened yogurt, generally reduced the amount of demineralization produced in dentin. This study helps establish a scientific basis for appropriate between-meal snacks for patients who are concerned about their dietary habits as a part of their overall preventive oral health plan.

Keywords: Cariogenic potential, snack foods, remineralization, demineralization, enamel, dentin, human intra-oral remineralization/demineralization model, diet, caries
Following a screening examination, subjects were provided an explanation of the proposed research, a written information summary statement, and each conveyed informed consent to participate in the study.

Either a first or second permanent molar requiring a crown restoration was selected for placement of a test appliance for each individual. These teeth were prepared for a final restoration and two polyvinyl-siloxane impressions were obtained. One impression was used for fabricating the test appliance, and the second was used to fabricate the final crown. The specific design of the appliances replicated those described previously by Jensen and Wefel. The "slot" receptacle area in the proximal surface of each appliance was positioned below the contact area with the adjacent tooth to simulate the area where proximal carious lesions are frequently found. Test sections of human enamel and dentin containing caries-like lesions were placed in these "slot" areas with acid-resistant varnish for two-week test periods.

This research project was designed as a ten-way cross-over study. Each test period consisted of two weeks during which subjects were given all test food portions to eat three times each day between meals. The subjects were asked to (1) maintain a similar diet during all test periods, (2) consume the snack foods at the same times between meal times, and (3) eat the snack foods in a normal fashion as described to them. Consumption times were recorded in a diet log during the test periods. This diet log was returned to the study coordinator at the end of each two-week test period. No test food or other snack was consumed during a control period.

Introduction

The cariogenic properties of various snack foods is extremely important in dietary counseling for patients who wish to minimize their vulnerability to dental caries. The Vipeholm study established the concepts that frequency of consumption of fermentable carbohydrates and their retentive properties were primary considerations in the development of dental caries in humans. Other studies have focused on the cariogenicity of snack foods. It is clear there should be a scientific basis for recommending foods that can be safely consumed as between-meal snacks. Plaque pH telemetry is still used today as a basis for labeling snack foods and medications that are hypoacidogenic. However, plaque pH models do not take into account the mineral changes that actually occur in tooth structure. In addition, such models lack the capability of identifying anti-cariogenic foods, which result in remineralization, or at least prevent demineralization of tooth structure.

For these reasons, intra-oral remineralization and demineralization models appear to be a much more powerful tool for examining the cariogenic potential of snack foods. Such models have previously been used to identify processed cheese food as a beneficial snack for both enamel and dentin caries.

Materials and Methods

Fifteen healthy volunteers were selected for participation in this study. Eight male subjects had a mean age of 38 years and seven female subjects had a mean age of 36 years. The DMFS for females was 41.2 compared to 51.1 for males.
The test snack foods used in this study are listed in Table 1. These snacks were provided to each test subject in pre-portioned individual doses for each snack to be consumed by the volunteer. At the end of each two-week test period, the appliance was removed for test section recovery and microradiographic analysis.

A second appliance was then placed and the following test session was begun. Artificial caries-like lesions were created in normal dentin and enamel. This was achieved by drilling specimens 3mm in diameter from extracted human teeth that had been stored in 3% buffered (neutral) formalin. The specimens were mounted using methyl methacrylate to form a rod. Enamel specimens were ground for 5-10 minutes using 600 grit silicon carbide paper to remove approximately 50 microns from the surface. They were then polished for 8 minutes using Gamma Alumina to produce a high luster. Dentin specimens were similarly treated with the exception that grinding and polishing was carried out for only four minutes each. The surface of each specimen was covered with an acid-resistant varnish except for a 1.0mm strip area in the center of the surface. This varnish protected area was maintained as sound tooth structure for both the dentin and enamel specimens.

Artificial “caries-like” lesions were formed by placing each specimen in 13ml of solution containing 0.1 mol/L lactic acid, 50% saturated HAP, and 2% carbopol adjusted to pH 5.00 at 37°C. Enamel lesions were created using a 64 hour time period and dentin lesions were created using a 34 hour time period.

Following lesion formation, a control section (100 microns thick) was removed from each specimen and saved for analysis for mineral content by microradiography. A second, adjacent “treatment” section from each specimen was cut and assigned an identification number. All sections were kept in sealed containers moistened with wet cotton at all times to maintain hydration.

A prepared treatment section was carefully trimmed to fit the “slot” of the appliance to be used. All surfaces of the section except the outer test edge were covered with acid-resistant varnish prior to placement in the test appliance. One dentin section and one enamel section was placed in the test “slot” for testing. The enamel section was always placed at the occlusal position relative to the dentin section. After testing in the human volunteers, each test section was removed for analysis by microradiography.12

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Table 1. Snack foods used in the study

<table>
<thead>
<tr>
<th>TEST FOOD</th>
<th>SERVING</th>
<th>BRAND</th>
<th>MANUFACTURER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Milk</td>
<td>8 oz.</td>
<td>Kemps</td>
<td>Marigold Foods</td>
</tr>
<tr>
<td>2% Milk</td>
<td>8 oz.</td>
<td>Kemps</td>
<td>Marigold Foods</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>8 oz.</td>
<td>Kemps</td>
<td>Marigold Foods</td>
</tr>
<tr>
<td>Chocolate Milk</td>
<td>8 oz.</td>
<td>Kemps</td>
<td>Marigold Foods</td>
</tr>
<tr>
<td>Strawberry Yogurt</td>
<td>8 oz.</td>
<td>Dannon</td>
<td>Dannon</td>
</tr>
<tr>
<td>Medium Cheddar Cheese</td>
<td>1 oz.</td>
<td>Crystal Farms</td>
<td>Crystal Farms</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>8 oz.</td>
<td>Speas Farms</td>
<td>Sundor Brands</td>
</tr>
<tr>
<td>Cola Drink</td>
<td>8 oz.</td>
<td>Coca-Cola</td>
<td>Coca-Cola</td>
</tr>
<tr>
<td>Orange Juice</td>
<td>8 oz.</td>
<td>Kemps</td>
<td>Marigold Foods</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td></td>
<td>(Normal Diet without snacks)</td>
</tr>
</tbody>
</table>
The control (pre-treatment) sections, as well as the post-treatment sections, both 100 microns thick, were placed together on a microscope slide with an aluminum step wedge. Microradiographs were then made using IMTEC type 1A plates and x-rays generated at approximately 35 Kvp and 25 mamps at a distance of 30 cm for 10 minutes and processed.

The microradiographs were analyzed using a Zeiss EOM microscope and custom software on an IBM computer. The lesion depth (to 83% mineral for enamel and to 43% mineral for dentin) was determined in the lesion area. Control (pre-treatment) Delta Z and experimental (post-treatment) Delta Z values were calculated as follows:

\[ \text{Delta Z value for each section was determined and the change in mineral content (Delta M) was also calculated by subtracting pre-treatment (control) Delta Z from the post-treatment (experimental) Delta Z for each specimen.} \]

Statistical analyses were done using ANOVA followed by Tukey’s protected-T-tests to identify groups that were statistically significantly different.

**Results**

All fifteen subjects completed the ten test-periods during the cross-over design of the study. The sections were analyzed after recovery. Only twelve complete sets of data without any missing values (broken or damaged sections) were available. One set of sections was totally missing from the appliance when it was recovered from.

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**Table 2. Tukey’s Protected T-tests for Enamel**

<table>
<thead>
<tr>
<th></th>
<th>Chocolate</th>
<th>Control</th>
<th>2% Milk</th>
<th>Whole Milk</th>
<th>Orange Juice</th>
<th>Skim Milk</th>
<th>Cheese</th>
<th>Apple Juice</th>
<th>Cola Drink</th>
<th>Yogurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate</td>
<td>0</td>
<td>-1.222</td>
<td>0.843</td>
<td>1.565</td>
<td>-3.564**</td>
<td>1.104</td>
<td>2.235*</td>
<td>-5.005**</td>
<td>-5.795*</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>-1.222</td>
<td>0.026</td>
<td>5*</td>
<td>2.783**</td>
<td>-2.342*</td>
<td>2.326*</td>
<td>3.456**</td>
<td>3.456**</td>
<td>-4.573**</td>
<td>1.222</td>
</tr>
<tr>
<td>2% Milk</td>
<td>0.843</td>
<td>2.065**</td>
<td>0.718</td>
<td>0</td>
<td>-4.407**</td>
<td>0.261</td>
<td>1.392</td>
<td>-5.848**</td>
<td>-6.638**</td>
<td>-0.843</td>
</tr>
<tr>
<td>Whole Milk</td>
<td>1.561</td>
<td>2.783**</td>
<td>0.718</td>
<td>0</td>
<td>-5.125**</td>
<td>-0.457</td>
<td>0.674</td>
<td>-8.566**</td>
<td>-7.356**</td>
<td>-1.561</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>1.104</td>
<td>2.326*</td>
<td>0.261</td>
<td>-0.457</td>
<td>4.668**</td>
<td>0</td>
<td>1.131</td>
<td>-6.109**</td>
<td>-6.899**</td>
<td>-1.104</td>
</tr>
<tr>
<td>Cheese</td>
<td>2.235*</td>
<td>3.456**</td>
<td>1.3915</td>
<td>0.674</td>
<td>5.798**</td>
<td>1.131</td>
<td>0</td>
<td>-7.240**</td>
<td>-8.030**</td>
<td>-2.235*</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>-5.005**</td>
<td>-3.784**</td>
<td>5.858**</td>
<td>-6.566**</td>
<td>-1.442</td>
<td>-1.422</td>
<td>0</td>
<td>-0.790</td>
<td>5.005**</td>
<td>0</td>
</tr>
<tr>
<td>Yogurt</td>
<td>0</td>
<td>1.222</td>
<td>0.843</td>
<td>-1.561</td>
<td>3.564**</td>
<td>-1.104</td>
<td>-2.235*</td>
<td>5.005**</td>
<td>5.795**</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: * = p<0.05  ** = p<0.01
the subject. Sections from two other subjects were either fractured or did not allow for accurate microradiographic analysis. Therefore, the statistical analyses were performed using the twelve sets of complete data.

The ANOVA for the mineral content changes of enamel lesions showed highly statistically significant differences with a p<0.0001. The Tukey's protected t-tests are shown in Table 2 (page 4).

Mean mineral content changes showed remineralization of enamel lesions in the test snack groups of chocolate milk, the control (no snacks), 2% milk, whole milk, skim milk, cheddar cheese, and yogurt.

Demineralization was demonstrated in the enamel lesions from the mean mineral content measurements from the snack groups of orange juice, apple juice, and the cola beverage when used as between meal snacks.

The ANOVA of the mineral content data for the dentin lesions also showed statistically significant differences with a p<0.0001. The Tukey's protected t-tests shown in Table 3 illustrate how the groups differed. Mean demineralization values were obtained from all groups except the cheddar cheese, skim milk, and orange juice.

Table 3. Tukey’s Protected T-tests for Dentin

<table>
<thead>
<tr>
<th></th>
<th>Chocolate</th>
<th>Control</th>
<th>2% Milk</th>
<th>Whole Milk</th>
<th>Orange Juice</th>
<th>Skim Milk</th>
<th>Cheese</th>
<th>Apple Juice</th>
<th>Cola Drink</th>
<th>Yogurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate</td>
<td>0</td>
<td>-1.166</td>
<td>-1.030</td>
<td>-0.199</td>
<td>0.551</td>
<td>0.684</td>
<td>1.228</td>
<td>-2.436</td>
<td>-4.454**</td>
<td>-2.513**</td>
</tr>
<tr>
<td>Control</td>
<td>-1.166</td>
<td>0</td>
<td>0.136</td>
<td>0.967</td>
<td>1.718</td>
<td>1.851</td>
<td>2.394*</td>
<td>-1.270</td>
<td>-3.287**</td>
<td>-1.347</td>
</tr>
<tr>
<td>2% Milk</td>
<td>-1.030</td>
<td>0.136</td>
<td>0</td>
<td>0.831</td>
<td>1.581</td>
<td>1.714</td>
<td>2.258*</td>
<td>-1.406</td>
<td>-3.424**</td>
<td>-1.482</td>
</tr>
<tr>
<td>Whole Milk</td>
<td>-0.199</td>
<td>0.967</td>
<td>0.831</td>
<td>0</td>
<td>0.750</td>
<td>0.883</td>
<td>1.427</td>
<td>-2.237*</td>
<td>-4.255**</td>
<td>-2.314*</td>
</tr>
<tr>
<td>Orange Juice</td>
<td>0.551</td>
<td>1.718</td>
<td>1.581</td>
<td>0.750</td>
<td>0</td>
<td>0.133</td>
<td>0.676</td>
<td>-2.988</td>
<td>-5.005**</td>
<td>-3.065**</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>0.684</td>
<td>1.850</td>
<td>1.714</td>
<td>0.883</td>
<td>0.133</td>
<td>0</td>
<td>0.543</td>
<td>-3.120**</td>
<td>-5.138**</td>
<td>-3.198**</td>
</tr>
<tr>
<td>Cheese</td>
<td>1.228</td>
<td>2.394*</td>
<td>2.258*</td>
<td>1.427</td>
<td>0.676</td>
<td>0.543</td>
<td>0</td>
<td>-3.66**</td>
<td>-5.681**</td>
<td>-3.74**</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>-2.436*</td>
<td>-1.270</td>
<td>-1.270</td>
<td>-2.237*</td>
<td>-2.988**</td>
<td>-3.121**</td>
<td>-3.66**</td>
<td>0</td>
<td>-2.017*</td>
<td>-0.077</td>
</tr>
<tr>
<td>Yogurt</td>
<td>-2.513</td>
<td>-1.347</td>
<td>-1.483</td>
<td>-2.314*</td>
<td>-3.065**</td>
<td>-3.198**</td>
<td>-3.741**</td>
<td>-0.077</td>
<td>1.940</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: * = p<0.05    ** = p<0.01
The percentage change of the lesion values were also analyzed using ANOVA for both the enamel and dentin sets of data. These data from both types of tissue also showed highly statistical differences with p<0.0001. Figure 1 illustrates the mean percentage mineral content change data for the enamel specimens. Demineralization is shown (positive values) for the apple juice, cola beverage and yogurt snack groups. The data for the percentage change of mineral content for the dentin lesions is illustrated in Figure 2.

Discussion
Many human intra-oral model systems have been used over the past century to attempt to estimate the cariogenic potential of various food substances.13-22 Various in vitro test models were used as early as 1890 by Miller23 to assess the caries-producing capacity of foods when mixed with human saliva and then incubated. Similarly, many enamel demineralization tests have been used in vitro to examine foods and their cariogenic properties.24-27 "Artificial mouths” have also been used in an attempt to duplicate the human oral environment in order to measure cariogenicity.28-30

Animal caries models have also been used extensively in an attempt to estimate the cariogenic properties of various foods.31-32 None of these models is capable of accurately duplicating human conditions. Some of the most useful models have been human intraoral plaque pH measurements that do not assess a measure of cariogenicity or mineral content change. Plaque pH
telemetry methods have been very useful in estimating cariogenic potential from acid challenge measurements. These data have been helpful in even the ranking of foods according to cariogenic potential. However, plaque pH model systems do not take into account the actual mineral loss from the hard tissues. It is not known how to apply the data from such models to dietary recommendations if the foods tested are not hypoacidogenic.

Human intra-oral demineralization studies were pioneered by Von der Fehr to produce "white-spot" lesions on the teeth of volunteers given nine daily rinses of 50% sucrose while abstaining from normal dental hygiene procedures. Enamel blocks have been placed in intra-oral appliances to measure iodine-permeability and microhardness measurements have been used as well as mineral changes assessed with microradiography in model systems. Such intra-oral model systems have been used to examine the remineralizing or protective effects of cheese or other dairy products. The single-section intra-oral models have also been used to demonstrate protective effects of processed cheese when added as a snack to the diet of human volunteers.

The intraoral demineralization and remineralization model with human subjects was used in this study to evaluate the cariogenic potential of between meal snacks much in the same way as the model has been used to evaluate fluoride dentifrices and other therapeutic products. Similarly, this model has been used to evaluate the effects of such human dietary habits as chewing gum.

It is noteworthy that all volunteers in the study reported here continued all of their normal oral hygiene practices (except flossing at the section site), used an ADA accepted fluoridated dentifrice, and drank optimally fluoridated water. This finding makes the resultant remineralization and demineralization data from this study relevant due to the normal human conditions and is not artificially skewed as a test system. This fact increases the confidence that recommendations regarding the use of snack foods used in this study are relevant.

The results of this study showed highly statistically significant differences in the mineral content changes in both enamel and dentin sections from these snack foods eaten between meals. The yogurt group was different from the cheese and cola groups, while the whole milk group was different from the apple juice and yogurt groups. The cola group was different from all other groups except that the yogurt group showed a wide amount of variability.

It is somewhat difficult to understand that different results were obtained from the orange juice group for enamel and dentin specimens since they were
in the same appliances adjacent to each other. Perhaps this difference can be explained by the difference in tissue susceptibility (dentin vs. enamel) during the use of the fluoridated toothpaste and water. It is clear the acid attack from both the fermentable carbohydrates and the dietary acid in the orange juice was countered by the remineralization process in the sample dentin sections.

On the other hand, the acid attack from the normal diet (control group) was not increased from the addition of the between meal snacks in the groups of chocolate milk, 2% milk, and whole milk since these results show no difference from the control. This situation may indicate apple juice, yogurt, and the cola beverage have significant cariogenic potential to the dentin and cheese is significantly anti-cariogenic in its properties. The effect of chocolate milk, 2% milk and skim milk was different from the control indicating they were not cariogenic to the dentin or at least a difference could not be detected by this model under these test conditions.

The percentage change in lesion areas obtained in this study present an alternative manner of examining the results. In general, the results were similar to the mineral content change data with a few exceptions. The enamel lesions in the yogurt group showed a mean increase in lesion area of 19.6%, while the mineral content data showed substantial remineralization of the caries-like lesion. One possible explanation may be focused on the composition of this food substance. The “milk” components (calcium/phosphate, casein, and phosphoproteins) undoubtedly contributed to net remineralization of the lesions. Possible mechanisms of anticariogenic action for dairy products have been presented and discussed elsewhere and are relevant here. These mechanisms include a wide range of dairy product components. This is true because the Delta Z of the control group was -158 while being -247 for the yogurt group. In other words, this regime is providing more mineral somehow to the lesions. On the other hand, this particular yogurt was sweetened and flavored (strawberry). The added fermentable carbohydrate in the yogurt must have provided enough of an additional “acid attack” to cause net demineralization. The protective effects of the milk components and the volunteer’s own “host protective mechanisms” were not sufficient enough to halt progression of the lesions. Interestingly enough, the lesion body showed a net increase in mineral content over the initial lesion. This also means there was probably a “re-distribution” of mineral within the lesion.

Lewinstein et al demonstrated enamel “re-hardening” greater than salivary stimulation alone on softened enamel when cheese, cheese-flavored with strawberry marmalade, and sucrose-sweetened cheese were consumed. Surface re-hardening occurred within a single 5 minute test exposure to softened enamel which was free of a typical plaque layer. That model system was, therefore, not designed to take into account the complex interactions that occur in the presence of plaque at interproximal sites.

The changes in Delta Z for enamel specimens in the orange juice group showed slight remineralization, while the percentage change data was significantly larger. The Delta Z for the specimens in the orange juice group were different (p<0.05) from the control group, while the percentage area changes were not significantly different. The area of the lesions did not change for the orange juice group from that of the control. The mineral loss was evidently caused from the dietary acids in the orange juice as well as its fermentable carbohydrate, but the host protective mechanisms were strong enough to prevent lesions from increasing in size under these test conditions and time period.

When percentage lesion area changes of the dentin lesions were compared to the Delta Z data, the patterns are no different. The Delta Z data from the orange juice group and skim milk group showed slight remineralization trends but were not statistically significantly different from the control group. The percent of lesion area change for the dentin lesions showed a slight increase in area, but were not significant.
The use of snack foods in normal dietary patterns is extremely important for caries prevention. Bowen et al stated from animal studies that “... milk may have modest cariostatic properties when ingested at the same time as a cariogenic challenge.” The data from this study certainly supports the position that cariostatic properties may exist for dairy products when used as between meal snacks. Caution should be taken in considering these substances for use under different conditions such as reduced salivary flow or xerostomie patients, use in infant bottles, or even just prior to sleeping when salivary flow ceases.

Conclusion

Results from this study can be used to help increase effectiveness in caries prevention by recommending that apple juice, cola beverage, and sweetened yogurt be eliminated as between-meal snack foods. If between-meal snacking is necessary, cheese and other milk products used in this study should be considered. The reduction in dentin demineralization and actual remineralization of enamel caries-like lesions suggest these products may actually have anti-cariogenic potential.
References


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