EFFECT OF CHEWING GUM CONTAINING CALCIUM-FLUORIDE ON SALIVARY AND PLAQUE IN SITU

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Abstract

The aim of this study was to examine the effect of chewing gum containing calcium-fluoride, on the salivary fluoride concentration, pH, buffering capacity, flow rate, calcium concentration and total stimulation and plaque pH and fluoride concentration. Ten subjects (aged 8-12 years, 5 male-5 female) refrained from toothbrushing for 2 days. On the third day, they rinsed their mouth for 1 min with 10 ml of a 10 % sucrose solution. They started to chew either parafilm or one and two sticks of chewing gums for 10 min. Measurements of plaque pH and F concentrations and salivary samples were evalua-ted. In all three experiment groups (parafilm group, one stick chewing gum and two sticks chewing gum groups), plaque pH, salivary flow rate and buffering capacity increased obviously. The most pronounced recovery of plaque pH was registered for two sticks of chewing gum and difference between the parafilm and chewing gum at 15 min was significant (p<0.05). Salivary pH values differences were statistically significant between the initial and 0-1 min at all groups (p<0.05). Salivary flow rate obviously increased at 10 min in all groups while buffering capacity showed significant differences bet ween one and two sticks at initial and 55-60 min intervals (p<0.05). Chewing gum containing CaF highly increased Ca^{++} concentrations of saliva while there were no effect on F- concentrations.

Key Words: Chewing Gum, pH, Calcium, Fluoride, Flow rate, Buffering capacity, Plaque, Salivary

Kalsiyum Florür İçeren Cikletin Tükürük ve Plak Üzerine Etkisinin İn Situ İncelenmesi

Bu çalışmadaki amaç kalsiyum-florür içeren cikletin, tükürük florür konsantrasyonu, pH'ı, tamponlama kapasitesi, akış hızı, kalsiyum konsantrasyonu, toplam stimülasyonu ve plak pH'ı ve florür konsantrasyonu üzerine etkisini incelemektir. Çalışmada, yaşları 8-12 arasında olan 5'i kız 5'i erkek olmak üzere toplam 10 denek kullanılmıştır ve denekler 2 gün süreyle dişlerini fırçalamamışlardır. Üçüncü günde ağızlarını 1 dakika boyunca 10 ml % 10'luk sükroz çözeltisiyle çalkalamışlardır. Sonra 10 dakika süreyle, parafilm veya 1 ya da 2 ciklet çiğnemişlerdir. Plak pH ölçümleri, florür konsantrasyonları ve tükürük örnekleri değerlendirilmiştir. Üç deney grubunda (parafilm grubu, bir tane ciklet çiğneyen ve iki tane ciklet çiğneyen grup) da plak pH'sı, tükürük akış hızı ve tamponlama kapasitesi belirgin biçimde artmıştır. Plak pH'sının en belirgin iyileşmesi 2 tane ciklet çiğneyenlerde görülmüştür ve 15 dakikalık çiğneme süresinde ciklet ile parafilm arasındaki fark anlamlı bulunmuştur (p<0.05). Bütün gruplardaki başlangıç ve 0-1 dakikalık grupları arasındaki tükürüğün, pH değerleri arasındaki fark istatistiksel olarak anlamlıdır (p<0.05). Tükürük akış hızı bütün gruplarda 10 dakikada açıkça artmış, tamponlama kapasitesi bir ve iki ciklet çiğneyenlerin başlangıç ve 55-60 dakika aralıklarında önemli farklılıklar göstermiştir (p<0.05). Kalsiyum ve florür içeren ciklet çiğnenmesi, tükürüğün kalsiyum konsantrasyonunu oldukça artırmıştır, oysa florür konsantrasyonu üzerine etki etmemiştir.

Anahtar Kelimeler: Ciklet, pH, Kalsiyum, Florür, Akış hızı, Tamponlama Kapasitesi, Plak, Tükürük

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Introduction

A number of studies have shown that the consumption of fermentable carbohydrates promotes a plaque pH drop to values for below the critical pH at which enamel starts to demineralize (1-5). It is well documented the use of a sugar-free chewing gum raises plaque pH (6-8). The use of a sugar-free chewing gum has been demineralized enamel specimens in vivo and stimulated salivary flow rate (9-12).

Chewing gums containing Fluoride (F) are used as a fluoride administration in the Scandinavian countries (13). Chewing gum containing 0.25 mg F, allow frequent topical application as the daily F intake expected to be relatively low (14). Ekstrand et al. found that repeated daily chewing of F chewing gums had an inhibitory effect on the acidogenicity of dental plaque in vivo (15). F containing chewing gums have been shown to release F into a saliva at a rate comparable to F tablets (14).

The aim of the present study was to evaluate the effects of a chewing gum containing calcium fluoride on pH, F and Ca⁺⁺ concentration of plaque and buffer capacity, flow rate of saliva in children in situ.

Experimental

Subjects

Ten children (5 girls, 5 boys), aged 8-12 years participated in the study. All children presented good oral hygiene (dmf (t) = 2.1) and had no periodontitis or gingivitis. They all lived in Ankara-Turkey, where there is less than 0.3 ppm F in drinking water (16). They didn't use any drugs or antibiotics during the last two weeks.

Procedures

The chewing gum used was Vivident Calcium+Fluoride® (Ca+F) (Perfetti, Turkey). The composition of gum is presented in Table I. The parafilm (American National Can, Chicago, IL, 60631) was used in control group. Five days before the beginning of this study and also during the saliva and plaque study, the subjects refrained from using fluoridated dentrifices, dental floss or other F products. They were instructed not to clean their teeth for plaque study for two days before the test and come to the laboratory at 9 a.m. without having eaten or drunk anything during the last 8 hours. After the plaque study they participated saliva study at 2 p.m. No eating or drinking was allowed for 1 hour before taking the saliva collections. All participants used each of 3 groups (parafilm, one stick Ca+F chewing gum, two sticks Ca+F chewing gum), with an interval of at least one week. Each plaque pH experiment started with 1 min mounthrinse with 10 ml of 10% sucrose solution, after ten minutes followed by chewing parafilm, one or two sticks Ca+F chewing gum. The sucrose rinse was followed by a ten min pause in order to reach a low plaque pH, directly after the chewing started.

Table 1: Composition of the gum

Compounds	%
Xylitol	4
Sorbitol	50
Natrium fluoride	0.014
Calcium lactate	0.898

Plaque pH

Eight subjects participated in this part of study. Plaque pH measurements were performed with irridium touch microelectrodes (diameter 0.1 mm, Beetrode NMPH 3; W.P. Instruments, New Hawen, CT, USA) and reference electrodes DRIREF 2 (Beetrode, W.P. Instruments, New Hawen, CT, USA) connected to battery run Orion SA 720 pH/ISE meters (Orion Research, Cambridge, MA, USA). A reference salt bridge was created by the test subject having one finger dipped into a 3M KCl solution also containing reference electrode (17). After the baseline (0 min) pH responses were recorded, the test sessions were initiated. An irridium touch microelectrode was inserted into the approximal plaque between second decidious molar and first permanent molar in both mandibular and maxillar quadrants. Each examination series, comprising pH measurement of all selected sites in a subject, took in our hands 1-2 min. Before and after each such examination series, the electrodes were immediately calibrated according to Scheie et al. (18). All examinations were done by one investigator. pH measurements were carried out before the chewing (0 min), during the chewing period (5, 10 min), and after the chewing period (15, 20, 30, 40, 55 and 70 min) and recorded (Figure 1). 10 min after the sucrose rinse the subjects chewed parafilm, one and two sticks gums.

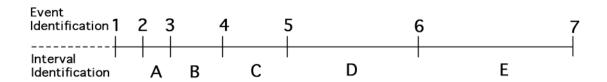


Figure 1: Sequence of events during test sessions

1: Record starved plaque pH and plaque sampling, 2: Rince with 10% sucrose solution, 3: Complete sucrose rinse and measure sucrose response, 4: Complete sucrose response measurement and measure test response, plaque sampling, 5: Complete chewing gum and measure test response, plaque sampling, 6: Plaque sampling, stop to measure plaque pH, 7: Plaque sampling, A: 1 min sucrose challenge, B: 10 min sucrose response record, C: 10 min gum chewing, D: 50 min measurement of gum chewing effect, E: 60 min no eating or drinking until plaque sampling period-waiting period. (Total time of the intervals is 130 min).

Plaque F Measurements

Ten subjects participated in this part of study. Prior to chewing gum, baseline Ca⁺⁺ and F levels plaque samples were collected from all accessible sites using a steril plastic spatula except for approximal surfaces where pH measured and lingual surfaces of lower incisor. After the sucrose challenge, subjects chewed parafilm and gums for ten minutes. Plaque was sampled after 10, 60 and 120 min chewing. The sampled plaque from each individual was transferred to preweighed small plastic porter (3x0.5 cm) and put into a preweighed 5 ml polypropylene tubes. The tubes were weighed after the plaque sampling and plaque wet weight was at least 2 mg. Plaque samples which were lower than 2 mg were excluded the study. A volume of 1 ml TISAB II buffer (Orion Research Inc, Boston, MA, USA) and 1 ml deionized distilled water (1:1) was mixed with plaque material. The tube was then vibrated using a vortex for 30 second (19). The sample was frozen at -18 °C until analysed. Before analysis the plaque suspension was homogenized by sonication for 5 second (Ultrasonic Homogenizer 4710, Cole Palmer Inst. Co., IL, USA). The F concentrations of the samples were measured using on ion-specific electrode (Orion 96-09, Orion Research, Inch. Cambridge, MA, USA).

Salivary Collection Procedure and Analyses

Ten subjects participated in this study every day at 2 p.m. The whole saliva was collected from each subject for the determination of baseline Ca⁺⁺ and F levels, pH, flow rate and buffering capacity. No food had been consumed during the previous hour. Prior to collection, it was given to the children to relax and they were informed about the collection procedure. The sample was collected into a graduate 2 ml dental plastic injector, which needle was removed and tip of it was sealed with parafilm and used as a plastic funnel. The salivary flow rates were expressed as ml/min. They chewed test gums and parafilm for ten minutes and at 1-2, 2-4, 4-6, 6-8, 8-10, 20-25, 35-40, 55-60 min saliva were sampled (Figure 2).

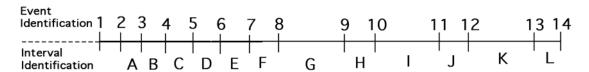


Figure 2: Sequence of events during test sessions

1: Collect saliva to determine baseline Ca and F levels, pH, flow rate and buffer capacity, 2: Initiate using test gum chewing, 3, 4, 5, 6, 7: Gum chewing, 8: Complete gum chewing, 9, 10, 11, 12, 13, 14: No chewing (waiting) period, A, B: 1 min; collect saliva to determine pH, flow rate, Ca and F levels, C, E, F: 2 min; collect saliva to determine pH, flow rate, Ca and F levels and buffer capacity, G, I: 10 min waiting period, J: 5 min; collect saliva to determine pH, flow rate, Ca and F levels, K: 10 min waiting period, H, L: 2 min; collect saliva to determine pH, flow rate, Ca and F levels and buffer capacity. (Total time of the intervals is 60 min).

The participants were instructed not to eat or drink anything or rinse their mouth during the sampling. After the sampling procedure, the samples were immediately analysed by Electrolyte Analyser (AVL company 988-4, Graz, Austria) and pH, Ca level were determined.

For determination of F concentrations, 0.750 ml saliva sample was transferred into 1.5 ml test tube (Eppendorf PSR, Sarstedt, Numbrecht, Germany) and frozen at -18 °C until analysed. Before analysis they allowed to room temperature and mixed with 0.750 ml TISAB II buffer (Orion Research Inc, Boston, MA, USA). The salivary F concentration was measured using the ion-specific electrode. The salivary buffer capacity was measured as the method of Krasse which is modified for small volumes (20). 0.5 ml saliva was mixed with 1.5 ml 5 mmol/L HCl, vigorously shaken and waited ten minutes to allow touch on air and then pH was measured.

Statistical Methods

Statistical evaluation of differences in salivary and plaque values were performed using one way ANOVA and t tests. The level of statistical significance was set at 0.05. Values for the Area Under Curve (AUC) (pH x time) of the response curve below 6.0 were transformed to logarithms before analyses because of the substantial heterogenity of variance of the data and analyzed using one way ANOVA.

Results

Plaque pH

The results regarding the plaque pH measurements were obtained from the human subjects by microelectrode method exhibited in Figure 3. The initial pH values of the related three groups were determined to be in the range of 6.75-6.96. The difference was not statistically significant (p>0.05). After using 10% sucrose mouthrince for 10 min the plaque pH value decreased in all groups (Figure 3). After chewing period the plaque pH values increased in a statistically significant manner over 15 min (parafilm 6.956 \pm 0.349, one stick Ca+F 7.23 \pm 0.20, two sticks Ca+F 7.67 \pm 0.72) (p<0.05). Although the obtained data was observed higher than the initial pH values, the difference was not statistically significant (p>0.05). When the difference between the initial pH values and at the measurement periods were compared with the three groups, the only statistically significant data was obtained at the 0 and 15 min between the parafilm and two Ca+F chewing groups (p<0.05). When the pH variation in three experimental groups related time was evaluated by plotting and the AUC was calculated by logarithmic values below pH 6, no statistically significant difference was found between the groups (Figure 4) (p>0.05).

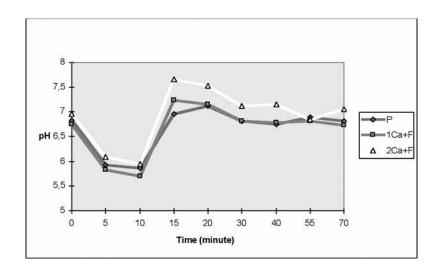


Figure 3. Variation of plaque pH versus time.

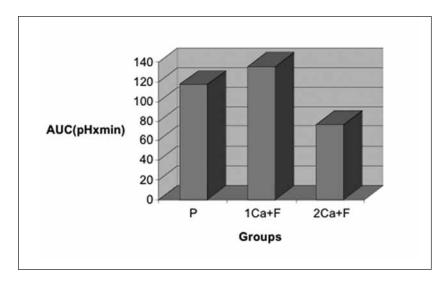


Figure 4. Variation in AUC values versus time.

Plaque F

The initial (0 min) and after the chewing period, plaque F values (at the 10, 60 and 120 min) of the subjects were exhibited in Figure 5. As the plaque samples having the weight below 2 mg were discarded from the experiments, the study was completed with 7 subject in parafilm, 7 subject in one Ca+F gum group and 8 subjects in two Ca+F gum groups. The F concentration variation between the groups related to time was not signi-ficant after the statistical evaluation (p>0.05).

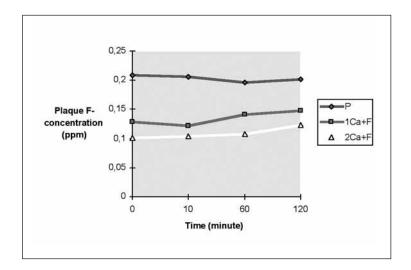


Figure 5. Variation in plaque F concentration versus time.

Salivary pH

The salivary pH values of the human subjects are shown in Figure 6. These values showed variation depending on time for all experimental groups and the difference was found to be statistically significant (p<0.05). But pH difference in saliva was insignificant among the groups (p>0.05). The maximum pH values were reached in parafilm chewing group at 6-8 min, (7.339 \pm 0.237), at 8-10 min in one Ca+F chewing group (7.482 \pm 0.086) and at 8-10 min in two Ca+F chewing groups (7.506 \pm 0.180). When the values of the three groups were compared, the difference between the unstimulated and 0-1 min was significant (p<0.05) only in parafilm and two Ca+F groups.

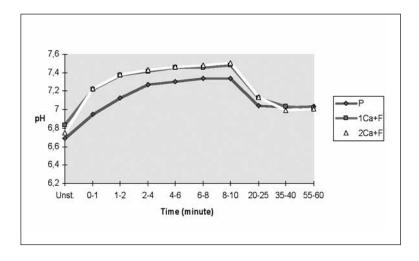


Figure 6. Variation in salivary pH versus time.

Buffering Capacity of the Saliva

Results are shown in Figure 7. Buffering capacity of the saliva were significantly increased from 5.51 to 6.15 in parafilm group, 5.72 to 6.57 in one stick group and 6.01 to 6.66 in two sticks group at the time interval of 4-6 min. In two Ca+F chewing groups, the 4-6 min value (6.66±0.29), 20-25 min value (6.01±0.53) and 55-60 min value (6.14±0.32) were above the initial value (5.76±0.44). These differences were found to be significant (p<0.05). When the buffering capacity of the saliva was examined in the three groups, it was concluded that their variations seem to be parallel. When the buffering capacity values of only initial and 55-60 min were compared, only the difference between one Ca+F chewing group and two Ca+F chewing groups were found to be significant (p<0.05).

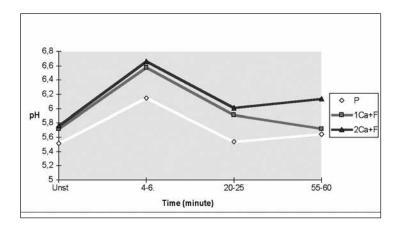


Figure 7. Variation in buffering capacity of saliva versus time.

Salivary Flow Rate

The salivary flow rate results of the subjects before and after chewing gum are exhi-bited in Figure 8.

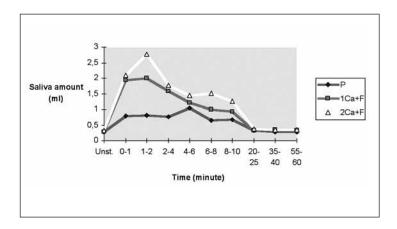


Figure 8. Variation in saliva flow rate versus time.

A significant increase in salivary flow rate was determined in all three groups following the chewing in 10 min (p<0.05). When the differences were evaluated up to the initial values in three experimental groups; the significant differences were obtained at 0-1, 1-2 and 2-4 min between parafilm and one Ca+F chewing group; at 0-1, 1-2, 2-4 and 8-10 min between parafilm and two Ca+F chewing groups and at 1-2, 6-8 min between one Ca+F and two Ca+F chewing groups (p<0.05).

Salivary Ca⁺⁺ concentration

The related data were shown in Figure 9. In parafilm chewing group, all of salivary Ca⁺⁺ data were measured lower than the initial value (1.20±0.298) and this difference was found statistically significant (p<0.05). In one Ca+F chewing group, all the chewing period values obtained higher than the initial measurement (1.11±0.32) and these differences were significant (p<0.05). Although at 20-25 min salivary Ca⁺⁺ values were obtained closer to initial values and at 35-40 and 55-60 min salivary Ca⁺⁺ values obtained lower than the initial values, the differences were found to be significant (p<0.05). In two Ca+F chewing gum groups, the data was obtained as similar as the other two groups and the difference was significant (p<0.05).

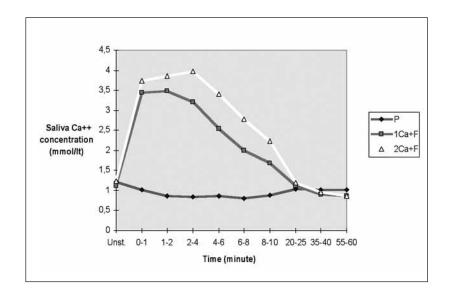


Figure 9. Variation in saliva Ca⁺⁺ concentration versus time.

When the variations of salivary Ca⁺⁺ concentration of one stick chewing gum group and two stick chewing gum groups were compared with parafilm group, the initial values at 0-1, 1-2, 2-4, 4-6, 6-8 and 8-10 min were found to be significant. On the other hand, the initial values at 6-8 and 8-10 min were statistically different when the one stick chewing gum and two stick chew-

ing gum groups were compared. The differences between the groups which were mentioned above found to be significant at the stated time points (p<0.05).

Salivary F Concentration

Data regarding salivary F concentration is obtained as shown in Figure 10. In parafilm chewing group initial F value (0.027±0.012) increased to 0.032±0.012 at 0-1 and 2-4 min after chewing and this difference was statistically significant (p<0.05). The values obtained afterwords have not shown any significant difference (p>0.05). In one Ca+F group, initial F value (0.030±0.014) have shown a significant increase at 0-1, 1-2, 2-4, 4-6 and 6-8 min after the chewing has started and this variation was statistically significant (p<0.05). In this group, maximum salivary F concentration was measured at 0-1 and 1-2 minutes as being 0.044±0.020. At other measurement times, lower values were obtained as similar to initial values. In two Ca+F chewing gum groups, initial F value (0.037±0.019) showed a significant increase after the chewing has started at 0-1, 1-2, 2-4 and 4-6 min in which the difference was determined as statistically significant (p<0.05). Maximum F concentration was gained at 2-4 min (0.066±0.030) in this group. The mea-sured values were decreased back to initial values for other time points and groups showed differences concerning salivary F concentration related to time. When two of the Ca+F gums were chewed, F concentration at 1-2 minutes found significantly different from parafilm chewed group (p<0.05). Other differences between the groups were found to be insignificant (p>0.05).

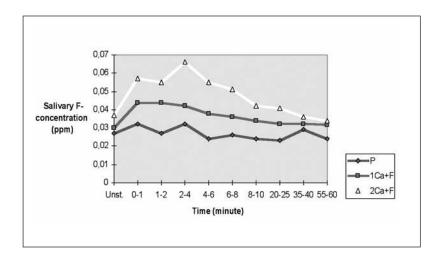


Figure 10. Variation in salivary F concentration versus time.

Total Stimulation Amount

Total stimulation amount formed in three experimental groups during chewing showed in Figure 11. Total stimulation amount in three groups were found to be different from each other and results were determined to be statistically significant (p<0.05). The highest values were obtained in two sticks of chewing gum.

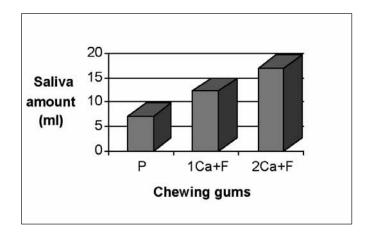


Figure 11. Variation in total stimulated saliva amount.

Plaque Weight

The mean plaque weight related to the subjects and their variation regarding time was determined as exhibited in Figure 12. The difference in plaque weight was not found to be important statistically (p>0.05).

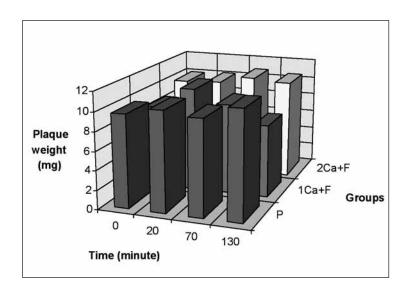


Figure 12. Variation in plaque weight versus time.

Discussion

In recent years, chewing gums were recommended as a caries-preventive method in most countries. F containing chewing gums have been found to give similar salivary F concentrations as the other F sources (14, 21). Chewing gums stimulate salivary flow, which facilitates the clearance of plaque, raises salivary and plaque pH, promotes enamel remineralisation and allows frequent topical application with relatively low dosage of F (22-24). The present findings have shown that fluoride chewing gums release most of its fluoride within the first 4 min of chewing (Figure 10). The salivary fluoride concentrations were attained to peak value at 2-4 min after chewing one and two sticks. The salivary fluoride levels found after chewing two sticks of gum (Figure 10), a dosage of 0.06 mg F was considerably lower than the levels reported from different fluoridated chewing gums (14, 25, 26). This may be due to the different F levels of the gums. On the other hand the initial salivary F concentrations in this study were also very low, probably that all our subjects lived in an area with low water F (< 0.3 ppm) and had no access to any F supplements (16). With the sampling technique used in the present study, the samples stimulated saliva will most likely contain a mixture from different local sites and result in an average fluoride concentration for the individuals (27).

Most of the calcium from the chewing gums was also released within the first 4 min of chewing as fluoride results (Figure 9). Chewing two sticks of Vivident Ca-F increased the salivary calcium levels to 3 times. However, there were no significant difference in calcium concentrations of saliva samples (except 8-10 min interval between one and two sticks of gum) (p>0.05).

The salivary flow rate is a primary factor in determining the time necessary for plaque pH recovery to resting leves. Saliva stimulation by chewing may be influenced by various characteristics of the chewed material, such as portion size, texture, flavour, stickness, as well as the duration of chewing. Another factor of importance for pH recovery is the buffering activity of saliva (8). The present result has shown that with all products, salivary flow rate peaked in the first minute of stimulation and then fell progressively with time. With the flavoured chewinggums there was little difference in their ability to stimulate flow (Figure 8) and by 6-8 min, two sticks of gums elicited significantly more saliva than did the gum base. These results are supported by other authors (10, 11, 28, 29). The cause of the decline in flow rate may be due to the reduction in gustatory stimulation due to loss of tastants from the gum, a reduction in mechanical stimulation due to the decrease in size of each gum with time, adaptation to the tastants, a reduction in the frequency or intensity of chewing time and a reduction with time in the secretory capacity of the saliva glands (11). On the other hand, the low concentration of fluoride in the saliva during the entire experimental period may be due to dilution effect of the stimulated saliva (27). The buffering capacity of the saliva between the groups were seen to be parallel the present study. The difference between the initial and 55-60 min levels were statistically significant in one and two sticks. At the two sticks group, buffering capacity of the saliva increased at 4-6 min, 20-25 min and 55-60 min. There are many difficulties in measuring the pH of whole saliva because when saliva is exposed to mouth air or to atmospheric air as it is spat into a collection tube, CO₂ will tend to be lost, causing a rise in pH (11). On the other hand, the stimulation of the salivary flow rate may at the same time have an effect on raising salivary pH (13). The salivary pH values showed a variation related to time in all experimental groups and the differences were statistically significant between groups (p<0.05). The increase in salivary pH on stimulation is may be due to the increase in flow rate.

The results regarding the plaque pH and F measurements obtained from the present study exhibited in Figure 3 and 5. The pH response to the sucrose challenge was not below 5.5 during the entire 10 minutes in this study. This is probably due to the increase stimulation effect of the sucrose solution on the salivary flow rate in children which promotes neutralization on plaque pH (30). In this study, it has been showed that fluoridated chewing gums and parafilm had a neutralizing effect by elevating plaque pH values towards neutrality after a sucrose rinse. Plaque pH recovery was most pronounced after 15 min gum chewing. The values started to decline to initial values after the end of chewing period and stayed at constant levels. Many studies showed that neutralizing effect could be evaluated by parafilm, sweeteners or fluoridated containing chewing gums (8, 13, 31, 32). This is probably due to gum chewing which stimulates the salivary flow rate leading a systemic effect on plaque pH and the clearance of plaque.

The plaque F- concentration has not been changed in all three groups in this study. Although Hattab et al. found that chewing two sticks of fluoridated chewing gums increased the plaque fluoride levels to 1.7 times, it may be due to difference between the drinking water F levels of the subjects and the difference of F levels of the gums (25).

Conclusion

The results from this study indicate that chewing gums is favourable to the plaque pH recovery, increasing salivary flow rate and buffering capacity. Ca+F chewing gums used in this study could highly increase the calcium concentration but has no effect on F concentration. The dose of F in the chewing gums should be arranged at effective levels.

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