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Original Article

Effect of Chewing Gum Containing CPP-ACP on Salivary Flow and Buffer Capacity: An in vivo Study

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Abstract

Objective: To evaluate salivary flow and buffer capacity by means of mechanical and chemical-mechanical stimuli, through the use of chewing gums. **Material and Methods:** The study was a cross-sectional study with 12 volunteers, divided into three groups, in three phases: Group A: paraffin gum; Group B: Chewing gum without sucrose, flavored (Trident®); Group C: Flavored chewing gum, without sucrose and amorphous calcium casein-phosphate phosphopeptide (Trident Total®). The stimulated total saliva was collected after 5 minutes of mastication of one of the products and the volume was expressed in mL / min. The same sample was submitted to pH measurement with the use of a digital potentiometer, where the results were classified in normal buffer capacity (final pH between 5.0 and 7.0) or low (final pH <4.0). The results were evaluated regarding the normality of the sample distribution (Shapiro-Wilk test), Analysis of Variance (ANOVA) and Tukey's test. **Results:** Chewing gums increased the salivary flow of the volunteers, when compared to the control group (paraffin) (1.53 mL / min), differing statistically from the group, although there was no difference between Trident® (2.09 mL / Min) and Trident Total® (2.06mL / min). Regarding the buffer capacity, the values obtained were 6.94 (paraffin), 6.99 (Trident®) and 6.93 (Trident Total®), with no difference between groups (p = 0.713). **Conclusion:** It was concluded that chewing gums, with and without CPP-ACP, increased the salivary flow in relation to the control group. In relation to buffer capacity the values obtained for chewing gums with and without CPP-ACP, are shown to be within the normal range.

Keywords: Saliva; Chewing Gum; Caseins.

Introduction

Saliva is the glandular secretion that bathes the oral cavity, responsible for the maintenance of oral homeostasis [1,2]. The importance of salivary flow in maintaining oral health is related to its protective properties: lubrication, digestion, antimicrobial action, bacterial agglutination, salivary pellicle formation, taste, water balance, cleaning, buffering and remineralizing action [1,2].

These properties and functions are closely related to the composition of saliva, which may suffer variations when there salivary stimulation. Oral conditions such as tooth decay, erosion, abrasion and dental attrition, candidiasis and erosion of the oral mucosa are changes frequently observed in patients with hyposalivation [2,3]. Saliva stimulated has the greatest potential to repair minerals lost by the teeth (remineralizing property) than unstimulated saliva and this is due to its higher pH, as well as calcium, phosphate, and proteins work together as an antisolubility factor and modulate demineralization and remineralization [3]. Therefore, if a chewing gum besides increase the salivary flow is source of these ions, the remineralization could be more effective [4].

To increase the salivary flow has been advocated the use of chewing gums, which promote an increase in salivary immediate and lasting flow, with the approach widely accepted by most patients and their use has been shown to be able to improve some functions / properties of saliva as a lubricant action, taste, cleanliness, buffering and remineralizing action [5]. Thus, a reduction in caries can be expected in patients undergoing chewing sugar less chewing gums [6].

Some agents have been incorporated into the gums in order to increase the remineralizing effect of saliva. The incorporation of CPP-ACP (casein phosphopeptide-amorphous calcium phosphate) known as Recaldent® has shown potential as anticaries agent, particularly in studies in situ model [7].

CPP-ACP can be used as supplementary strategy to enhance precipitation mineral at enamel initial erosions, whereas the saliva is able to increase the hardness of demineralized enamel after use gum containing CPP-ACP [8]. Regarding the in vivo studies, a significant difference was found in the calcium concentration in saliva after use gum with CPP-ACP [9]. However, there is still a discussion whether the protective effect can be attributed entirely to the substance used, or increased salivary flow may be responsible for the reduction of dental caries [10].

Although the anticariogenic effect of CPP-ACP has been demonstrated in different models such as in situ studies [7, 11] in children [8,12], as well as its anti-erosive potential [8,13-15]; there is a lack of these findings in adults. Thus, the objective of this study was to evaluate if the increase of the salivary flow caused by the mechanical stimulus could be optimize the buffering effect of CPP-ACP.

Material and Methods

Ethical Aspects

This study was approved by the Ethics Committee of the Federal University of Amazonas (Protocol N. 02317712.0.0000.5020).

Experimental Design

The study involved a cross-over design with twelve volunteers divided into three groups. The total period of the study comprised 3 stages with an interval of thirty days between each phase (wash-out). Sample size calculation was based on a previous study [14], built on α-error of 5 % and β- error of 20 %.

The treatments groups were: Group A: 1g of unflavoured paraffin (Parafilm® - BEMIS, USA); Group B: a unit (8g) of flavored sugar free chewing gum, without CPP-ACP (Trident® peppermint, Bauru-SP, Brazil); Group C: a unit (8g) of flavored sugars free chewing gum, with CPP-ACP (Trident Total® - Recaldent, 18.8 mg CPP–ACP/gum, peppermint, Bauru-SP, Brazil).

Research Subjects

Participated twelve young-adults, dental students, of both sexes (9 females and 3 males, age 20 - 25 years old), non-smokers, in good general and oral health (not oral pathology such as untreated caries, erosion lesions, active periodontal disease or others), which did not use dentures or orthodontic appliances (inclusion criteria); who did not use medications that interfere with salivary flow, namely, diuretics, laxatives, antacids, anorexic, antihypertensives, antidepressants, antipsychotics, sedatives, antihistamines, anticholinergics, antiparkinsonian, as well as those who are under stress situations and systemic conditions such as rheumatoid arthritis, Sjogren's syndrome, chemotherapy, radiation therapy in head and neck, infection by the human immunodeficiency virus (HIV), hepatitis C, diabetes mellitus, hypothyroidism, menopause, nephritis, and neurological diseases (exclusion criteria). All the volunteers selected had salivary flow and buffer capacity within normal values.

Collection of Saliva and Salivary Flow

Total and stimulated saliva was collected between 8:00 and 9:00 am, at least one hour after consumption of drinks or foods. Participants were asked to sit and relax for a few minutes. Each subject received one of the products (1 g of film paraffin or sucrose-free chewing gum, flavored or chewing gum flavored, sucrose-free and casein phosphopeptide-amorphous calcium phosphate), according to the experimental design. The substrate was retained in the mouth so that it was softened and saliva produced in this time (30 seconds) was swallowed. Thereafter, the substrate was chewed and all the saliva produced in a time of 5 minutes was collected in a measuring cylinder. The amount of saliva produced was measured and the rate of secretion expressed in milliliters per minute. The salivary flow rate was then classified as normal (1-2 mL / min.); decreasing (0.7 - <1.0 mL / min) and hyposalivation (<0.7 mL / min.) [16].

Buffering Capacity Assessment

Saliva sample was the same as obtained for the analysis of salivary flow. The pH was measured directly using a potentiometer (Orion™ Versa Star Pro™ pH, Massachusetts, USA)

coupled to a pH electrode (Thermo Scientific Orion, 8102BNUWP, Massachusetts, USA). The instrument calibration was performed with standard pH 4.0 and 7.0. Was then added 4 mL of saliva 80μL of 0.1 N hydrochloric acid, the sample was stirred and after 10 minutes resting, the final pH was measured. After reading, the results were classified as follows: The normal buffer capacity (final pH between 5.0 and 7.0) or low buffer capacity (final pH <than 4.0) [17,18].

Statistical Analysis

The data were presented by descriptive statistics, which are calculated the mean and standard deviation (SD). The data were evaluated for normal distribution using the Shapiro-Wilk test at 5% significance level. When comparing the means we used the analysis of variance (ANOVA) and Tukey test. The software used in the analysis was the Epi-Info Version 7 software for Windows developed and freely distributed by the CDC (www.cdc.org/epiinfo). The significance level set in the tests was 5%.

Results

Masticatory stimuli (chemi-mechanical) caused by the use of chewing gum increased salivary flow rate of volunteers when compared to the control group (mechanical stimuli) ($p = 0.024$), although without presenting difference between the gums with and without CPP-ACP (Table 1). It has further been found that the values are characterized as normal salivary flow.

Table 1.Distribution according to the average salivary flow (mL / min.) into different groups.

Groups	n	Mean	Standard Deviation	Amplitude
Paraffin	12	1.53 ǂ	0.57	0.80 – 2.60
Trident®	12	2.09 ‡	0.53	1.60 – 3.20
Trident total®	12	2.06 ‡	0.53	1.40 – 3.10

p = 0.024 (ANOVA); Different codes indicate statistical difference at the 5% level of significance by the *Tukey* test.

After examining the buffer capacity of the collected saliva it was observed that there was no difference between the groups, showing that gum with CPP-ACP has not improved the saliva buffer capacity compared to gum without CPP-ACP. The observed values of the different groups were presented with normal buffer capacity (Table 2).

Table 2. Distribution according to the mean buffering capacity for the diferente groups.

Groups	n	Mean	Standard Deviation	Amplitude	p-value*
Paraffin	12	6.94	0.22	6.37 – 7.25	
Trident®	12	6.99	0.19	6.68 – 7.28	0.713
Trident total®	12	6.93	0.25	6.61 – 7.40	

*ANOVA.

Discussion

The present study analyzed if the increase in salivary flow caused by the mechanical stimulus could optimize the buffering effect of CPP-ACP.

The results made it possible to observe that the masticatory stimuli (chemi-mechanical) obtained by the use of chewing gum to increase salivary flow of volunteers, compared with the control group (mechanical stimuli). The literature has shown positive results with use of chewing gum to stimulate salivary flow [5,19]. Our results suggest that the increase of the salivary flow by mechanical stimulation can be enhanced by chewing gums by combining with flavorings that allow the stimulation of chemoreceptors [20]. The data presented are supported by studies comparing chewing gums with and without flavor, which show that flavored gums produce a greater increase in salivary flow [20,21]. Besides of this, it has been estimated that 85% of salivary flow is related to gustatory stimulation, while 15% to masticatory stimulation [22]. Indeed, some authors [23] observed that the therapy with chewing gum there has been an increase of salivary flow even after 90 minutes as compared to measurement of unstimulated salivary flow and that salivary glands responded sharply in presence of a taste stimulus present in the gum. The flavor of chewing gum can influence the salivary flow rate and the results showed that the salivary flow was significantly increased in all 5 flavors of chewing gum (mint, cinnamon, watermelon, strawberry and apple) [24]. In this sense, the results of this study corroborate those obtained in the literature, since the masticatory stimulus (chemi-mechanical) by chewing gum increased salivary flow compared to the control group (mechanical), differing statistically the same ($P = 0.024$), although without presenting difference between Trident® and Trident Total® (Recaldent).

Regarding the saliva buffering capacity, it was observed in this study there was no difference between the groups, showing that the Trident Total® not improved the saliva buffer capacity compared to the others products used. These results can be explained by the fact that the saliva stimulated is able to promote an increase in bicarbonate concentration and therefore increases the buffering capacity [4], therefore, this may justify the value obtained for control group.

Other studies found significant difference in the calcium and phosphorus concentration of saliva before and after chewing CPP-ACP containing chewing gum [9]. In addition, a systematic review concluded that there is sufficient clinical evidence to demonstrate enamel remineralization and caries prevention through the regular use of CPP-ACP-containing products [25], being considered as one of the most evidence-based remineralization technologies for clinical use [26].

In the present study, the buffer capacity obtained after use of chewing gum containing CPP-ACP (Trident Total®) did not differ gum without CPP-ACP and the control group, which may be explained by methodological limitations, since only pH was measured, while other studies have evaluated the phenomena of demineralization and remineralization through clinical research involving analyzes of calcium and phosphate ions. In our study, the subjects were not submitted to erosive or cariogenic challenges, which on the other hand represents a contribution on the use of CPP-ACP gums in adults under normal conditions.

Conclusion

It was concluded that chewing gums, with and without CPP-ACP, increased salivary flow in relation to the control group. Regarding buffer capacities, the values obtained for chewing gums with and without CPP-ACP showed to be within the normal range, as well as the control group.

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