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Procjena koncentracije kalcija i fosfata u slini prije i poslije žvakanja žvakaće gume s CPP – ACPom

Evaluation of Salivary Calcium and Phosphorous Concentration Before and After Chewing CPP-ACP Containing Chewing Gum

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Sažetak
Svrha: Željelo se procijeniti kolika je koncentraciju kalcija i fosfata u slini prije žvakanja žvakaće gume bez šećera s dodatkom kazein-fosfopeptida i amorfnog kalcijeva fosfata (CPP—ACP-a) pod imenom Recaldent™ te procijeniti količinu i koliko dugo ostaju određene koncentracije kalcija i fosfata u slini nakon žvakanja gume s CPP—ACP-om. **Materijali i metode:** Provedeno je kliničko nerandomizirano ispitivanje u skupini od 30 nasumce odabranih studenata u dobi između 18 i 25 godina. Od svih je skupljena nestimulirana slina cijeđenjem prije žvakanja gume s CCP—ACP-om i u točno određenim intervalima nakon 20-minutnog žvakanja. U uzorcima se mjerila koncentracija kalcija i fosfata. Podaci su analizirani SPSS ver.17, softverom s postavkama ponovljenog mjerenja ANOVA-om, studentskim parnim t-testom i postotnom razlikom. **Rezultati:** Srednja vrijednost kalcija u slini znatno se povećala neposredno nakon (Ai) žvakanja žvakaće s CCP—ACP-om, sa srednjom razlikom od 22 posto u usporedbi s osnovnom vrijednošću (B). Povećanje koncentracije kalcija u odnosu na osnovnu vrijednost mjerilo se dva sata nakon žvakanja, kada je opaženo sniženje koncentracije fosfata u slini i do jedan sat nakon žvakanja žvakaće u usporedbi s osnovnom vrijednošću (B). **Zaključak:** CPP kao ACP-nositelj znatno povećava koncentraciju kalcija u slini i tako olakšava remineralizaciju.

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Ključne riječi
CPP–ACP, kazein fosfopeptid-amorfni kalcijev fosfat nanokompleks; žvakaća guma; fosfati; kalcij; slina

Uvod

Oralna šupljina poseban je ekosustav s mnoštvom funkcija u kojem je jako mnogo mikroorganizama i poseban je smještaj izloženoga mineraliziranog tkiva. Ima vlastiti obrambeni sustav za borbu protiv bolesti (1). Među kroničnim bolestima karijes drugi po učestalosti pogađa ljudsku rasu (2). Slina ima važnu zadaću u održavanju oralnoga zdravlja. Ona oplakuje svoj ekosustav i sadržava mnogo organskih i anorganskih tvari koje su sastavni dio različitih enzima. Anorganske komponente su kalcij, fluoridi, magnezij, natrij, klor, kalij, bikarbonati i drugo (3). Važna zadaća sline jest održavati i zaštititi tvrda tkiva zuba tako da im se osigura izvor kalcijevih i fosfatnih iona (4). Ti ioni utječu na pokretačku silu precipitacije ili raspodjele kalcijeva hidroksilapatita (HAP-a), osnovnog elementa za građu mineraliziranog zubnog tkiva (5). Ioni također imaju ključnu ulogu u posteruptivnom sazrijevanju cakline i olakšavaju remineralizaciju početnih karijesnih lezija (4).

Demineralizacija i remineralizacija odlučujuće djeluju na tvrdoću i čvrstoću zubne cakline. Borba da se zubi zadrže snažnima i zdravima ovisi o njihovu omjeru. U razvoju ka-

Introduction

Oral cavity is a distinct ecosystem, which performs a wide range of functions, harbors a plethora of microorganisms and is unique in accommodating exposed mineralized tissues. It has its own inbuilt defensive mechanisms to fight against oral diseases (1). Among the diseases, dental caries is the second most common chronic disease affecting the human race (2).

Saliva plays an important role in maintaining oral health. Saliva that bathes its ecosystem possesses a large number of components which include both organic and inorganic constituents and various enzymes. The inorganic components are calcium, phosphate, fluoride, magnesium, potassium, chlorine, sodium, bicarbonates etc (3). An important role of saliva is to maintain and protect the hard tissues of the tooth by providing a source of calcium and phosphate ions (4). These ions influence the driving force for the precipitation or dissolution of calcium hydroxyapatite (HAP), the principal inorganic component of dental hard tissue (5). They play a key role in post-eruptive maturation of enamel and they facilitate the remineralization of incipient carious lesions (4).

rijesa, na omjer između demineralizacije i remineralizacije, utječe slina koja olakšava prijenos iona, bakterija i razgradivih ugljikohidrata na izloženim površinama zuba (6).

Remineralizacija je prirodni proces popravljanja početne neurušene karijesne lezije i oslanja se na kalcij i fosfatne ione potpomognute fluoridima kako bi se stvorile nove površine na postojećim ostacima kristala u potpovršinskoj leziji koja ostaje nakon remineralizacije. Pojačana remineralizacija *bijele mrlje,* uz pomoć protoka stimulirane sline (primjerice, žvakaćim gumama bez šećera), ilustrira njezin dinamični zaštitni učinak (7).

Nekoliko posljednjih desetljeća prati se učinak fluorida na smanjenje karijesa (8). Nedavno je predložen novi pristup u pojačanju remineralizacije na temelju mliječnih derivata s kazein- fosfopeptidima (CPP-a) i amorfnog kalcijeva fosfata (ACP-a) (9). Nanočestični kompleksi kazein-fosfopeptida i amorfnog kalcijeva fosfata (CPP–ACP-a) također su pokazali protukarijesni učinak, kako na laboratorijskim životinjama tako i tijekom humanih pokusa *in situ* (10, 11). CPP–ACP lokalno djeluje tako da povećava ACP u plaku koji puferira slobodne kalcijeve i fosfatne ione te tako pomaže u superzasićenosti u zubu sprječavajući demineralizaciju i pomažući remineralizaciju (12).

Učinkovitost žvakaće gume sa sorbitolom kao protukarijesnim sredstvom zapravo je učinak protoka sline, a ne neposredni utjecaj na zasićenost mineralima u lokalnim područjima oko zuba. Koncentracija kalcijeva fosfata u slini i plaku može također povećati zasićenost zubnim mineralima i povećati kariostatski učinak.

Koncentracija kalcijeva fosfata u slini i plaku može povećati zasićenost minerala zuba te se može pretpostaviti pojačani kariostatski učinak žvakaćih guma obogaćenih tim trima spojevima (13).

Chow i suradnici (14) pokazali su 1998. da žvakanje žvakaćih guma s monokalcijevim fosfatnim monohidratom (MCPM-om), te dikalcijevim (DCPA-om) i tetrakalcijevim fosfatom (TTCP-om) pojačava salivaciju te da obje žvakaće gume povećavaju zasićenost sline kad je riječ o mineralima zuba.

Klinički dokaz da žvakaće gume Recaldent™ [kazein-fosfopeptid i amorfni kalcijev fosfat (CPP–ACP)] mogu pomoći u remineralizaciji potvrđen je u istraživanjima *in situ* koje je provela uglavnom jedna skupina istraživača (15). Zato je potrebna neovisna potvrda tih nalaza drugim metodama koje bolje oponašaju kliničke uvjete. To je razlog da smo u našem istraživanju procijenili promjene u koncentraciji kalcija i fosfata u slini nakon žvakanja gume bez šećera i s dodatkom CPP–ACP-a te odredili količinu i koliko dugo traje razina koncentracije kalcija i fosfata u slini nakon žvakanja gume bez šećera s dodatkom CPP–ACP-a.

Demineralization and remineralization have a crucial impact on the hardness and strength of tooth enamel. The battle to keep teeth strong and healthy is dependent upon the ratio between demineralization and remineralization. In the development of dental caries, the relationship between demineralization and remineralization is influenced by the presence of saliva, which facilitates the transportation of ions, oral bacteria, and fermentable carbohydrates to the exposed surfaces of the teeth (6).

Remineralization is the natural repair process for non-cavitated lesions, and relies on calcium and phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization. Enhanced remineralization of white spot lesions by stimulated salivary flow (e.g. from chewing a sugar-free gum) illustrates dynamic protective effects of saliva (7).

Over the last few decades, fluoride has shown to reduce caries (8). Recently, a novel approach to enhance remineralization based on a milk derivative containing casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP) has been suggested (9). Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) nanocomplexes have also been demonstrated to have anticariogenic properties in both laboratory animal and human *in situ* experiments (10,11). The proposed mechanism of anticariogenicity for the CPP-ACP is that they localize ACP in dental plaque which buffers the free Ca and P ion activities thereby helping to maintain a state of super saturation with respect to tooth enamel by depressing demineralization and enhancing remineralization (12).

The effectiveness of sorbitol containing chewing gums as anticaries agents is apparently the result of their effect on salivary flow, and hence their influence on the saturation of tooth mineral in the local environment of the teeth. Calcium phosphate concentrations in saliva and in plaque can also increase tooth mineral saturation and an increased cariostatic effect might be anticipated with the gum enriched by these agents (13).

Chow et al (1998), (14) demonstrated that chewing gums containing monocalcium phosphate monohydrate (MCPM) dicalcium phosphate (DCPA) and tetracalcium phosphate (TTCP) enhances salivation, and found that both gums produced a pronounced elevation in the saturation of saliva with respect to tooth mineral.

Clinical evidence that Recaldent™ [Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP)] containing chewing gum has remineralization potential has been shown in many *in situ* model studies conducted mainly by one research group (15). There is a need for independent confirmation of these findings by other methods that more closely model clinically relevant conditions. Hence, the present study was conducted to evaluate the changes in the salivary concentration of calcium and phosphorous after chewing CPP-ACP containing sugar free chewing gum and to determine the extent and duration of salivary calcium and phosphorous levels after chewing CPP-ACP containing sugar-free chewing gum.

Materijali i metode

Odabir ispitanika

Provedeno je nerandomizirano kliničko istraživanje na Odjelu za stomatološko javno zdravstvo Pacifičkoga stomatološkog fakulteta i bolnice u Udaipuru u Rajasthanu u Indiji.

Nasumce je bilo odabrano 30 ispitanika među preddiplomskim i poslijediplomskim studentima Pacifičkoga stomatološkog fakulteta, a izabrani su oni koji su bili voljni sudjelovati. Bili su u dobi između 18 i 25 godina. Istraživanje je odobrilo Etičko povjerenstvo te visokoškolske ustanove. Prije početka istraživanja svi su sudionici potpisali informirani pristanak.

Kriteriji uključivanja i isključivanja

U istraživanje su bili uključeni dobrovoljni ispitanici u dobi od 18 do 25 godina, bez karijesnih lezija i patoloških promjena na oralnoj sluznici ili gingivnim/parodontnim tkivima.

Iz istraživanja su bili isključeni svi s poznatom alergijom na bilo koji proizvod za oralnu higijenu, terapijska sredstva ili zubne materijale ili s alergijama na mliječne proteine, zatim oni s fluorozom, pušači ili ako kronično uzimaju antibiotike koji utječu na količinu protoka sline.

Metoda

Provedeno je eksperimentalno istraživanje na petero ispitanika kako bi se ispitalo je li moguće provesti istraživanje te radi kalibracije istraživača tako da se upoznaju s laboratorijskim postupcima analize sline. Ti sudionici nisu bili uključeni u konačno istraživanje.

Prikupljanje uzoraka sline

Svim je ispitanicima rečeno da ne jedu i ne piju najmanje jedan sat prije prikupljanja uzoraka. Kako bi se kontrolirale dnevne oscilacije, uzorci nestimulirane sline uzimani su prije podne između 8 i 12,30 sati, a sudionici cijelo to vrijeme nisu smjeli jesti niti piti.

Na dan skupljanja uzoraka sline ispitanici su došli u ordinaciju i sjeli u stomatološki stolac. Zamoljeni su da prije postupka usta temeljito isperu vodom. Za uzimanje uzoraka u mirovanju i nestimulirane sline koristili smo se cijeđenjem (16). Sudionici su se nagnuli naprijed, s glavom prema dolje, kako bi se slina mogla cijediti kroz lijevak u čiste, sterilne, na ledu ohlađene epruvete. Od svakoga je tijekom tri do četiri minute uzeto dva do tri mililitra sline. Taj je uzorak označen riječju *prije* (osnovna razina–B). Odmah nakon prikupljanja sline epruvete su začepljene i spremljene u hladnjak do analize. Svaki pojedinac dobio je dva komadića žvakaće gume bez šećera s dodatkom CPP–ACP-a (Recaldent™, Cadbury, Japan, Limited, 1,5 g/komadić), morao ga je žvakati 20 minuta i zatim ispljunuti.

Odmah nakon žvakanja ponovno je uzet uzorak sline. Postupak je bio isti, a na epruvetama je napisano *neposredno nakon* (Ai). Prikupljanje uzorka sline ponavljalo se nakon 30 minuta (A30), jedan sat (A1), dva sata (A2) i tri sata (A3) nakon žvakanja gume s CPP–ACP-om. Sve testne epruvete hermetički su začepljene i odložene u hladnjak sve do analize.

Materials and Method

Subject recruitment

A non-randomized clinical study was conducted at the Department of Public Health Dentistry, Pacific Dental College and Hospital, Udaipur, Rajasthan, India.

Among the undergraduate and postgraduate students from Pacific Dental College and Hospital, who were willing to participate in the study, 30 subjects were randomly selected. They were in the age group of 18 – 25 years. Ethical clearance was obtained from the Ethical Committee of Pacific Dental College and Hospital, Udaipur. Written informed consent was obtained from the study subjects, prior to the beginning of the study.

Inclusion and Exclusion criteria

Subjects aged 18 – 25 years without any carious lesions; any pathological changes in the oral mucosa or gingival/periodontal tissue and who were willing to participate were included in the study.

Subjects with any known allergy to oral hygiene products, therapeutic agents or dental materials or those who have milk protein allergies, those with fluorosis, those who smoke and those with chronic use of antibiotics, all of which affects the salivary flow rate were excluded from the study.

Method

A pilot study was conducted on a group of 5 subjects in order to know the feasibility of the study and for the calibration of the investigator so as to get acquainted with the laboratory procedure of salivary analysis. These subjects were not included in the final study.

Collection of saliva sample

All the participating subjects were instructed not to eat or drink anything at least 1 hour before the collection of the saliva samples. To control the circadian variation, unstimulated saliva samples were collected between 8.00am – 12.30pm, during which period they were asked not to eat or drink.

On the day of sample collection, the subjects were comfortably seated on dental chairs. They were asked to rinse their mouth thoroughly with water before collection of the saliva. Draining method[16] was used for the collection of resting or unstimulated saliva samples. The subjects were asked to lean forward with their head tilted downwards allowing the saliva to drain or drool into clean, sterile, ice chilled test tubes via the funnels. 2 - 3 ml of saliva was collected from each subject in approximately 3 - 4 minutes. This sample was labeled as 'Before sample (Baseline - B). Immediately after collection, the test tubes were closed by stoppers and stored in the refrigerator, until the analysis.

Then, each individual was given 2 pellets of sugar free chewing gum containing CPP-ACP (Recaldent™, Cadbury Japan Limited, 1.5 gm /pellet) and was asked to chew it for a period of 20 minutes and then spit the gum.

Immediately after chewing the gum, collection of saliva was done by following the same procedure as mentioned above and labeled as 'After immediately (Ai). Collection of saliva samples was repeated at 30 minutes (A30), 1 hour (A1),

Svaki dan su prikupljeni, označeni i uskladišteni uzorci sline triju ispitanika. Nakon toga odneseni su u laboratorij gdje je analizirana koncentracija kalcija i fosfata.

Analiza sline

Analiza uzoraka sline obavljena je na Biokemijskom odjelu Stomatološkog fakulteta i bolnice Pacifičkog sveučilišta.

U svakom uzorku određena je koncentracija kalcija i fosfata (mg/dl) korištenjem kita s reagensima za određivanje kalcija i fosfata u serumu (ACCURARE, Lab-Care Diagnostics Indija PVT. LTD, Indija)(17) i poluautomatski analizator (Chem–5 Plus v2, Erba Diagnostics Manheim Gmbh, Njemačka)(1, 17, 18).

Korišteni analizator radio je na osnovi atomske apsorpcijske spektrofotometrije. Dobivene vrijednosti složene su tablično i statistički analizirane.

Statička analiza

Podaci su analizirani SPSS-ovom verzijom 17. programskog paketa (SPSS Inc., Chicago, IL, SAD) izračunom aritmetičke sredine, standardne devijacije i postotne razlike. Normalna razdioba podataka provjerena je Kolmogorov–Smirnovljim testom. U ponovljenom mjerenju ANOVA je korištena za usporedbu koncentracije kalcija i fosfata u slini prije žvakanja žvakaće gume s CPP–ACP-om i nakon toga. Studentov parni t-test rabio se za uparenu, unutarskupnu usporedbu koncentracije kalcija i fosfata u slini prije žvakanja žvakaće gume s CPP– ACP-om i nakon toga u određenim vremenskim intervalima.

Zaključeno je da postoji statistički značajna povezanost između skupina ako je p-vrijednost bila manja od 5 posto ($p \leq 0,05$).

Rezultati

Tablica 1 prikazuje usporedbu aritmetičke sredine razina kalcija i fosfata mjerenih u točno određenim različitim vremenskim intervalima. Koncentracija kalcija u slini prije žvakanja gume s CPP–ACP-om (B) bila je 5,87±1,50 (arit. sredina ± SD), a koncentracije poslije žvakanja u različitim vremenskim razmacima iznosile su: 7,17±1,62 (Ai), 6,82±1,59 (A30), 6,34±1,59 (A1), 5,97±1,57 (A2) i 5,84±1,50 (A3). Razlika u srednjim vrijednostima kalcija u slini bila je statistički značajna (p=0,000).

Razlike u srednjim vrijednostima koncentracije fosfata nisu bile statistički značajne (p=0,13), što je pokazalo ponovljeno mjerenje s pomoću ANOVE. Koncentracija fosfata prije žvakanja gume s CPP–ACP-om (B) bila je 8,23±1,84 (arit. sredina ± SD), a koncentracije fosfata u slini poslije žvakanja gume s CPP–ACP-om u različitim vremenskim intervalima bile su: 7,16±1,89 (Ai), 7,55±1,89 (A30), 8,00±1,85 (A1), 8,20±1,84 (A2) i 8,22±1,83 (A3).

Tablica 2, Slika 1 i Slika 2 pokazuju razliku u srednjim vrijednostima koncentracija kalcija i fosfata prije žvakanja žvakaće gume s CPP–ACP-om i nakon toga u različitim intervalima mjerenja. Srednja vrijednost koncentracije kalcija

2 hours (A2) and 3 hours (A3) after chewing CPP-ACP containing chewing gum. These test tubes were closed by stoppers and stored in the refrigerator, until the analysis. In one day, saliva samples from 3 subjects were collected, labeled and stored. These samples were then taken to the laboratory and analyzed for calcium and phosphorus concentration.

Salivary Analysis

Analysis of the samples was done on the same day at the Department of Biochemistry, Pacific Dental College and Hospital.

Each saliva sample was then tested for calcium and phosphorus concentration (mg/dl) by using serum calcium and serum phosphorus reagent kits[17] (ACCURARE, Lab-Care Diagnostics India PVT. LTD, India) and Semi-auto analyzer[1,17,18] (Chem – 5 Plus v2, Erba Diagnostics Manheim Gmbh, Germany). This autoanalyzer works on the principle of atomic absorption spectrophotometry. The values obtained were tabulated and subjected to statistical analysis.

Statistical analysis

Data were analyzed using SPSS version 17 software (SPSS Inc., Chicago, IL, USA) using mean, standard deviation and percentage difference. The normal distribution of the data was checked using the Kolmogorov-Smirnov test. For repeated measuring, Anova was used in order to compare the calcium and phosphorus concentrations of saliva before and after chewing CPP-ACP containing chewing gum. Students paired t-test was used for pair-wise intra (within) group comparison of the salivary calcium and phosphorous levels before and at different intervals after chewing CPP-ACP containing sugar free chewing gum. A significant relationship was assumed to exist between the groups if the p value was found to be less than 5% ($p \leq 0.05$).

Results

Table 1 shows the comparison of mean calcium and phosphorus levels at different time intervals. The calcium concentrations of saliva before (B) chewing CPP-ACP containing chewing gum was 5.87±1.50 (mean±SD), and the calcium concentrations observed after chewing CPP-ACP chewing gum at different time intervals were as follows (mean±SD); 7.17±1.62, 6.82±1.59, 6.34±1.59 and 5.97±1.57, 5.84±1.50 for Ai, A30, A1, A2 and A3 respectively. The difference in the mean calcium concentrations of saliva were found to be statistically significant (p=0.000).

However, the difference in the mean phosphorus concentrations of saliva was not statistically significant (p=0.13) as shown by repeated measuring by Anova. The phosphorus concentrations of saliva before (B) chewing CPP-ACP containing chewing gum was 8.23±1.84 (mean±SD), and the phosphorus concentrations observed after chewing CPP-ACP chewing gum at different time intervals were as follows (mean±SD); 7.16±1.89, 7.55±1.89, 8.00±1.85, 8.20±1.84, 8.22±1.83, for Ai, A30, A1, A2 and A3 respectively.

Table 2, Figure 1 and Figure 2 depict the mean difference of salivary concentration of calcium and phosphorus before

Tablica 1. Usporedba srednjih vrijednost koncentracije kalcija i fosfata u slini u različitim točno određenim intervalima
Table 1 Comparison of mean salivary concentration of calcium and phosphorus at different time intervals

Parameteri • Parameters	Vremenski interval • Time interval	Srednja vrijednost • Mean	SD	F-vrijednost • F value	p-vrijednost • p value
Kalcij • Calcium (mg/dl)	B	5.87	1.50	3.82	0.000*
	Ai	7.17	1.62		
	A30	6.82	1.59		
	A1	6.34	1.59		
	A2	5.97	1.57		
	A3	5.84	1.50		
	Total	6.33	1.61		
Fosfati • Phosphorus (mg/dl)	B	8.23	1.84	1.73	0.13
	Ai	7.16	1.89		
	A30	7.55	1.89		
	A1	8.00	1.85		
	A2	8.20	1.84		
	A3	8.22	1.83		
	Total	7.89	1.86		

Test: ponovljeno mjerenje ANOVA-om • Test used: Repeated measure Anova; * Značajnost p≤0,05 • Significance at p≤0.05
B – prije žvakanja žvakaće gume s CPP–ACP-om • Before chewing the CPP-ACP gum
Ai – odmah nakon žvakanja žvakaće gume s CPP–ACP-om • Immediately after chewing the CPP-ACP gum
A30 – 30 minuta nakon žvakanja žvakaće gume s CPP– CP-om • 30 minutes after chewing the CPP-ACP gum
A1 – jedan sat nakon žvakanja žvakaće gume s CPP–ACP-om • 1 hour after chewing the CPP-ACP gum
A2 – dva sata nakon žvakanja žvakaće gume s CPP–ACP-om • 2 hours after chewing the CPP-ACP gum
A3 – tri sata nakon žvakanja žvakaće gume s CPP–ACP-om • 3 hours after chewing the CPP-ACP gum

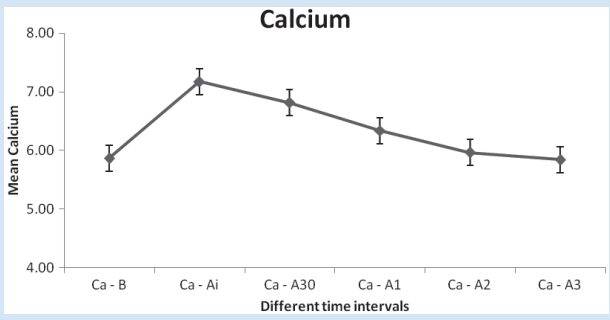
Tablica 2. Usporedba srednjih vrijednost koncentracija kalcija i fosfata u slini prije žvakanja žvakaće gume s CPP–ACP-om i u točno određenim intervalima te nakon toga
Table 2 Comparison of mean salivary concentrations of calcium and phosphorus between before and at intervals after chewing CPP-ACP containing chewing gum

Parameteri • Parameters	Vremenski interval • Time interval	Srednja vrijednost ± SD • Mean ± SD	Razlika srednje vrijednosti • Mean difference	% razlike od srednje vrijednosti • % difference from mean	t- vrijednost • t value	P-vrijednost • P value
Kalcij • Calcium (mg/dl)	B	5.87±1.50	-	-	-	-
	Ai – B	7.17±1.62	1.30	22	22.62	0.001*
	A30 - B	6.82±1.59	0.95	16	19.00	0.001*
	A1 - B	6.34±1.59	0.47	8	12.00	0.001*
	A2 - B	5.97±1.57	0.10	2	5.94	0.001*
	A3 - B	5.84±1.50	-0.03	- 0.5	4.78	0.001*
Fosfati • Phosphorus (mg/dl)	B	8.23±1.84	-	-	-	-
	Ai - B	7.16±1.89	-1.07	-13	22.89	0.000*
	A30 - B	7.55±1.89	-0.68	-8	10.17	0.000*
	A1 - B	8.00±1.85	-0.23	-3	5.31	0.000*
	A2 – B	8.20±1.84	-0.03	-0.4	1.66	0.11
	A3 - B	8.22±1.83	-0.01	-0.1	0.64	0.53

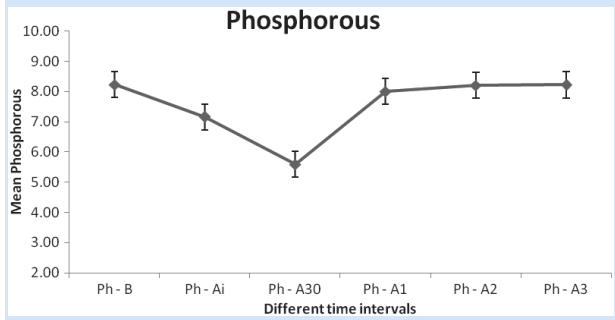
Test: usporedni t-test • Test used: Paired t-test; * Značajnost p≤0,05 • Significance at p≤0.05
B – prije žvakanja žvakaće s CPP–ACP-om • Before chewing the CPP-ACP gum
Ai – odmah nakon žvakanja žvakaće s CPP–ACP-om • Immediately after chewing the CPP-ACP gum
A30 – 30 minuta nakon žvakanja žvakaće s CPP–ACP-om • 30 minutes after chewing the CPP-ACP gum
A1 – jedan sat nakon žvakanja žvakaće s CPP–ACP-om • 1 hour after chewing the CPP-ACP gum
A2 – dva sata žvakanja žvakaće s CPP–ACP-om • 2 hours after chewing the CPP-ACP gum
A3 – tri sata žvakanja žvakaće s CPP–ACP-om • 3 hours after chewing the CPP-ACP gum

u slini znatno se povećala odmah nakon (Ai) žvakanja gume s CPP–ACP-om, uz postotnu razliku u srednjim vrijednostima od 22 posto u usporedbi s osnovnom koncentracijom (B). Nakon što je prestalo žvakanje, koncentracija kalcija u slini postupno je opadala tijekom svih kasnijih mjerenja

and at different intervals after chewing CPP-ACP containing chewing gum. The mean calcium concentration of saliva increased significantly immediately after (Ai) chewing CPP-ACP containing chewing gum with the mean difference of 22% when compared to the baseline (B). Thereafter,



Slika 1. Srednje vrijednosti koncentracije kalcija u slini prije žvakanja gume s CPP–ACP-om i u različitim točno određenim intervalima nakon 20-minutnog žvakanja
Figure 1 shows the mean calcium concentration of saliva before and at different intervals after chewing CPP-ACP containing chewing gum for 20 minutes.



Slika 2. Koncentracija fosfata u slini prije žvakanja gume s CPP–ACP-om i u različitim točno određenim intervalima nakon 20-minutnog žvakanja
Figure 2 shows the mean phosphorus concentration of saliva before and at different intervals after chewing CPP-ACP containing chewing gum for 20 minutes.

(A30, A1, A2 i A3). Statistički značajno povećanje koncentracije kalcija u slini, u usporedbi s početnom (B), zabilježeno je tijekom prva dva sata nakon žvakanja gume s CPP–ACP-om, a srednja koncentracija kalcija u slini tri sata nakon žvakanja bila je neznatno niža od osnovne koncentracije (B) – smanjenje je iznosilo 0,5 posto.

Srednja vrijednost koncentracije fosfata u slini jako se snizila odmah nakon (Ai) žvakanja žvakaće gume s CPP–ACP-om, s 13-postotnom razlikom srednje vrijednosti u odnosu prema osnovnoj početnoj (B). Odmah nakon prestanka žvakanja, koncentracija fosfata postupno je rasla tijekom svakog mjerena (A30, A1, A2 i A3). Značajno smanjenje koncentracije fosfata u slini, u usporedbi s osnovnom koncentracijom (B), uočeno je do jedan sat nakon žvakanja gume s CPP– ACP-om ($p<0,05$). Premda je količina fosfata u slini rasla i tri sata nakon žvakanja gume s CPP– ACP-om, razlika nije bila statistički značajna u intervalima od dva (A2) i tri (A3) sata.

Rasprava

Potrebna je dovoljna količina sline kako bi se zaštitilo oralno tkivo. Prisutnost kalcija, fosfata i ostalih anorganskih iona, posebice fluorida, omogućuje slini važnu zaštitnu ulogu u zadržavanju integriteta zubnih tkiva. Okolina bogata kalcijem i fosfatima olakšava remineralizaciju početnih karijesnih lezija i demineralizirane cakline (4).

Ravnoteža između demineralizacije i remineralizacije ovisi o koncentraciji kalcija i fosfata u slini, te o razini salivarne alkalne fosfataze. Kako bi utjecala na demineralizaciju i remineralizaciju zuba, slina mora biti zasićena kalcijem i fosfatima (19).

Remineralizacijski potencijal žvakaćih guma s kalcijem i fosfatima u različitim oblicima dokazali su korištenjem CPP–ACP-a godine 2001. istraživači Shen i Reynolds (11) te Iijima i suradnici tri godine kasnije – 2004.(20). Itthagarun i kolege su 2005. (21), uporabom dikalcijeva fosfata i CPP–ACP-a s ureom, te Suda i njegov tim (2006.)(22) kori-

the mean calcium concentration of saliva decreased gradually at all other intervals (A30, A1, A2 and A3). A statistically significant increase in the calcium concentration of saliva as compared to the baseline (B) was observed for up to 2 hours after chewing CPP-ACP containing gum, whereas the mean calcium concentration of saliva 3 hours after chewing CPP-ACP chewing gum was significantly lower compared to the baseline (B), with a decrease of 0.5%.

The mean phosphorus concentration of saliva decreased significantly immediately after (Ai) chewing CPP-ACP containing chewing gum with the mean difference of 13% when compared with the baseline (B). Thereafter, the mean phosphorus concentration of saliva increased gradually for all other intervals (A30, A1, A2 and A3) after chewing CPP-ACP containing chewing gum. A significant decrease in the phosphorus concentration of saliva compared to the baseline (B) was observed for up to 1 hour after chewing CPP-ACP containing gum ($p < 0.05$). Although the mean phosphorus concentration of saliva increased up to 3 hours after chewing CPP-ACP containing chewing gum, this difference was not statistically significant at 2 hours (A2) and 3 hours (A3) after chewing the gum ($p>0.05$).

Discussion

A sufficient amount of saliva is necessary to protect the oral tissues. The presence of calcium, phosphate and other inorganic ions, particularly fluoride, permits saliva to play an important protective role in maintaining the integrity of dental tissues. Calcium- and phosphate- rich environment facilitates remineralization of incipient carious lesions or demineralized zones of enamel (4).

The balance between demineralization and remineralization depends on the salivary calcium and phosphorus concentration as well as on the level of the salivary alkaline phosphatase. It is important that saliva be saturated with calcium and phosphorous to have an effect on demineralization and remineralization of teeth (19).

The remineralization potential of chewing gum containing calcium and phosphorus in various forms has been proved by Shen and Reynolds (2001),(11) and Iijima et al (2004), (20) using CPP-ACP, Itthagarun et al (2005), (21) using dicalcium

štenjem kalcijeva laktata dodanog u žvakaću gumu s ksilitolom dokazali su isto.

Kazein-fosfopeptid (CPP) sa slijedom klastera Ser(p)–Ser(p)–Ser(p)–Glu-Glu ima i u metastabilnoj otopini pokazao je zapanjujuću mogućnost stabilizacije amorfnog kalcijeva fosfata (ACP). Zahvaljujući mnogostrukim fosfoseriliranim ostacima, CPP se veže i stvara nanoklastere ACP-a, sprječavajući njihov rast do kritične veličine potrebne za stvaranje jezgri mineralizacije i transformaciju faze (11). Kazeinfosfopeptidi (CPP) imaju važnu zadaću kao nosači ACP-a, lokalizirajući visokotopljivu fazu kalcijeva fosfata na površini zuba. Ta lokalizacija održava velik koncentracijski gradijent kalcijevih i fosfatnih iona u potpovršinskom sloju cakline (23). Reynolds (24) je 2003. u svojem istraživanju usporedio remineralizacijski potencijal CPP–ACP-a s drugim oblicima kalcija. CPP-ACP je postigao najvišu razinu remineralizacije potpovršinske lezije u caklini, neovisno o tome koliko se dugo i često žvakalo. Korištenje 1,0-postotne otopine CPP–ACP-a dva puta na dan povisilo je za 144 posto razinu kalcija i za 160 posto razinu anorganskog fosfata (7). Ti rezultati pokazuju da postoji protukarijesni učinak CPP–ACP-a, pri čemu CPP stabilizira i lokalizira ACP na površini zuba te tako puferira pH plaka, smanjuje demineralizaciju i poboljšava remineralizaciju cakline (10).

U našem istraživanju prikupljeni uzorci sline procijenjeni su korištenjem fotometra (Chem – 5 Plus v2, Erba Diagnostics Manheim Gmbh, Njemačka) koji je pokazao znatne razlike u koncentraciji kalcija i fosfata u slini prije žvakanja žvakaće gume bez šećera s dodatkom CPP– ACP-a u odnosu na koncentracije mjerene u točno određenim intervalima nakon 20-minutnog žvakanja.

Budući da su preliminarna istraživanja Shena i suradnika (11) 2001. godine upućivala na to da se CPP–ACP nije mogao otkriti u bolusu žvakaće nakon 10 minuta, u našem je istraživanju ispitanicima rečeno da žvaču 20 minuta.

Slobodan ionizirani kalcij važan je u slučaju napada karijesa jer ta frakcija kalcija uspostavlja ravnotežu između kalcijeva fosfata i tvrdih tkiva zuba te okolne tekućine (19). Stomatolozi se slažu da izlučivanje sline i izlučene tvari itekako utječu na jakost pojedinih napada karijesa. Ashley (25) je 1975. opazio da se koncentracija kalcija u slini snižava ako postoji pojačana karijesna aktivnost. Suprotno tome su 2008. Sharabi i njegovi kolege (18) ustanovili da nema značajne povezanosti između kalcija, fosfata, alkalne fosfataze i karijesnog procesa. Visoke razine fosfata povezivale su se s niskom stopom karijesa, ali je zabilježeno i suprotno (19).

U našem istraživanju srednja vrijednost koncentracije kalcija (mg/dl) u slini znatno se povećavala dva sata nakon žvakanja žvakaće gume s CPP–ACP-om, u usporedbi s početnom osnovnom koncentracijom.

Suprotno rezultatima našeg istraživanja, Reynolds i suradnici (24) su 2004. istaknuli da se CPP može otkriti na površini zuba i tri sata nakon konzumiranja žvakaće gume s CPP–ACP-om i ksilitolom.

I u našem se istraživanju srednja vrijednost koncentracije (mg/dl) fosfata također snižavala do jedan sat nakon žvakanja žvakaće gume s CPP–ACP-om, u usporedbi s početnom osnovnom koncentracijom.

phosphate and CPP-ACP with urea, and Suda et al (2006), (22) using calcium lactate added to xylitol chewing gums.

Casein phosphopeptides (CPP) containing the cluster sequence Ser(p)-Ser(p)-Ser(p)-Glu-Glu have a remarkable ability to stabilize amorphous calcium phosphate (ACP) in metastable solution. Through the multiple phosphoseryl residues, the CPP binds to form nanoclusters of ACP, preventing their growth to the critical size required for nucleation and phase transformation[11]. The casein phosphor-peptides (CPP) have an important role as an ACP carrier localizing the highly soluble calcium phosphate phase at the tooth surface. This localization maintains high concentration gradients of calcium and phosphate ions in the subsurface enamel (23).

In a study by Reynolds (2003), (24), that compared the remineralization potential of CPP-ACP and other forms of calcium, CPP-ACP produced the highest level of enamel subsurface lesion remineralization, independent of chewing frequency or duration.

The two times daily use of the 1.0% CPP-ACP solution resulted in a 144% increase in calcium level and a 160% increase in inorganic phosphate level[7]. These results suggested that an anticariogenic mechanism for the CPP-ACP exists, where the CPP stabilizes and localizes ACP at the tooth surface, thereby buffering plaque pH, depressing enamel demineralization and enhancing remineralization (10).

In the present study, saliva samples collected from the subjects were evaluated by the use of photometer (Chem – 5 Plus v2, Erba Diagnostics Manheim Gmbh, Germany) which showed highly significant change in the calcium and phosphorus concentration of saliva before and at different intervals after chewing CPP-ACP containing sugar-free chewing gum for 20 minutes.

Since preliminary studies of Shen et al (2001)[11] indicated that no CPP-ACP could be detected in the gum bolus after 10 min of chewing, the subjects in the present study were instructed to chew the gum for 20 minutes.

The free ionized calcium is important to the event in caries attack, since this fraction of calcium takes part in the equilibrium between the calcium phosphate of the dental hard tissue and its surrounding liquid (19). There is a general argument among dental professionals that the salivary secretions and substances secreted with the saliva influence to a high degree, the strength of an individual caries attack. Ashley (1975), (25) observed that salivary calcium concentration decreased with increasing carious activity. However, Shahrabi et al (2008), (18) found no significant relationship between calcium, phosphate, alkaline phosphatase and caries process. High phosphate levels have been associated with low caries rates, but the reverse relationship has also been seen (19).

In the present study, the mean calcium concentration (mg/dl) of saliva increased significantly up to 2 hours after chewing CPP-ACP containing gum when compared to the baseline, while the mean calcium concentration (mg/dl) of saliva 3 hours after chewing CPP-ACP chewing gum was significantly lower when compared to baseline.

In contrast to the results of the present study, Reynolds et al (2003), (24) demonstrated that CPP could still be detected on the tooth surface 3 hours after consuming xylitol gum containing CPP-ACP.

Nestimulirana slina dobro je zasićena fosfatima (26). Koncentracija fluorida opada čim se protok sline počne povećavati (14, 19, 26). Prosječna postotna razlika u zastupljenosti fosfata u nestimuliranoj slini u odnosu na simuliranu iznosi, prema rezultatima Edraga (26) iz godine 1992., 29 posto, a prema rezultatima Lagerlofa i suradnika dobivenima 1994. (19) iznosila je 25 posto.

Brzo opadanje koncentracije fosfata u slini nakon žvakanja žvakaće gume s CPP–ACP-om, uočeno u ovom istraživanju, može se objasniti većim protokom sline nakon žvakanja gume s CPP–ACP-om. To se slaže s opažanjima Vogela i njegovih kolega tijekom 2000. godine (13).

U mnogim istraživanjima istaknuta je velika uloga kalcija u remineralizaciji caklinske površine. Ta visoka razina zasićenosti pripisuje se CPP–ACP-u u žvakaćoj gumi (10, 11). Morgan i suradnici (2008.)(27) smatraju da žvakanje žvakaće gume bez šećera s CPP–ACP-om statistički značajno utječe na radiografsku dijagnostiku aproksimalnih lezija.

Povećana koncentracija kalcija u slini, nakon žvakanja žvakaće gume s CPP–ACP-om, uočena u ovom istraživanju, može pomoći u remineralizaciji površine zuba kao dodatak mehaničkom čišćenju tijekom žvakanja.

Na kraju, u ovom se istraživanju ističe pozitivni remineralizacijski potencijal žvakaće gume bez šećera s dodatkom CPP–ACP-a, što je potkrijepljeno znatno povišenim razinama koncentracije kalcija u slini, premda je kvantiteta i kvaliteta kliničkih ispitivanja nedovoljna za zaključke o dugoročnom učinku derivata kazeina, posebice CPP–ACP-a u sprječavanju karijesa *in vivo* i terapije preosjetljivosti zuba ili suhih usta (28).

Korištenje žvakaćih guma bez šećera prihvaćeno je kao dodatak postupcima za održavanje oralne higijene. Dio je protukarijesnog programa. Žvakanje žvakaćih ne samo da djeluje kao stimulator salivacije, nego može biti koristan donositelj fluorida, klorheksidina i kalcijeva fosfata na površine zuba (29).

Provedeno istraživanje o procjeni koncentracije kalcija i fosfata u slini prije žvakanja žvakaće gume bez šećera s CPP–ACP-om i nakon toga, dalo je ohrabrujuće rezultate. Tako je postalo jasno da se bolje treba razraditi utjecaj žvakaćih guma s CPP–ACP-om te djelovanje na karijes i njegovu prevenciju. Potrebno je obaviti detaljnija istraživanja na većem uzorku i s duljim vremenskim razmacima procjene kako bi se dobili pouzdaniji podaci o žvakaćim gumama s CPP–ACP-om.

Potrebno je potvrditi, osim određivanja optimalne koncentracije kalcija i fosfata u slini potrebnih za remineralizaciju površina zuba, i količinu, učestalost te trajanje korištenja žvakaćih guma bez šećera s CPP–ACP-om da bi bile korisne u remineralizaciji zubnih površina.

Zaključak

Žvakaće gume bez šećera s dodatkom CCP–ACP-a tijekom dva sata povećavaju koncentraciju kalcija u slini, a smanjuju koncentraciju fosfata te se tako raspoloživa količina kalcija za cijelu denticiju može iskoristiti za remineralizaciju zuba. Iz navedenoga je jasno da kazein-fosfopeptid i amorfni kalcijev fosfat značajno povećavaju koncentraciju kalcija u slini i tako poboljšavaju remineralizaciju.

Also, the mean phosphorus concentration (mg/dl) of saliva in the present study, decreased significantly up to 1 hour after chewing.

Unstimulated saliva is well saturated with phosphorus (26). The concentration of phosphorus falls as the flow rate increases (14,19,26). The average percentage difference of phosphorus between the unstimulated and stimulated saliva was shown to be 29% by Edgar (26) and 25% by Lagerlof et al (19).

Therefore, immediate decrease in the salivary concentration of phosphorus after chewing CPP-ACP containing chewing gum observed could be attributed to the increase in the salivary flow. This observation of the present study is supported by Vogel et al (13).

Many studies have suggested that calcium plays an important role in remineralization of enamel surface. This level of super saturation of saliva with calcium can be attributed to CPP-ACP in the chewing gum (10,11). Morgan et al (27) showed that chewing the CPP-ACP sugar free gum demonstrated a statistically significant difference in radiographically diagnosed approximal carious lesions.

Therefore, the increase in the salivary calcium concentration observed in present study af may aid in remineralization of tooth surfaces in addition to the mechanical cleansing while chewing.

In conclusion, the present study indicates a positive remineralization potential of sugar-free gum by the inclusion of CPP-ACP, as it is shown to significantly elevate the calcium concentration of saliva. The quantity and quality of clinical trial evidence is insufficient to make conclusions regarding the long term effectiveness of casein derivatives in preventing caries *in vivo* and treating dentin hypersensitivity or dry mouth (28).

Sugar free chewing gum has been increasingly accepted as an addition to oral hygiene procedures and as part of caries prevention. Chewing gum not only acts as a salivary stimulus but may also be a useful vehicle for delivering agents such as fluoride, chlorhexidine and calcium phosphorus on to the tooth surfaces (29).

The pilot study conducted to assess the calcium and phosphorus concentration of saliva before and after chewing CPP-ACP containing sugar free chewing gums seems to be encouraging. With regard to the findings of our study, a need arises to clearly understand the role of CPP-ACP containing chewing gums and their relation to dental caries prevention. Hence, a more sophisticated trial is required with larger sample and a long term evaluation.

Since the optimum calcium and phosphorus concentration of saliva required for remineralization of tooth surface still needs further research, the quantity, frequency and duration of use of CPP-ACP containing sugar-free chewing gums to be useful in aiding remineralization of tooth surface needs to be verified.

Conclusion

Sugar free chewing gums when supplemented with CPP-ACP aid in increasing the calcium concentration of the saliva for a period of 2 hours and the phosphorus concentration of saliva is decreased, thereby supplying an increased amount of calcium to the entire dentition, which can be useful in remineralization of the tooth surface. Consequently, Casein phosphopeptide as an Amorphous Calcium Phosphate carrier significantly increases the calcium concentration of the saliva, thereby facilitating remineralization.

Abstract

Objective: To estimate the salivary concentration of calcium and phosphorous before and after chewing sugar free chewing gum containing Casein phosphopeptide-Amorphous calcium phosphate (CPP-ACP) namely Recaldent™ and to determine the extent and duration to which the salivary levels of calcium and phosphorus remain retained in saliva after chewing the CPP-ACP containing gum. **Materials and Method:** A non-randomized clinical trial was conducted among 30 randomly selected students aged 18 – 25 years. Unstimulated saliva was collected by draining method before and at different intervals after chewing CPP-ACP chewing gum for 20 minutes. These samples were tested for calcium and phosphorous concentration. Data were analyzed using SPSS version 17 software using Repeated measure Anova and Students paired t-test and percentage difference. **Results:** The mean calcium concentration of saliva increased significantly immediately after (Ai) chewing CPP-ACP containing chewing gum with the mean difference of 22% when compared to the baseline (B). A significant increase in the calcium concentration of saliva as compared to the baseline (B) was observed for up to 2 hours after chewing the gum, whereas a decrease in the phosphorus concentration in saliva for up to 1 hour after chewing the gum was observed when compared to baseline (B). **Conclusion** - CPP as an ACP carrier significantly increases the calcium concentration of the saliva, thereby facilitating remineralization.

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Key words
CPP-ACP; Casein Phosphopeptide-Amorphous Calcium Phosphate Nanocomplex; Chewing Gum; Calcium; Phosphorus; Saliva

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