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The effects of home-use and in-office bleaching treatments on calcium and phosphorus concentrations in tooth enamel

An in vivo study

Flávia Lucisano Botelho do Amaral, DDS, MS, ScD; Robson Tetsuo Sasaki, DDS, MS; Tatiana Cristina Ricci da Silva, BS; Fabiana Mantovani Gomes França, DDS, MS, ScD; Flávia Martão Flório, DDS, MS, ScD; Roberta Tarkany Basting, DDS, MS, ScD, PhD

Researchers have conducted in vitro studies of dental bleaching to evaluate the effect of different concentrations, protocols and agents on the microhardness,¹⁻¹¹ mineral content,⁵ micromorphology^{3,8} and surface roughness^{3,9} of bleached dental enamel. Although in vitro models are able to predict the behavior of bleaching agents on dental surfaces by the use of artificial saliva under highly controlled conditions, they are not able to simulate the complex process that occurs in the oral environment, including the presence of human saliva and its dynamic effect on tooth demineralization and remineralization.¹⁰ Thus, some investigators¹¹⁻¹⁷ have proposed in situ evaluations of the effects of bleaching agents on enamel and dentin structure that simulate intraoral conditions in a more realistic way.

In an attempt to confirm the results from the in vitro and in situ studies in the literature, investigators have conducted clinical studies to evaluate the effects of bleaching agents on enamel surface roughness by means of a profilometer analysis

ABSTRACT



Background. Because the effects of dental bleaching on enamel needs to be clarified in vivo, the authors conducted a study to determine calcium and phosphorus concentrations in enamel after the application of different bleaching treatments.

Methods. The authors applied four agents (10 percent and 20 percent carbamide peroxide [both recommended for home use], 38 percent and 35 percent hydrogen peroxide [both applied in the dental office]) to the enamel of 80 participants, who were divided into four groups of 20. The authors collected enamel microbiopsy specimens from incisors before (baseline), during (seven, 14 and 21 days) and after (seven and 14 days) the bleaching treatments. They analyzed calcium and phosphorus concentrations by using a spectrophotometer.

Results. The authors analyzed data by using the Friedman test and the Kruskal-Wallis test, followed by the Dunn test ($\alpha = .05$). There were no statistical differences between the evaluation results, regardless of which bleaching gel was used, for determining the concentration of either calcium or phosphorus.

Conclusions. Home-use and in-office bleaching gels did not alter the concentrations of calcium and phosphorus concentrations on the enamel surface in vivo.

Clinical Implications. In vivo, different dental bleaching techniques did not alter the inorganic composition of enamel.

Key Words. Tooth bleaching; clinical protocols; enamel; dental materials; research.

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Dr. Amaral is a professor, Department of Cariology and Biochemistry, Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, Rua José Rocha Junqueira 13, Ponte Preta, Campinas, São Paulo, 13045-755, Brazil, e-mail flbamamaral@gmail.com. Address reprint requests to Dr. Amaral. Dr. Sasaki is a professor, Department of Anatomy, Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil.

Mrs. Silva is a technical biologist, Laboratory of Materials Testing, Biochemistry and Physiology, Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil.

Dr. França is a professor, Department of Restorative Dentistry, Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil.

Dr. Flório is a professor, Department of Preventive and Social Dentistry, Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil.

Dr. Basting is a professor, Department of Restorative Dentistry, Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil.

of enamel replicas,^{9,18} an analysis of enamel replicas by means of scanning electron microscopy¹⁹⁻²¹ or by measurement of the enamel microhardness in bleached teeth extracted for orthodontic reasons.²² However, to our knowledge, no researcher has yet described the *in vivo* quantification of calcium and phosphorus content in enamel before, during and after bleaching with the home-use and in-office agents.

Brudevold and colleagues²³ proposed a methodology for collecting samples from enamel without causing any injuries to the dental structure, a procedure called “enamel microbiopsy.” Recently, this methodology was used to detect the presence of lead content in the primary tooth enamel of preschool-aged children.^{24,25} These microbiopsies could be helpful when determining the effects of dental bleaching on the mineral content of enamel in a clinical situation, in which the teeth are constantly submitted to the remineralizing-demineralizing influence of human saliva.

Thus, in our study we aimed to determine, *in vivo*, the concentration of calcium and phosphorus in enamel that underwent home-use and in-office dental bleaching with agents of different concentrations, measuring the concentrations before, during and after the bleaching treatment.

METHODS

Experiment design. The factors we studied were bleaching techniques and agents at four levels: home-use carbamide peroxide 10 percent, home-use carbamide peroxide 20 percent, in-office hydrogen peroxide 35 percent and in-office hydrogen peroxide 38 percent. We performed six evaluations during three periods: before the bleaching treatment (baseline); during the bleaching treatment at seven, 14 and 21 days; and after the bleaching treatment at seven and 14 days. The sample consisted of 80 participants whom we randomly assigned to four groups of

20 each according to each bleaching technique and agent. We obtained the response variable by means of enamel microbiopsies, in which we determined calcium and phosphorus concentrations in milligrams per milliliter by using a spectrophotometer. Table 1 provides descriptions of the bleaching techniques and agents used in the study.

Ethical aspects and selection of participants. After the research ethics committee of the Dental School and São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil, approved the study protocol, we enrolled 94 healthy participants from the São Leopoldo Mandic Institute and Research Center (18 male and 76 female, aged 18-42 years). These participants were free of caries and periodontal disease, and they each had an indication for dental bleaching after being examined by professors from the restorative dentistry post-doctoral program. We required that they have six maxillary and six mandibular anterior teeth and that they not have restorative material covering more than one-sixth of each buccal surface. We excluded from the study pregnant or breastfeeding women, as well as patients with previous dentin hypersensitivity to thermal stimulus, tetracycline-stained teeth or both. According to these criteria, and after they underwent a health history collection and a clinical examination, we

TABLE 1

Agents used in the study, according to bleaching technique.

BLEACHING TECHNIQUE	BLEACHING AGENT	MANUFACTURER	COMPOSITION*	pH	LOT NO.
Home Use	Opalescence PF 10%	Ultradent Products, South Jordan, Utah	10 percent carbamide peroxide, 0.5 percent potassium nitrate, 0.11 percent fluoride ion (1,000 parts per million)	7.1	B51JR
	Opalescence PF 20%	Ultradent Products	20 percent carbamide peroxide, 0.5 percent potassium nitrate, 0.11 percent fluoride ion (1,000 ppm)	7.2	B3NVC
In Office	Pola Office With 35% HP	SDI, Bayswater, Victoria, Australia	Liquid: 35 percent hydrogen peroxide, distilled water, stabilizers; powder: thickener, catalyst, pigments, potassium nitrate	2.6	083011, 082776, 082547
	Opalescence Boost PF 38% HP	Ultradent Products	38 percent hydrogen peroxide, 3 percent potassium nitrate, 1.1 percent fluoride ion	6.6	B3VFR, B563J

* The exact percentage of these additives is proprietary.

selected 80 participants. These participants received detailed information about the aims and methods of the research and signed a written form indicating their informed consent.

For the two weeks before and throughout the entire experimental phase, the participants brushed their teeth with the same brand of toothpaste (Colgate Máxima Proteção Anticáries, Colgate-Palmolive, São Paulo) and the same type of toothbrush (Oral B Classic, Procter & Gamble, São Paulo).

Study protocols. Home-use bleaching protocol. Before applying the bleaching agent, we obtained alginate impressions (Jeltrate Alginate Impression Material, Dentsply Caulk, Milford, Del.) of both arches of each participant from which to prepare stone molds (Gesso, Rio, Rio de Janeiro, Brazil). We made no preparations with reservoirs because we found no difference in effectiveness (as noted by Javaheri and Janis²⁶) or higher rates and higher intensity of gingival inflammation (as noted by Kirsten and colleagues²⁷) with or without the reservoir. We made an individual mold for each participant, using a 0.4-millimeter-thick flexible polymer in a vacuum plasticizer (Bio-Art Equipamentos Odontológicos, São Carlos, São Paulo, Brazil). Participants applied the home-use bleaching agents (Opalescence PF 10% [Ultradent Products, South Jordan, Utah] or Opalescence PF 20% [Ultradent Products]) for 21 days, according to the manufacturer's directions.

In-office bleaching protocol. Three clinicians (F.L.B.A., F.M.G.F. and R.T.B.) applied a resin dam (OpalDam Kit, Ultradent Products, or Gingival Barrier, SDI, Bayswater, Victoria, Australia) to isolate the gingiva from the teeth and used a lip retractor to protect the lips.

The bleaching agent Pola Office With 35% HP (SDI) consists of a powder and a liquid. The three clinicians mixed these before use in the proportion of one spoonful of powder to five drops of liquid so that the mixture attained a gel consistency. The bleaching agent Opalescence Boost PF 38% HP (Ultradent Products) is supplied in two syringes containing different gels: one with liquid hydrogen peroxide and the other with a chemical activator. To combine the two gels, the clinicians joined the syringes and shook them back and forth 20 times to mix them thoroughly. After mixing, the clinicians separated the syringes and applied the mixture, by using one of the syringes, in a thin layer on the buccal surfaces of the teeth (from second premolar to second premolar). After eight minutes, they removed the gel with gauze. No heat or special lamps were involved in completing the process. The clinicians performed this

application protocol three times at each session. After the last application, they washed the teeth with distilled and deionized water and removed the resin dam and lip retractor.

The clinicians performed the in-office bleaching treatment protocols during one session per week for three weeks (corresponding to 21 days of treatment).

Although the various products' manufacturers provided information about the pH of each of the agents, we evaluated it ourselves by using a fresh portion of the agents either extruded from the syringe (for the home-use agents) or recently mixed (for the in-office agents). We performed a measurement in triplicate by using a pH meter (MS Tecnopon Equipamentos Especiais, Piracicaba, São Paulo, Brazil) (Table 1).

Enamel microbiopsies. To determine the concentrations of calcium and phosphorus, we obtained a sample from the dental enamel after each evaluation period by using a technique called "enamel microbiopsy," which Brudevold and colleagues²³ proposed and Gomes and colleagues²⁴ modified. One of the clinicians (F.L.B.A.) isolated the working area with cotton rolls before performing the biopsy. She placed an adhesive tape (3M Scotch Premium Electrical Tape, Sumaré, São Paulo, Brazil) with a circular perforation (diameter, 1.6 mm) firmly on the labial surface of one of the maxillary or mandibular incisors to demarcate the biopsy site. She etched the sampling site once according to the following procedure: she applied 5 microliters of 1.6 moles per liter hydrochloric acid in 70 percent glycerol (volume/volume) to this area for 20 seconds and simultaneously agitated the drop gently with the pipette tip. The clinician then transferred the biopsy solution to tubes (Safe-Lock Eppendorf Tubes, Eppendorf do Brasil, São Paulo) already containing 200 μ L of ultrapurified water. Next, she rinsed the surface once with 5 μ L of 70 percent glycerol for 10 seconds, which she also transferred to the centrifuge tube. Last, she removed the tape and washed the tooth with water for 30 seconds and dried it with an air spray to prepare it for the application of a topical fluoride. We obtained biopsy samples at six time points: before the bleaching treatment (baseline), during the bleaching treatment (at seven, 14 and 21 days) and after the bleaching treatment (at seven and 14 days).

Chemical analysis. A biologist (T.C.R.S.) performed the chemical analyses, although she did not perform them immediately, and to avoid evaporation and loss of sample volume, we froze samples until the moment of their use. At the moment of calcium and phosphorus determina-

tion, the biologist defrosted and vortexed the samples, using one-half of the sample volume for calcium analysis and the other one-half for phosphorus analysis.

We measured phosphorus according to the methodology proposed by Fiske and Subbarow.²⁸ We assayed each enamel etch sample in triplicate and loaded reactions in a spectrophotometer (ELISA, ELx800uv, Absorbance Microplate Reader, BioTek Instruments, Winooski, Vt.) plate for reading. Reaction mixtures consisted of 30 μ L of the sample, 200 μ L of ultrapurified water and 50 μ L of molybdic acid solution (ammonium molybdate at 2.5 percent [weight/volume] in 4 normal sulfuric acid), which we vortexed thoroughly. After 10 minutes, we added 20 μ L of reducing agent and vortexed the mixture again. After 20 minutes, we measured absorbance at 630 nanometers. The biologist calibrated the plate reader with standards containing known concentrations of phosphorus (1, 2, 4 and 8 micrograms per milliliter).

We determined calcium concentrations by means of colorimetric reagent arsenazo end point analysis, in which arsenazo, in the presence of calcium ions in an acidic pH environment, yields a colored complex whose color intensity is directly proportional to the calcium concentration in the tested sample. Reaction mixtures consisted of 10 μ L of the sample and 200 μ L of Calcium-Arsenazo III reagent (K051, Bioclin, Santa Branca, Belo Horizonte, Minas Gerais, Brazil), which we vortexed and incubated at 370°C for two minutes. Next, we measured absorbance at 630 nm in a spectrophotometer.

Statistical analysis. Because the assumptions of the analysis of variance were not met, the clinician conducting the statistical analysis (F.M.G.F.) used the nonparametric Kruskal-Wallis test for the comparison among the bleaching agents in each evaluation, and she used the nonparametric Friedman test for the

TABLE 2

Phosphorus concentration of bleaching agents in tooth enamel, according to technique and evaluation interval.

EVALUATION INTERVAL	BLEACHING AGENTS' MEDIAN (MINIMUM-MAXIMUM) VALUES OF PHOSPHORUS CONCENTRATION IN TOOTH ENAMEL IN MILLIGRAMS PER MILLILITER, ACCORDING TO TECHNIQUE			
	Home Use		In Office	
	Carbamide peroxide 10% (n = 16)	Carbamide peroxide 20% (n = 15)	Hydrogen peroxide 35% (n = 19)	Hydrogen peroxide 38% (n = 15)
Baseline	1.55 ^{Aa} (0.65-8.35)	0.97 ^{Aa} (0.65-6.76)	2.71 ^{Aab} (0.67-3.81)	1.06 ^{Aa} (0.65-3.15)
Treatment Day 7	1.62 ^{Aa} (0.65-8.26)	1.19 ^{Aa} (0.65-8.35)	2.61 ^{Aab} (0.65-4.77)	1.00 ^{Aa} (0.68-4.34)
Treatment Day 14	1.29 ^{Aa} (0.80-8.35)	1.16 ^{ABa} (0.66-5.52)	3.23 ^{Aab} (0.65-4.54)	0.80 ^{Ba} (0.65-2.51)
Treatment Day 21	1.27 ^{ABa} (0.65-1.66)	1.05 ^{ABa} (0.65-1.62)	2.69 ^{Aab} (0.65-5.06)	0.86 ^{Ba} (0.65-2.13)
Posttreatment Day 7	0.97 ^{Ba} (0.65-2.01)	1.17 ^{ABa} (0.81-1.99)	2.11 ^{Ab} (0.67-3.59)	1.07 ^{ABa} (0.65-1.96)
Posttreatment Day 14	1.26 ^{Ba} (0.65-2.15)	0.83 ^{Ba} (0.65-1.59)	2.98 ^{Aa} (1.13-4.62)	1.00 ^{Ba} (0.65-1.81)
* Medians followed by the same superscripted letters (capital letters in the horizontal rows, lowercase letters in the vertical columns) are not statistically significantly different ($P > .05$), according to the Kruskal-Wallis test and the Friedman test, respectively.				

comparisons between the evaluations of each bleaching agent. She performed multiple comparisons by using the Dunn test. The level of significance was $\alpha = .05$.

RESULTS

Of the 80 participants who began the dental bleaching study, 15 were excluded because of extreme tooth sensitivity or an inability to attend all sessions of microbiopsy analyses (four from the home-use carbamide peroxide 10 percent group, five from the home-use carbamide peroxide 20 percent group, one from the in-office hydrogen peroxide 35 percent group and five from the in-office hydrogen peroxide 38 percent group). Therefore, for the calcium and phosphorus evaluation, we considered only the participants for whom we had all samples from baseline, seven, 14 and 21 days of bleaching and seven and 14 days posttreatment.

Table 2 describes the median, minimum and maximum values of phosphorus concentration (mg/mL) according to the technique and bleaching agent and the evaluation interval. We verified that there was no statistically significant difference in phosphorus concentrations among the evaluation times, regardless of which bleaching gel was used. This indicates that the length of the bleaching time does not affect the amount of phosphorus in dental enamel. The

TABLE 3

Calcium concentration of bleaching agents in tooth enamel, according to technique and evaluation interval.

EVALUATION INTERVAL	BLEACHING AGENTS' MEDIAN (MINIMUM-MAXIMUM) VALUES OF CALCIUM CONCENTRATION IN TOOTH ENAMEL IN MILLIGRAMS PER MILLILITER, ACCORDING TO TECHNIQUE			
	Home Use		In Office	
	Carbamide peroxide 10% (n = 16)	Carbamide peroxide 20% (n = 15)	Hydrogen peroxide 35% (n = 19)	Hydrogen peroxide 38% (n = 15)
Baseline	10.63 ^{Aa} (3.47-21.42)	4.32 ^{ABa} (3.07-13.95)	7.05 ^{ABa} (1.07-13.45)	3.69 ^{Ba} (1.88-8.10)
Treatment Day 7	9.86 ^{Aa} (4.55-25.44)	4.60 ^{ABa} (1.11-10.87)	9.95 ^{ABa} (1.35-17.48)	3.92 ^{Ba} (0.47-20.70)
Treatment Day 14	8.21 ^{Aa} (3.45-22.32)	5.07 ^{ABa} (3.31-12.58)	10.19 ^{Aa} (2.57-18.55)	3.59 ^{Ba} (0.53-29.18)
Treatment Day 21	4.48 ^{ABa} (1.56-34.46)	5.08 ^{ABa} (2.41-10.22)	8.63 ^{Aa} (3.18-15.84)	2.99 ^{Ba} (0.95-5.05)
Posttreatment Day 7	5.67 ^{Aa} (2.46-42.18)	5.07 ^{ABa} (1.75-9.73)	7.52 ^{Aa} (3.26-12.63)	2.94 ^{Ba} (0.43-6.30)
Posttreatment Day 14	5.57 ^{ABa} (0.93-9.33)	4.19 ^{ABa} (2.75-7.17)	9.12 ^{Aa} (1.73-13.97)	4.39 ^{Ba} (0.84-5.80)

* Medians followed by the same superscripted letters (capital letters in the horizontal rows, lowercase letters in the vertical columns) are not statistically significantly different ($P > .05$), according to the Kruskal-Wallis test and the Friedman test, respectively.

only difference we observed was in the phosphorus concentration at the day 7 and day 14 posttreatment times for the group that underwent in-office bleaching with hydrogen peroxide 35 percent, but these values were statistically similar to the baseline value. When comparing the bleaching gels, we verified that at the day 14 treatment time, the enamel bleached with hydrogen peroxide 38 percent had the lowest phosphorus concentration compared with the enamel bleached with the carbamide peroxide 10 percent and the hydrogen peroxide 35 percent, but it had the highest phosphorus amount at the day 14 posttreatment time. At the day 21 treatment time, we observed a lower phosphorus concentration in enamel bleached with the hydrogen peroxide 38 percent than in enamel bleached with the hydrogen peroxide 35 percent. At the day 7 posttreatment time, the phosphorus content of the enamel bleached with the carbamide peroxide 10 percent was lower than that of the enamel bleached with the hydrogen peroxide 35 percent.

Table 3 describes the median, minimum and maximum values of calcium concentration (mg/mL) according to technique and bleaching agent and evaluation interval. We verified that there was no statistically significant difference in calcium concentration among the time points,

regardless of the bleaching gel. This indicated that the evaluation times did not interfere with the calcium content of bleached enamel.

Among the tested bleaching agents, we verified that the group receiving the hydrogen peroxide 38 percent bleaching treatment had the lowest quantities of calcium in enamel, quantities that were statistically different from those at baseline, day 7 and day 14 treatment times and the day 7 posttreatment time in teeth treated with the carbamide peroxide 10 percent bleaching, and from those at the day 14 and day 21

treatment times and the day 7 and day 14 posttreatment times in teeth that underwent the hydrogen peroxide 35 percent bleaching treatment. Therefore, we were unable to compare calcium concentration among groups during and after the bleaching treatments because there were differences at baseline. However, the values in the group that received the hydrogen peroxide 38 percent bleaching treatment were statistically similar at all evaluation times to those in the group that received the carbamide peroxide 20 percent bleaching treatment.

DISCUSSION

Regarding calcium and phosphorus concentrations, we verified that there were no differences among them at the three evaluation times (baseline, during bleaching and after bleaching) when we analyzed each technique and bleaching agent individually. When compared with results of other in vivo methodologies, the results of this study corroborate those of Metz and colleagues²² (who did not observe any microhardness alterations in dental enamel that underwent bleaching with a 15 percent carbamide peroxide agent), as well as the findings from Cadenaro and colleagues⁹ (who did not find morphological alterations on the surface of enamel bleached with 38 percent hydrogen peroxide and

35 percent carbamide peroxide gels, as ascertained by means of profilometric analysis and scanning electronic microscopy). Also, our results confirm those of a previous *in situ* study,¹⁷ in which the investigators verified that there were no alterations in microhardness, morphology or calcium and phosphorus mineral content of bleached enamel in an analysis conducted by means of energy-dispersive spectroscopy. This most likely is caused by the protective effect of saliva, which promotes dilution, supplements calcium and phosphorus ions for enamel remineralization, and has a buffering capacity.

Justino and colleagues¹² showed the role of saliva in preventing the demineralizing effect of bleaching gel, reporting that the amount of calcium on the tooth was highest on the first day of bleaching, both *in vitro* and *in situ*. However, they found that the adverse effects of carbamide peroxide on enamel were evident in specimens bleached *in vitro* but not in specimens bleached *in situ*. This condition was not confirmed in our study, because the amount of calcium in dental enamel was no different during bleaching and 14 days after bleaching, and it was similar to the baseline concentration. Considering the home-use bleaching agents, the saliva may or may not have had a role in enamel remineralization, as participants were instructed to leave the gel on for at least two hours per night, alternating with periods of nonuse. However, with the *in-office* technique, we took the microbiopsy samples immediately after the gel application—that is, preventing saliva from having any effect on enamel. Despite this, values were statistically similar across all the evaluations. Our results suggest that the length of time for which the gel was in contact with the dental structure (in this study, a total of 24 minutes) was not enough to promote alterations of calcium and phosphorus content in the enamel.

Published reports in the literature have described the possible influence that incorporating fluoride into the bleaching agent has on enamel remineralization. However, research findings have verified that during *in vitro* dental bleaching, fluoride from bleaching material is not able to prevent enamel demineralization.²⁹⁻³¹ In our study, we noted that this fluoride effect may be possible, as mineral content was similar before, during and after bleaching. Even though the 35 percent hydrogen peroxide gel does not contain fluoride, it created no verified differences in the calcium and phosphorus content of bleached enamel. Thus, further research is needed regarding the role of bleaching gel's fluo-

ride content in tooth remineralization. Also, the more acidic pH of some bleaching agents has been associated with significant mineral loss, according to an analysis of enamel microhardness.³² In our study, even for the agent with the lowest pH (35 percent hydrogen peroxide, with a pH of 2.6), we observed no alterations in calcium and phosphorus content of enamel bleached with that agent during or 14 days after the bleaching procedure.

When we compared the dental bleaching techniques and gel concentrations, we verified that during the 14-day bleaching period, the phosphorus content of enamel bleached with the 38 percent hydrogen peroxide *in-office* gel was lower than that of enamel bleached with the 35 percent hydrogen peroxide (*in-office*) and the 10 percent carbamide peroxide (*home-use*) agents. At the day 21 evaluation, we found a significantly lower phosphorus concentration in enamel bleached with the 38 percent hydrogen peroxide gel (*in-office* technique) than in enamel bleached with the 35 percent hydrogen peroxide gel (also an *in-office* technique). One could hypothesize that the higher percentage of hydrogen peroxide enhanced the product's oxidant potential and made it more deleterious. But in our clinical study, this comparison was difficult because each participant received only one bleaching agent and there could be an inter-participant variability. The same observation applies to the data in Table 3, which show that the calcium concentration was lower in enamel bleached with 38 percent hydrogen peroxide, but this difference also was evidenced at this group's baseline evaluation. Smidt and colleagues¹⁷ reported that variation in the calcium and phosphorus concentrations may be due to interparticipant variability. They indicated that the variability arose because the participants may not have followed the directions for the use of the *home-use* bleaching agents strictly or because each participant had different environmental and physiological intraoral conditions.

Despite the methodology proposed in this study for the determination of the calcium and phosphorus concentration in enamel, it did not detect significant differences during the course of the bleaching treatments. We should note that in the choice of the technique and bleaching gel and the bleaching agent's concentration, other factors are just as important, such as each technique's efficacy, possible associated sensitivity and convenience (costs and benefits, the option of using bleaching gel at home or having it applied in the dental office).

It also is important to emphasize that there

were difficulties in the execution of the proposed methodology during the determination of calcium concentration. Alterations in the final volume of samples were necessary to enable us to determine the calcium content by means of colorimetric arsenazo end point analysis, because the volume of the collected sample was lower than the manufacturer's recommendation. This consideration, as well as the lack of evaluations done by means of the microbiopsy technique because of the baseline differences, made exact comparisons difficult and demonstrated the need for further clinical investigations regarding calcium content of bleached enamel.

CONCLUSION

We conclude that, within the limitations of this clinical investigation, calcium and phosphorus concentrations in dental enamel were not different before, during or after bleaching procedures with the home-use (10 percent and 20 percent carbamide peroxide) and in-office (35 percent and 38 percent hydrogen peroxide) agents. ■

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1. Basting RT, Rodrigues AL Jr, Serra MC. The effects of seven carbamide peroxide bleaching agents on enamel microhardness over time. *JADA* 2003;134(10):1335-1342.
2. Sasaki RT, Barbosa MC, Flório FM, Basting RT. Enamel microhardness and shear bond strength after treatment with an 18 percent carbamide peroxide bleaching varnish. *Am J Dent* 2007;20(5):324-328.
3. Sasaki RT, Arcanjo AJ, Flório FM, Basting RT. Micromorphology and microhardness of enamel after treatment with home-use bleaching agents containing 10 percent carbamide peroxide and 7.5 percent hydrogen peroxide. *J Appl Oral Sci* 2009;17(6):611-616.
4. Grobler SR, Majeed A, Moola MH. Effect of various tooth-whitening products on enamel microhardness. *SADJ* 2009;64(10):474-479.
5. Berger SB, Cavalli V, Ambrosano GM, Giannini M. Changes in surface morphology and mineralization level of human enamel following in-office bleaching with 35 percent hydrogen peroxide and light irradiation. *Gen Dent* 2010;58(2):e74-e79.
6. Cavalli V, Rodrigues LK, Paes-Leme AF, et al. Effects of the addition of fluoride and calcium to low-concentrated carbamide peroxide agents on the enamel surface and subsurface (published online ahead of print Jan. 4, 2011). *Photomed Laser Surg* 2011;29(5):319-325. doi:10.1089/pho.2010.2797.
7. Cavalli V, Rodrigues LK, Paes-Leme AF, et al. Effects of bleaching agents containing fluoride and calcium on human enamel. *Quintessence Int* 2010;41(8):e157-e165.
8. Fu B, Hoth-Hannig W, Hannig M. Effects of dental bleaching on micro- and nano-morphological alterations of the enamel surface. *Am J Dent* 2007;20(1):35-40.
9. Cadenaro M, Breschi L, Nucci C, et al. Effect of two in-office whitening agents on the enamel surface in vivo: a morphological and non-contact profilometric study. *Oper Dent* 2008;33(2):127-134.

10. Attin T, Schmidlin PR, Wegehaupt F, Wiegand A. Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review (published online ahead of print July 16, 2008). *Dent Mater* 2009;25(2):143-157. doi:10.1016/j.dental.2008.05.010.
11. Basting RT, Rodrigues Júnior AL, Serra MC. The effect of 10 percent carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin in situ. *Oper Dent* 2001;26(6):531-539.
12. Justino LM, Tames DR, Demarco FF. In situ and in vitro effects of bleaching with carbamide peroxide on human enamel. *Oper Dent* 2004;29(2):219-225.
13. Basting RT, Rodrigues AL, Serra MC. Micromorphology and surface roughness of sound and demineralized enamel and dentin bleached with a 10 percent carbamide peroxide bleaching agent. *Am J Dent* 2007;20(2):97-102.
14. Barbosa CM, Sasaki RT, Flório FM, Basting RT. Influence of in situ post-bleaching times on resin composite shear bond strength to enamel and dentin. *Am J Dent* 2009;22(6):387-392.
15. Bittencourt ME, Trentin MS, Linden MS, et al. Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations. *JADA* 2010;141(3):300-306.
16. Araujo Fde O, Baratieri LN, Araújo E. In situ study of in-office bleaching procedures using light sources on human enamel microhardness. *Oper Dent* 2010;35(2):139-146.
17. Smidt A, Feuerstein O, Topel M. Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ. *Quintessence Int* 2011;42(5):407-412.
18. Cadenaro M, Navarra CO, Mazzoni A, et al. An in vivo study of the effect of a 38 percent hydrogen peroxide in-office whitening agent on enamel. *JADA* 2010;141(4):449-454.
19. Bitter NC. A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface in vivo. *Gen Dent* 1998;46(1):84-88.
20. Leonard RH Jr, Eagle JC, Garland GE, Matthews KP, Rudd AL, Phillips C. Nightguard vital bleaching and its effect on enamel surface morphology. *J Esthet Restor Dent* 2001;13(2):132-139.
21. Türkun M, Sevgican F, Pehlivan Y, Aktener BO. Effects of 10 percent carbamide peroxide on the enamel surface morphology: a scanning electron microscopy study. *J Esthet Restor Dent* 2002;14(4):238-244.
22. Metz MJ, Cochran MA, Matis BA, Gonzalez C, Platt JA, Pund MR. Clinical evaluation of 15 percent carbamide peroxide on the surface microhardness and shear bond strength of human enamel. *Oper Dent* 2007;32(5):427-436.
23. Brudevold F, Reda A, Aasenden R, Bakhsos Y. Determination of trace elements in surface enamel of human teeth by a new biopsy procedure. *Arch Oral Biol* 1975;20(10):667-673.
24. Gomes VE, Rosário de Sousa ML, Barbosa F Jr, et al. In vivo studies on lead content of deciduous teeth superficial enamel of preschool children. *Sci Total Environ* 2004;320(1):25-35.
25. Costa de Almeida GR, de Sousa Guerra C, de Angelo Souza Leite G, et al. Lead contents in the surface enamel of primary and permanent teeth, whole blood, serum, and saliva of 6- to 8-year-old children (published online ahead of print Feb. 25, 2011). *Sci Total Environ* 2011;409(10):1799-1805. doi:10.1016/j.scitotenv.2011.01.004.
26. Javaheri DS, Janis JN. The efficacy of reservoirs in bleaching trays. *Oper Dent* 2000;25(3):149-151.
27. Kirsten GA, Freire A, de Lima AA, Ignacio SA, Souza EM. Effect of reservoirs on gingival inflammation after home dental bleaching. *Quintessence Int* 2009;40(3):195-202.
28. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925;66:375-400.
29. de Oliveira R, Paes Leme AF, Giannini M. Effect of a carbamide peroxide bleaching gel containing calcium or fluoride on human enamel surface microhardness. *Braz Dent J* 2005;16(2):103-106.
30. da Costa JB, Mazur RF. Effects of new formulas of bleaching gel and fluoride application on enamel microhardness: an in vitro study. *Oper Dent* 2007;32(6):589-594.
31. Tschoppe P, Neumann K, Mueller J, Kielbassa AM. Effect of fluoridated bleaching gels on the remineralization of predemineralized bovine enamel in vitro (published online ahead of print Dec. 11, 2008). *J Dent* 2009;37(2):156-162. doi:10.1016/j.dent.2008.11.001.
32. Magalhães JG, Marimoto AR, Torres CR, Pagani C, Teixeira SC, Barcellos DC. Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity (published online ahead of print July 25, 2011). *Acta Odontol Scand* 2012;70(2):122-126. doi:10.3109/00016357.2011.600704.