In Situ Clinical Effects of New Dentifrices Containing 1.5% Arginine and Fluoride on Enamel De- and Remineralization and Plaque Metabolism

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Abstract

- **Objective**: The primary objective of the three studies reported in this paper was to evaluate the effects of new dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride for their ability to promote remineralization of demineralized enamel, and to prevent mineral loss from sound enamel specimens. A secondary objective was to determine the effects on plaque metabolism with respect to the conversion of arginine to ammonia and sucrose to lactic acid.
- **Methods**: In Study 1, an intraoral remineralization/demineralization clinical model was used to assess the ability to promote remineralization of enamel of two dentifrices containing 1.5% arginine and 1450 ppm fluoride, as sodium monofluorophosphate (MFP), relative to a positive control with dicalcium phosphate dihydrate (Dical) and 1450 ppm fluoride, and a negative control with Dical and 250 ppm fluoride. One of the arginine-containing dentifrices contained Dical, and the other contained calcium carbonate as the source of insoluble calcium. Microradiography and image analysis were used to measure mineral changes. The study used a double-blind crossover design with a two-week treatment period. Each treatment period was preceded by a one-week washout period. Each product was used twice a day for two weeks. In the two other studies, the ability of dentifrices containing 1.5% arginine and fluoride to prevent demineralization of sound enamel blocks was assessed using an intraoral demineralization/remineralization clinical model and a double-blind crossover design with a five-day treatment period. A one-week minimum washout period preceded each treatment phase. Microhardness was used to assess mineral changes. Cariogenic challenges were administered by dipping each intraoral retainer into a 10% sucrose solution four times per day. Each product was used twice per day during the treatment period. Plaque was harvested from the specimens to measure the ability of the plaque to convert arginine to ammonia (Studies 2 and 3) and sucrose to lactic acid (Study 3) at the end of each treatment period. In Study 2, a dentifrice containing 1.5% arginine, Dical, and 1450 ppm fluoride as MFP was compared to a matched positive control containing 1450 ppm fluoride and to a matched negative control containing 250 ppm fluoride. In Study 3, a dentifrice containing 1.5% arginine, calcium carbonate, and 1000 ppm fluoride as MFP was compared to a matched positive control containing 1000 ppm fluoride and to a matched negative control containing 0 ppm fluoride.
- **Results**: In Study 1, the percent mineral changes were +18.64, +16.77, +4.08, and –24.95 for the 1.5% arginine/Dical/1450 ppm fluoride, the 1.5% arginine/calcium carbonate/1450 ppm fluoride, the positive control, and negative control dentifrices, respectively. Study validation was successfully achieved by showing that the positive control was statistically significantly better that the negative control in promoting remineralization ($p = 0.0001$). The two arginine-containing test products were statistically significantly better than the positive control ($p < 0.05$). No significant difference was observed in efficacy between the two arginine-containing products, indicating that efficacy in promoting remineralization was independent of the choice of Dical or calcium carbonate as the source of insoluble calcium. In Study 2, the percent demineralization values were –8.50, +1.67, and +12.64 for the 1.5% arginine/ Dical/1450 ppm fluoride, the positive control, and negative control dentifrices, respectively. Study validation was successfully achieved by showing that the positive control was statistically significantly better at preventing demineralization than the negative control ($p < 0.0001$). The arginine-containing dentifrice was shown to be statistically significantly better at preventing enamel demineralization than the positive control ($p < 0.0001$). Plaque metabolism measures for plaque exposed to the three treatments gave the following values for ammonia production after an arginine-sucrose challenge, expressed in nanomoles per milligram plaque: 162.7; 105.4; and 115.9 for the 1.5% arginine/Dical/1450 ppm fluoride, positive control, and negative control dentifrices, respectively. No statistically significant differences were observed between the three treatments, but the arginine-based dentifrice showed directionally higher ammonia production than both the positive and negative controls. In Study 3, the percent demineralization values were +1.16, +4.96, and +15.34, for the 1.5% arginine/calcium carbonate/1000 ppm fluoride, the positive control, and negative control dentifrices, respectively. Study validation was successfully achieved by showing that the positive control was statistically significantly better at preventing demineralization than the negative control ($p < 0.0001$). The arginine-containing dentifrice was shown to be statistically significantly better at preventing enamel demineralization than the positive control ($p < 0.05$). Plaque metabolism measures for plaque exposed to the three treatments gave the following values for ammonia production after an arginine-sucrose challenge, expressed in nanomoles per milligram plaque: 99.6; 56.2; and 42.2 for the 1.5% arginine/calcium carbonate/1000 ppm fluoride, the positive control, and negative control dentifrices, respectively. Plaque treated with the arginine-

containing dentifrice produced significantly more ammonia than the positive and negative control dentifrices ($p < 0.05$). No significant difference in ammonia production was observed between the two controls. Lactic acid production after a sucrose challenge gave the following values, expressed as nanomoles per milligram plaque: 4.06; 5.12; and 4.64 for the 1.5% arginine/calcium carbonate/1000 ppm fluoride, the positive control, and negative control dentifrices, respectively. No significant difference was observed between the three treatments, but the arginine-based treatment showed directionally lower lactic acid production.

- **Results**: The results of these three studies show that dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride have a significantly improved ability to promote remineralization and prevent demineralization of enamel relative to dentifrices containing the same level of fluoride alone. Two different sources of insoluble calcium were evaluated, Dical and calcium carbonate. Dentifrices with Dical and with calcium carbonate, each in combination with 1.5% arginine and fluoride, provided superior efficacy as compared to matched dentifrices with fluoride alone, and the two products demonstrated comparable efficacy in promoting remineralization. The results of these studies demonstrate that the addition of 1.5% arginine to Dical-and calcium carbonate-based fluoride dentifrices provides superior efficacy in preventing demineralization and promoting remineralization, and, further, indicate that the arginine-containing dentifrices enhance the ability of plaque to metabolize arginine to ammonia.

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Introduction

Dental caries is a prevalent and ubiquitous oral health problem. In simplest terms, dental caries involves the loss of tooth mineral as a result of attack by acids produced through the fermentation of dietary sugars by acid-producing (cariogenic) bacteria. Caries can affect children at a very early age[1-3] and will continue to afflict most individuals throughout adolescence and adulthood,[4-6] thus presenting a significant oral health and public health concern on a global basis.[7-9] Many decades of scientific research have greatly increased our understanding of dental caries, and the application of this knowledge has led to the successful implementation of fluoride-based therapies to help prevent and arrest caries development and progression.[10] Nonetheless, dental caries remains a prevalent disease, largely because of its complex etiology and multi-factorial nature. The existence of dental caries, however, does not alone merit it as a major health issue. The disease must have a measurable impact or cost to society if it is to be elevated to the level of a major healthcare issue. The US Surgeon General's report in 2000 clearly identified the importance of good oral health as an integral part of general health and well being.[11] Healthy and strong teeth are important attributes of good oral health.

There are various ways of expressing the costs of caries to society, the most tangible, of course, being those costs associated with dental restorations. Roughly 60% of dental healthcare costs to dental insurers in the US are associated with dental restorations, and this translates into roughly $45 billion per year.[12] This does not take into account people without dental insurance. For populations of the world who cannot afford or do not have ready access to a dentist the cost is less tangible, but nevertheless important. Pain and suffering associated with caries is a true cost because it diminishes the quality of life. The global prevalence and associated costs have not escaped the attention of academia, government health organizations, the dental profession, and companies associated with developing oral care products. As a result, developing more effective strategies for the prevention of caries remains a key area of interest.

In the area of preventative treatments, most caries preventive regimens utilize fluoride which is, without question, a highly successful caries preventive agent. The dramatic decline in caries prevalence and severity observed over the last several decades has been attributed to fluoride's widespread use.[12] Indeed, the widespread use of fluoride dentifrices has been widely acknowledged by academic experts, the dental profession, and professional health organizations to be the single most important factor contributing to the decline observed in caries over the past several decades.[13,14]

Caries is a disease that is caused by prolonged contact of dental plaque with the tooth surface, accompanied by frequent ingestion of dietary sugars. The caries process is a cyclical and dynamic process with biological origins. Frequent ingestion of sugar, along with incomplete plaque removal associated with poor oral hygiene habits or improper brushing, are key to the progression of caries.[7,15] Cariogenic bacteria that reside naturally in dental plaque, as part of the bacterial community, utilize sugars for energy and use the acid by-product of sucrose fermentation to proliferate in the biofilm and gain a competitive advantage, as many non-pathogenic communal bacteria do not contain protective mechanisms to survive prolonged and frequent exposure to acids.[16] The acidic environment is also harmful to the tooth. Normally, the fluid in contact with the tooth is neutral in pH and supersaturated with respect to enamel. When the pH falls following a sucrose challenge and the consequent formation of acid at the plaque-tooth surface interface, the plaque fluid becomes undersaturated with respect to enamel and the tooth mineral begins to dissolve. Saliva plays a protective role by serving as a source of calcium and phosphate, and helps restore the plaque pH to a more neutral state after a cariogenic challenge so that repair processes may commence.[17-19] Frequent ingestion of sugar creates a shift in the plaque community from one supporting a healthy tooth environment to a more pathogenic state favoring the cariogenic bacteria. This exposes the tooth to longer periods of undersaturation, and shifts the mineral balance in favor of net mineral loss from the tooth.

The mode of action of fluoride has a favorable benefit on the mineral balance.[20,21] Its main role in preventing caries is to modulate the calcium phosphate chemistry at the tooth surface, but it does not influence the biological origin of caries. Specifically, it helps prevent demineralization of the tooth surface under acidic conditions, and helps promote remineralization at neutral pH when the caries challenge is no longer present.[22-28] A limitation of fluoride is that it does little to influence the primary

cause of caries, *i.e.*, acid production by cariogenic bacteria in dental plaque. Because of this, fluoride, as well as other technologies that rely solely on protective-repair mechanisms of the tooth mineral, cannot be expected to provide complete protection.

A promising approach to enhancing the efficacy of fluoride is to combine it with a technology that targets the cause of caries; that is, one that is capable of reducing the overall cariogenic potential of dental plaque. The cariogenic potential of plaque has both chemical and biological components. Nature provides a blueprint on how to lessen the cariogenic potential of plaque. Saliva is a key natural defense system used by the oral cavity to help protect against caries. Aside from saliva's ability to wash away and dilute acids, it contains both chemical and biological protective factors that help modulate the cariogenic potential of plaque. As noted, saliva is neutral in pH and rich in calcium and phosphate, which helps maintain supersaturation with respect to tooth mineral to aid in remineralization and prevention of demineralization. From a biological perspective, saliva is a key source of nitrogen-based metabolites, such as arginine and urea, which are derived from the breakdown of peptides and proteins by salivary enzymes.[17-19] Arginine is metabolized by arginolytic bacteria using the arginine deiminase system to produce energy in the form of adenosine triphosphate, and ammonia and carbon dioxide.[29] The important feature of this pathway is the production of ammonia which neutralizes acids and promotes a more alkaline pH that is unfavorable to cariogenic bacteria. Thus, by utilizing arginine as a survival mechanism against acidic conditions created by cariogenic bacteria, the arginolytic bacteria help to maintain a neutral pH, a condition in which cariogenic bacteria, such as *S. mutans*, are poor competitors in the biofilm, and their ability to dominate the plaque community and cause harm to the tooth is reduced.

Kleinberg has developed a highly effective fluoride-free anticaries technology based on the protective benefits provided by saliva. This technology is based upon a combination of arginine, calcium carbonate, and a cariostatic anion, such as bicarbonate, to deliver anticaries benefits.[19,30] This technology has been proposed to reduce the cariogenic potential of plaque by providing calcium to help maintain supersaturation under conditions of acid challenge, bicarbonate for buffering capacity, and arginine as a metabolic substrate for alkali production. Colgate-Palmolive has broadened the scope of this patented technology by combining arginine with an insoluble calcium compound and fluoride to provide a dentifrice with clinically proven superior anticaries benefits.[31,32]

This article summarizes the results of three intraoral caries clinical studies that demonstrate the enhanced efficacy of dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride in promoting remineralization and preventing demineralization of enamel as compared to matched dentifrices with fluoride alone. Complementary measurements of the ability of arginine-containing dentifrices to enhance arginine metabolism of plaque provide further insight into how this new technology may work to provide an improved benefit when combined with fluoride.

Materials and Methods

Table I provides an overview of the study details for the three studies reported in this article. This overview includes the study design, subject characteristics, study location, test dentifrices, appliance type, enamel specimen type, intraoral treatment, outcome measures, and statistics. Based upon previous remin/demin and demin/remin studies, it has been established that these study designs have residual standard deviations of 30 units and 10 units, respectively. The studies reported in this article were powered to detect differences among treatments of one standard deviation with an 80% probability, which requires minimum sample sizes of 30 and 10 subjects for the remin/demin and demin/remin studies, respectively. In all studies, each treatment phase was preceded by a minimum of a one-week washout period, during which subjects brushed their teeth twice daily with a Colgate adult soft bristle toothbrush and non-fluoride silica-based dentifrice. The duration of this washout period was based upon previous studies which showed no evidence of carryover effects. Each study utilized a randomized, double-blind, crossover design, with a balanced order presentation to further minimize potential carryover effects. Treatment times were based upon previous studies in which clinically relevant product differences were differentiated. All of the dentifrices were over-wrapped and coded to blind the studies. In each study, subjects were required to meet all the inclusion and exclusion criteria outlined in the protocol, and to read and sign an informed consent prior to starting the study. Each study protocol was reviewed by, and received ethical approval from, the appropriate Institutional Review Board.

Study 1

Design. Study 1 utilized an intraoral remineralization/ demineralization clinical model in which subjects wore an intraoral appliance consisting of a lower partial mandibular denture (Figure 1). The intraoral appliances each contained enamel-thin sections that had an artificially induced caries lesion. Mineral changes in the enamel-thin sections were measured by microradiography and image analysis to determine the percent mineral change or net remineralization of the artificially induced lesions. This study was conducted to determine if dentifrices containing 1.5% arginine, an insoluble calcium compound as either calcium carbonate or Dical, and 1450 ppm fluoride as MFP, significantly enhance remineralization, as compared to a dentifrice containing an insoluble calcium compound and 1450 ppm fluoride alone, and to determine if the two arginine-containing dentifrices were equally effective. This study included four treatment periods to test the following dentifrices: 1.5% arginine with 1450 ppm fluoride as MFP in a Dical base (test dentifrice); 1.5% arginine with 1450 ppm fluoride as MFP in a calcium carbonate base (test dentifrice); 1450 ppm fluoride as MFP in a matched Dical base (positive control); and 250 ppm fluoride as MFP in a matched Dical base (negative control).

Procedure for Preparing Enamel-Thin Sections. Details of the preparation and use of enamel-thin sections have been given in several previous publications.[33,34] Enamel blocks approximately 3 mm in width were cut from sound extracted human molars or canine teeth that were free of large cracks, white spots, or dis-

Table I
Summary of Study Details

Study Number	Study Design	Subject Characteristics	Study Location	Test Dentifrice	Appliance Type	Enamel Specimens	Intraoral Treatment	Outcome Measures	Statistics
1	Four cell, double-blind, randomized crossover	30 healthy male and female subjects; aged 18–70; minimum of 20 natural uncrowned teeth (excluding third molars); lower partial mandibular denture with enough space to fit 2 specimens	Colgate-Palmolive Global Technology Center, Mumbai, India	1. 1.5% arginine with 1450 ppm fluoride as MFP in calcium carbonate base 2. 1.5% arginine with 1450 ppm fluoride as MFP in dicalcium phosphate base 3. 1450 ppm fluoride as MFP in dicalcium phosphate base 4. 250 ppm fluoride as MFP, dicalcium phosphate base	Lower partial mandible denture	Acid demineralized human enamel-thin sections	Twice-daily brushing with dentifrice treatment for 2 weeks	% Mineral change 100*(before – after)/before from microradiography	Two-factor (subject and treatment) ANOVA to determine if significant differences exist; Tukey's multiple comparison test for pair-wise differences of treatments
2	Three cell, double-blind, randomized crossover	16 healthy male and female subjects; aged 18–65; minimum of 20 natural uncrowned teeth (excluding third molars)	Colgate-Palmolive Global Technology Center, Piscataway, NJ, USA	1. 1.5% arginine with 1450 ppm fluoride as MFP in dicalcium phosphate base 2. 1450 ppm fluoride as MFP in dicalcium phosphate base 3. 250 ppm fluoride as MFP in dicalcium phosphate base	Upper palatal retainer	Sound bovine enamel	Twice-daily brushing with dentifrice; treatment for 5 days; 4 × daily *ex vivo* sucrose challenge	Primary: % change in enamel micro-hardness = 100* (before – after)/ before Knoop indentation before and after treatment) Secondary: Ammonia production from harvested plaque	Two-factor (subject and treatment) ANOVA to determine if significant differences exist; Tukey's multiple comparisons test for pair-wise differences of treatments
3	Three cell, double-blind, randomized crossover	18 healthy male and female subjects; aged 18–65; minimum of 20 natural uncrowned teeth (excluding third molars)	Colgate-Palmolive Global Technology Center, Piscataway, NJ, USA	1. 1.5% arginine with 1000 ppm fluoride as MFP in calcium carbonate base 2. 1000 ppm fluoride as MFP in calcium carbonate base 3. 0 ppm fluoride in calcium carbonate base	Upper palatal retainer	Sound bovine enamel	Twice-daily brushing with dentifrice; treatment for 5 days; 4 × daily *ex vivo* sucrose challenge	Primary: % change in enamel micro-hardness = 100* (before – after)/ before) (Knoop indentation before and after treatment) Secondary: Ammonia and lactic acid concentrations from harvested plaque	Two-factor (subject and treatment) ANOVA to determine if significant differences exist; Tukey's multiple comparisons test for pair-wise differences of treatments

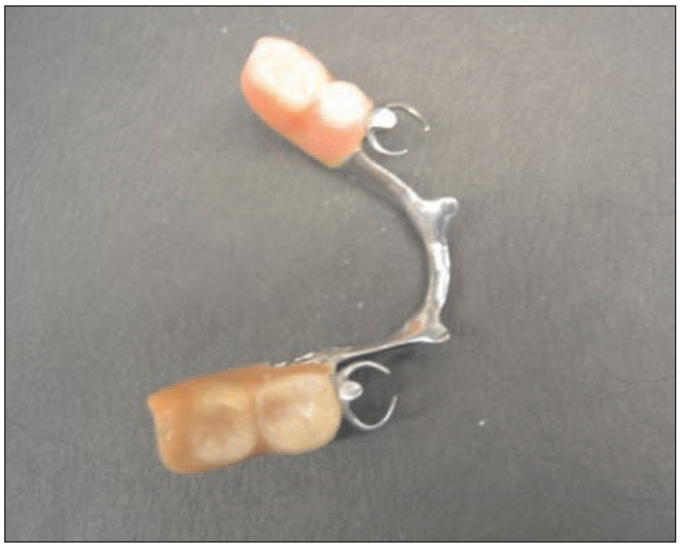

Figure 1. *Intraoral lower mandible partial denture appliance used in remineralization/demineralization study (Study 1).*

coloration. They were then cleaned by scrubbing with a toothbrush and a diluted liquid detergent, and sterilized for four hours using ethylene oxide. The blocks were then mounted in a specimen holder using cyanoacrylate adhesive, and were sectioned with a Leica 1600 Microtome saw (Leica, Bannockburn, IL, USA) to a 150 micron thickness. The thin sections were then embedded in a polyester film together with a nickel-plated marker to ensure consistent area measurement throughout the study. Specimens were stored at room temperature during preparation. In the last step of the preparatory phase, caries-like lesions were formed in the exposed enamel edges of the thin sections by immersing them in 0.1 N acetic acid (Sigma, St. Louis, MO, USA), pH 4.6, for 48 hours at 37°C. The enamel-thin sections were then removed from the demineralizing solution, rinsed with de-ionized water, and air dried at room temperature.

Microradiography and Measurement of Mineral Density Changes of Enamel-Thin Sections. Microradiography was used on the enamel-thin sections to obtain mineral density changes. Mineral density changes were measured from radiographs of

the enamel-thin sections before lesion formation (sound), after lesion formation (untreated), and after treatment with the dentifrice being tested (treated). Image analysis was used to obtain the mineral density profiles. Lesion areas before and after treatment were calculated by subtracting the sound profile from the profile of the untreated and treated profiles to generate difference profiles. The area under the curve of a difference profile represents the lesion area. A custom-designed program was used to overlay the profiles and measure the lesion areas. Mineral changes are expressed as percentage change from the initial lesion size after treatment, as given by the formula below:

Mineral Change = Lesion area before treatment
– Lesion area after treatment
% Mineral Change = Mineral change/
(Lesion area before treatment) X 100

Placement of Enamel-Thin Sections into Lower Partial Mandibular Denture. Depending on the available space for the enamel-thin sections, holes were drilled into the left or right side of the lower partial mandibular denture, slightly larger than the size of the specimen. Two enamel-thin sections were then mounted at this site and held in place by use of a light-cured, non-fluoride dental composite.

Clinical Procedure. Thirty healthy subjects in Mumbai, India, aged 18–70 years, with at least 20 natural teeth and a partial mandibular denture, were recruited into this study. After the one-week washout period, the subjects placed the lower partial denture with the implanted enamel-thin sections into their mouths. The subjects were instructed to keep the appliance in the mouth for 24 hours a day during the two-week treatment phases. With the appliance in the mouth, the subjects were instructed to brush their teeth and the specimens with the assigned dentifrice twice per day (morning and evening before going to bed), for one minute each time, followed by a ten-second rinse with tap water. Subjects were allowed to remove the appliance after meals to clean them. Cleaning was permitted by rinsing the appliance under tap water only. No other oral care product was used during the course of the clinical study.

After each experimental two-week treatment period, the subjects returned the appliances and the specimens were removed for analysis. The subjects then began a one-week washout period before the next two-week treatment period.

Statistical Analysis. The primary measured response was the change in lesion area (% mineral change) before and after treatment. A two-factor ANOVA with the subject and treatment as factors was performed. A difference among treatments was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to validate the study (comparison of positive versus negative control). If the test method was validated, a second two-factor ANOVA, excluding the negative control, was conducted to compare the two test products versus the positive control. A difference among treatments was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to determine which treatments were significantly different from each other.

Study 2

Design. Study 2 utilized an intraoral demineralization/remineralization clinical model in which mineral changes (before and after each treatment) were measured by microhardness. Subjects wore an upper palatal retainer (Figure 2) containing four prepared bovine enamel specimens. These enamel blocks were covered with metal wire mesh to accumulate plaque during the treatment period. This study was conducted to determine if a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride prevents demineralization significantly more effectively than a matched dentifrice containing 1450 ppm fluoride alone. This study included three treatment periods to test the following dentifrices: 1.5% arginine with 1450 ppm fluoride as MFP in a Dical base (test dentifrice); 1450 ppm fluoride as MFP in a Dical base (positive control); and 250 ppm fluoride as MFP in a Dical base (negative control).

Procedure for Preparing Bovine Enamel Blocks. To prepare the bovine enamel specimens, each bovine tooth was cut into blocks with a final measurement of approximately 5 mm by 5 mm. The blocks were then cleaned, rinsed with distilled water, and sterilized by ethylene oxide for four hours. When not in use during the preparatory phase, blocks were stored in distilled water in a refrigerator.

In the grinding step, the blocks were flattened using a variable speed grinder/polisher with three retaining rings and 15 μ diamond polishing disc (Buehler, Lake Bluff, IL, USA). The polishing disc was wetted with water, and three specimen carriers, which are capable of holding 49 blocks each, were placed on the disc. The blocks were placed dentin-side down in the specimen carriers, and the dentin was ground flat for 2.5 minutes at 100 rpm as approximately three liters of water were poured slowly onto the center of the disc. After grinding, the blocks were removed and visually examined for flatness, and the procedure was repeated until the blocks appeared flat by visual inspection. After this procedure, the blocks were placed enamel-side down in the specimen carriers, and the enamel was ground flat for five minutes at 100 rpm as approximately four liters of water were

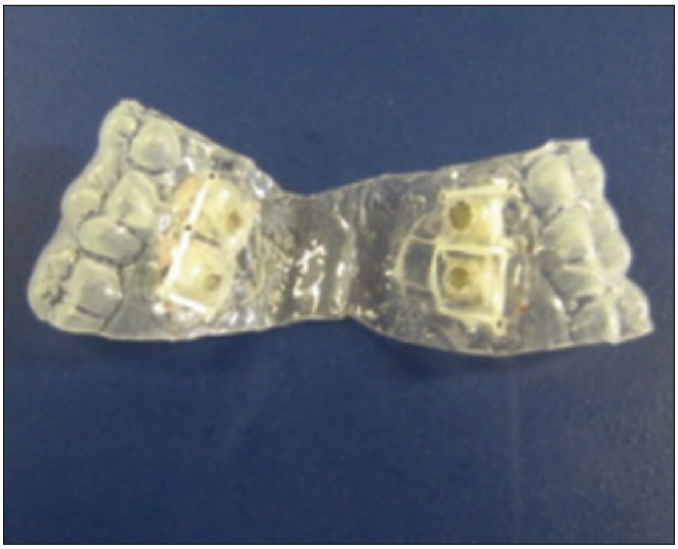

Figure 2. *Intraoral retainer used in demineralization/remineralization studies (Studies 2 and 3).*

poured slowly onto the center of the disc. After grinding, the blocks were removed and visually examined for flatness.

The blocks that passed the visual inspection were then polished using the same apparatus. A polishing cloth was fitted to the polishing disc, and a diamond suspension, METADI® 6 µ (Buehler, Lake Bluff, IL, USA), was sprayed to evenly cover the cloth. Blocks were placed enamel-side down, as previously described, and the system was run for ten minutes at 100 rpm, adding more diamond suspension as needed. The blocks and the polishing cloth were then rinsed with two liters of water over a two-minute time period. The blocks were then polished for another ten minutes, using the same procedure and second polishing suspension, Masterprep 0.05 µ (Buehler, Lake Bluff, IL, USA). The blocks were then washed by pouring 25 ml of 10% w/w Alconox solution (VWR, West Chester, PA, USA) onto the polishing cloth and running it for one minute before the blocks had a final rinse using four liters of water over another five-minute time period. The blocks were then removed and sonicated with distilled water for ten minutes, which was repeated until all appearance of foaming, suds, and cloudiness was gone. The final height of the blocks was determined by a micrometer.

Microhardness Testing of Bovine Enamel Blocks. The microhardness of the enamel blocks was determined using a Micromet 5101 Micro-hardness Tester with Knoop Diamond Indenter and a 50 gram load (Buehler, Lake Bluff, IL, USA). Baseline indents were required to be symmetrical, and readings no greater than 55–60 microns. Blocks were gently buffed with a dry microcloth before testing to both dry the block and remove any surface contaminant. The stage micrometers were zeroed and a block was located with a corner at 0,0. Under low-power magnification, the block was set in place in order to find a clean area. Once found, the indenter was placed over the block and released. After 15 seconds, the indenter was picked up, the micrometer was adjusted in the x direction another 0.01 mm, and another indent was dropped. This was repeated until five indents were made. The indents were then measured using a higher power lens, and the average was obtained for baseline microhardness (M1). After each five-day treatment period, the blocks were reassessed for microhardness (M2) as described above. Percent changes in indentation length 100*(M2 – M1)/M1 were used to determine changes in enamel hardness, as they are directly correlated with mineral content.

Preparation of Retainers for the Intraoral Study. Customized retainers were prepared by first casting an impression of the upper maxillary palate of each subject. Once the impressions were made, a sheet of 0.020 cm thick vacuum forming plastic material (Buffalo Dental Mfg. Syosset, NY, USA) was molded to fit across each subject's roof of the mouth, and to form to molar teeth on either side of the mouth.

Placement of Bovine Enamel Blocks into Retainer. Holes were punched into each of the retainers in order to expose the surface of the bovine enamel blocks to the treatment being used. Two blocks, measuring approximately 4 mm by 4 mm by 1 mm, were placed on both the right and left side of the retainer. They were secured into the retainers by drilling several small holes on the sides of the punched out holes, and stitching dental floss in back of the blocks. A thin sheet of soft dental orthodontic tray wax (Kerr, Romulus, MI, USA) was placed across back of the blocks to secure them into place. Before placing the blocks into the retainer, they were covered with a sheet of wire mesh. The wire mesh was used to accumulate plaque over the five-day treatment period.

Total Ammonia Production from *In Situ* Formed Plaque Samples. The plaque that accumulated on the blocks was collected before the microhardness measurement after treatment (M2), pooled, and stored at –20°C until analysis. The plaque assay procedure was adapted from a previously published method.[35] In the procedure, plaque was kept on ice and the concentration was normalized to 1 mg/ml in 1 phosphate buffered saline, pH 7.4 (Gibco, Grand Island, NY, USA). The samples were mixed with a vortex and then sonicated to break up plaque clusters and homogenize the sample. Each plaque sample was then challenged with sucrose (VWR, West Chester, PA, USA) and arginine (Sigma, St. Louis, MO, USA) to give a final concentration of 0.1% and 5 millimoles, respectively. The samples were incubated in a 37°C shaking water bath for 30 minutes before ammonia production was analyzed. A diagnostic ammonia assay kit (Diagnostic Chemicals Limited, Oxford, CT, USA) was used to quantify the ammonia produced in the plaque.

Clinical Procedure. Sixteen healthy subjects from an established pool of subjects in a clinical database in Piscataway, New Jersey, USA, aged 18-65, with a minimum of 20 natural teeth, were recruited into this study. After the one-week washout period, the subjects placed the custom retainer with the four bovine enamel blocks onto the upper maxillary palate, and they were instructed to brush their teeth, and not the retainer containing the bovine enamel blocks, with the assigned dentifrice twice per day (morning and evening before going to bed) for one minute each time, followed by a 10-second rinse with tap water. Subjects were also instructed to dip their appliances into a 10% sucrose solution four times per day at approximately 9:00 a.m., 11:00 a.m., 4:00 p.m., and 7:00 p.m. for ten minutes each time. The upper retainer was worn for 24 hours per day for each five-day treatment period. Subjects were allowed to remove the appliance only during meal time and to clean them. Cleaning was permitted by rinsing the retainer under tap water only. No other oral care product was used during the course of the clinical study.

After each experimental five-day treatment period, subjects returned their retainers, and the bovine enamel blocks were removed for analysis. The subjects then began a nine-day washout period before the next five-day treatment period.

Statistical Analysis. For the microhardness measurements, the primary response was the percent change in indentation length before and after treatment. The secondary outcome was the ammonia production. A two-factor ANOVA, with the subject and treatment as factors, was conducted on both the primary and secondary outcomes to determine whether significant differences existed between treatments. A difference was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to validate the study outcome (comparison of positive versus negative control) and determine which treatments were significantly different from each other.

Study 3

Design. This study followed the intraoral demineralization/remineralization clinical model used in Study 2. The only difference in the analyses of the samples was that plaque that accumulated on the enamel blocks was analyzed for lactic acid production, in addition to ammonia production. This study was conducted to determine if a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1000 ppm fluoride prevents demineralization and delivers anticaries benefits significantly more effectively than a matched dentifrice containing 1000 ppm fluoride alone. This study included three treatment periods to evaluate the following dentifrices: 1.5% arginine with 1000 ppm MFP in a calcium carbonate base (test dentifrice); 1000 ppm MFP in a calcium carbonate base (positive control); and 0 ppm MFP in a calcium carbonate base (negative control). Eighteen healthy subjects from the established pool in the clinical database in Piscataway, New Jersey, USA, aged 18–65, with similar age and oral profiles, were recruited into this study.

Total Lactic Acid Production from *In Situ* Formed Plaque Samples. The plaque that accumulated on the blocks was collected before the microhardness measurement after treatment, pooled, and stored at –20°C until analysis. The plaque sample was first resuspended in ice cold 0.03% trypticase soy broth (TSB; Difco, Becton, Dickinson and Company, Sparks, MD, USA) to a final concentration of approximately 0.03–0.04 mg of plaque per ml of TSB. Sucrose then was added to each plaque sample to a final concentration of 10% before incubation for ten minutes at 37°C with mild shaking. After the ten-minute incubation time, the samples first were heated to 80°C for five minutes to kill the bacteria and to release all acids, then cooled on ice water for an additional five minutes. After this cooling, the samples were centrifuged and the supernatant was filtered. The lactate (lactic acid anion) concentration in the supernatant was measured using capillary electrophoresis.

The conditions used to analyze the plaque samples using the capillary electrophoresis were adapted from a previously published method.[36] The separations were carried out on a fused-silica capillary with a 50 cm effective length X 50 µm internal diameter. The optimized buffer system consisted of 20 mM 2,6-pyridine dicarboxylic acid and 0.5 mM hexadecyltrimethyl ammonium bromide, pH 5.66 (Sigma, St. Louis, MO, USA). Because organic acids have little or no ultraviolet (UV) absorbance, detection was accomplished by using 2, 6-pyridine dicarboxylic acid as a background electrolyte (BGE). In this indirect detection method, the BGE has strong UV absorptive properties and produces a high background absorption in the UV detector. In the absence of non-absorbing analytes, the background signal is constant. When ionic analytes are introduced, they displace UV-absorbing additive ions on a charge-to-charge basis, resulting in a negative peak relative to the high UV absorption baselines. With the analysis, the sample was injected by pressure for ten seconds at 0.5 psi. The separation was performed at –25 kV, and the capillary was thermostated at 25°C. The wavelength for indirect UV detection was selected at 254 nm, and the signal with negative peaks was inverted to obtain a more familiar electropherogram to integrate and process.

To correct for injection errors, each sample was run with the incorporation of a 1.5 mM sodium nitrate internal standard, and a calibration curve was constructed using sodium lactate standards (Sigma, St. Louis, MO, USA). The concentration of lactate present in the plaque sample was determined based upon the ratio of lactate/nitrate peak area and the initial plaque weight.

Statistical Analysis. For the microhardness measurements, the primary response was the percent change in indentation length before and after treatment. The secondary outcomes were the ammonia and lactic acid concentrations. A two-factor ANOVA with the subject and treatment was conducted on both the primary and secondary outcomes to determine whether significant differences existed between treatments. A difference was considered significant if a 95% confidence level was achieved. If a significant difference was detected, a Tukey's multiple comparison test was used to determine which treatments were significantly different from each other.

Results

Study 1

This was an intraoral remineralization/demineralization study comparing four dentifrices: 1.5% arginine with 1450 ppm fluoride in a calcium carbonate base; 1.5% arginine with 1450 ppm fluoride in a Dical base; 1450 ppm fluoride in a Dical base (positive control); and 250 ppm fluoride in a Dical base (negative control)

Twenty-nine of thirty panelists successfully completed the study. The inability of one subject to complete the study was not related to product use, rather a result of personal reasons unrelated to the study.

The results of the study are summarized in Table II. Use of all three dentifrices containing 1450 ppm fluoride as MFP resulted in positive mineral changes in the enamel-thin sections, demonstrating that the enamel was remineralized. This is in contrast

Table II

The Effect of Two Dentifrices Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1450 ppm Fluoride on Remineralization of Demineralized Enamel, Expressed as Average Percent Mineral Change, Compared to Control Dentifrices Containing 1450 ppm and 250 ppm Fluoride, Respectively (Study 1)

Products Tested	Product Designation	Percent Mineral Change (± SD)
Dical toothpaste with 250 ppm fluoride as MFP	Negative control	– 24.6 ± 58.2[a]
Dical toothpaste with 1450 ppm fluoride as MFP	Positive control	+ 4.1 ± 29.3[a,b]
Calcium carbonate toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test Dentifrice 1	+16.8 ± 26.6[a,b]
Dical toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test Dentifrice 2	+18.6 ± 37.2[a,b]

[a] The negative control was statistically significantly different from the positive control and from the two test products ($p < 0.0001$).

[b] The two test products were statistically significantly different from the positive control ($p < 0.05$).

to the 250 ppm fluoride (as MFP) negative control where two weeks of product use resulted in net mineral loss in the enamel-thin sections.

Using the full dataset, a two-factor ANOVA using the subject and treatment as factors indicated that the treatment effect was highly significant ($p < 0.0001$). In order to validate the study, the results for the positive control were compared to those of the negative control using a Tukey's multiple comparison test. The positive control was shown to be significantly better than the negative control at promoting remineralization ($p = 0.0001$), which demonstrates study validity. With the study validated, a second two-factor ANOVA was conducted on a dataset excluding the negative control. This analysis indicated that the treatment effect was highly significant ($p = 0.01$). The two test products were compared to the positive control using a Tukey's multiple comparison test. Both of the dentifrices containing arginine (calcium carbonate and Dical variants) were shown to be significantly better than the positive control at remineralizing the enamel-thin sections ($p < 0.05$). There was no statistically significant difference with respect to remineralization of the enamel-thin sections between the two dentifrices containing 1.5% arginine.

Study 2

This intraoral demineralization/remineralization study compared three dentifrices: 1.5% arginine with 1450 ppm fluoride in a Dical base; 1450 ppm fluoride in a Dical base (positive control); and 250 ppm fluoride in a Dical base (negative control).

Twelve of the originally recruited sixteen subjects successfully completed the three treatment phases in this study. The other four subjects did not complete the study for reasons related to inconvenience or discomfort in wearing the upper palatal retainer for the entire course of the study. Product use was not a reason for discontinuing the study.

The results in Table III show that the dentifrice containing 1450 ppm fluoride as MFP in a Dical base (positive control) was significantly better than the dentifrice containing 250 ppm fluoride as MFP in a Dical base (negative control) at preventing demineralization ($p < 0.0001$), thus demonstrating that the study was successfully validated. In this study, the larger the percent demineralization value, the greater is the amount of demineralization or mineral loss. Compared to the positive control dentifrice containing 1450 ppm fluoride alone, the arginine-containing dentifrice was significantly better in preventing demineralization ($p < 0.0001$). In addition, the arginine-containing dentifrice was the only dentifrice that had a net mineral gain or remineralization, which indicates an increase in enamel hardness after use. These results demonstrate that the new dentifrice containing 1.5% arginine and 1450 ppm fluoride in a Dical base is significantly more effective in preventing enamel loss than a matched dentifrice with 1450 ppm fluoride alone.

Table IV shows the results for the amount of ammonia produced from the collected plaque samples following the arginine-sucrose challenge. While there was a numerical increase in ammonia produced by plaque collected after use of the arginine-containing test dentifrice compared to the matched positive control dentifrice containing 1450 ppm fluoride alone, the result was not statistically significant. No difference in ammonia production was observed between the dentifrices containing 250 ppm fluoride (negative control) and 1450 ppm fluoride (positive control).

Study 3

This was an intraoral demineralization/remineralization study comparing three dentifrices: 1.5% arginine with 1000 ppm fluoride in a Dical base; 1450 ppm fluoride in a Dical base (positive control); and 250 ppm fluoride in a Dical base (negative control).

All eighteen of the subjects successfully completed the study. This intraoral demineralization/remineralization study was also validated by demonstrating that the positive control dentifrice,

Table III

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1450 ppm Fluoride in Preventing Enamel Demineralization, Expressed as Average Percent Demineralization, Compared to Control Dentifrices Containing 1450 ppm and 250 ppm Fluoride, Respectively (Study 2)

Products Tested	Product Designation	Percent Demineralization (± SD)[c]
Dical toothpaste with 250 ppm fluoride as MFP	Negative control	12.6 ± 12.5[a]
Dical toothpaste with 1450 ppm fluoride as MFP	Positive control	1.7 ± 7.7[a,b]
Dical toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test	–8.5 ± 5.6[b]

[a] The negative control was statistically significantly different from the positive control ($p < 0.0001$).

[b] The test product was statistically significantly different from the positive control ($p < 0.0001$).

[c] A negative value indicates a net mineral gain or remineralization.

Table IV

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1450 ppm Fluoride on Total Ammonia Production from *In Situ* Plaque Samples Formed During a Demineralization/Remineralization Study (Study 2) Compared to Control Dentifrices Containing 1450 and 250 ppm Fluoride, Respectively

	Product Designation	Ammonia (nmol per mg Plaque) (Mean ± SD)
Dical toothpaste without 250 ppm fluoride	Negative control	115.9 ± 67.5
Dical toothpaste with 1450 ppm fluoride as MFP	Positive control	105.4 ± 76.2
Dical toothpaste with 1450 ppm fluoride as MFP and 1.5% arginine	Test	162.7 ± 92.8[a]

[a] The test product was not statistically significantly different from the positive control ($p = 0.13$).

containing 1000 ppm fluoride as MFP in a calcium carbonate base, was significantly more effective at preventing demineralization ($p < 0.0001$) than the negative control dentifrice containing 0 ppm fluoride in a calcium carbonate base (Table V). The results also demonstrate that the arginine-containing dentifrice with 1000 ppm fluoride in a calcium carbonate base was significantly more effective in preventing demineralization than the matched positive control dentifrice with fluoride alone ($p < 0.05$).

Table VI shows the amount of ammonia produced from the *in situ* formed plaque samples following the arginine-sucrose challenge. After tooth brushing with the new arginine-containing dentifrice, plaque samples produced significantly more ammonia than plaque samples after brushing with the matched positive control dentifrice containing 1000 ppm fluoride alone ($p < 0.05$). As observed in Study 2, there were no significant differences in ammonia production from plaque samples after brushing with the dentifrice without fluoride (negative control) and with the dentifrice containing 1000 ppm fluoride (positive control). The results suggest that fluoride level does not impact the arginolytic activity of plaque.

In addition to determining the production of ammonia, the study determined the amount of lactic acid produced by *in situ* formed plaque samples. Although the difference in lactic acid production was not statistically significant compared to the positive control or the negative control, the arginine-containing dentifrice produced the least amount of lactic acid (Table VII).

Discussion

In these three studies, intraoral caries clinical models were used to test the ability of a new dentifrice containing 1.5% arginine, an insoluble calcium compound, and fluoride to promote remineralization and prevent demineralization of enamel. Two formula variants were assessed, one using Dical and the other using calcium carbonate as the source of the insoluble calcium compound. The arginine level used in these formulations was 1.5%, which is the same level of arginine used in a non-fluoride dentifrice containing arginine, bicarbonate, and calcium carbonate that was shown to be as effective as a sodium fluoride dentifrice in preventing cavity formation in a two-year conventional caries clinical trial.[30] Further, *in situ* dose response studies, using similar intraoral clinical protocols to those described here, have shown that increasing the arginine level above 1.5% does not provide an additional caries benefit.[37]

Intraoral caries clinical models were used to evaluate this new dentifrice because the technology is designed to work on multiple steps of the complex caries process to deliver its caries protection benefits. Intraoral caries models are the methods of choice for evaluating dental formulations with complex modes of action, because the efficacy of such products is reliant on both the chemical and biological dynamics of the oral environment. The scientific literature suggests that intraoral models have distinct advantages over *in vitro* methods in predicting efficacy outcomes, such as the outcome of conventional caries clinical trials, because they are better able to capture real life

Table V

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1000 ppm Fluoride in Preventing Enamel Demineralization, Expressed as Average Percent Demineralization, Compared to Control Dentifrices Containing 1000 ppm and 0 ppm Fluoride, Respectively (Study 3)

Products Tested	Product Designation	Percent Demineralization (± SD)
Calcium carbonate toothpaste without fluoride	Negative control	15.3 ± 15.6[a]
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP	Positive control	5.0 ± 6.3[a,b]
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP and 1.5% arginine	Test	1.2 ± 5.3[b]

[a] The negative control was statistically significantly different from the positive control ($p < 0.0001$).
[b] The test product was statistically significantly different from the positive control ($p < 0.05$).

Table VI

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1000 ppm Fluoride on Total Ammonia Production from *In Situ* Plaque Samples Formed During a Demineralization/Remineralization Study (Study 3) Compared to Control Dentifrices Containing 1000 ppm and 0 ppm Fluoride, Respectively

Products Tested	Product Designation	Ammonia (nmol per mg Plaque) (Mean ± SD)
Calcium carbonate toothpaste without fluoride	Negative control	42.2 ± 32.6
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP	Positive control	56.2 ± 36.3[a]
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP and 1.5% arginine	Test	99.6 ± 69.0[a]

[a] The test product was statistically significantly different from the positive control ($p < 0.05$).

Table VII

The Effect of a Dentifrice Containing 1.5% Arginine, an Insoluble Calcium Compound, and 1000 ppm Fluoride on Total Lactic Acid Production from *In Situ* Plaque Samples Formed During a Demineralization/Remineralization Study (Study 3) Compared to Matched Control Dentifrices Containing 1000 ppm and 0 ppm Fluoride, Respectively

Product Tested	Product Designation	Lactate (nmol per mg Plaque) (Mean ± SD)
Calcium carbonate toothpaste without fluoride	Negative control	4.6 ± 5.2
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP	Positive control	5.1 ± 7.4
Calcium carbonate toothpaste with 1000 ppm fluoride as MFP and 1.5% arginine	Test	4.1 ± 6.7

conditions, such as product usage, the effect of saliva and its flow, and the bacterial/dental plaque component of caries.[33,34,38,39] This point is critically important to the evaluation of this new arginine-containing dentifrice because: 1) the fluoride component, MFP, relies on the dynamics of the mouth and the action of salivary enzymes to generate free fluoride ion, which is the active form of fluoride; 2) the arginine component relies on the dynamics of the mouth and the action of dental plaque, with its diversity of interdependent bacterial species, to generate ammonia and modulate plaque pH; and 3) the calcium component relies on the dynamics of the mouth to influence the degree of saturation of plaque fluid with respect to enamel. While *in vitro* methods can play a role in product testing, these critical efficacy factors cannot be adequately accounted for in *in vitro* methods limiting their value.

Two different intraoral caries models were used to assess the performance of dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride. The first model used is often referred to as the intraoral remineralization/demineralization model because it measures the relative ability of dentifrices to promote remineralization of partially demineralized thin sections of enamel. The second model is referred to as the intraoral demineralization/remineralization model. In this model, the relative ability of a dentifrice to prevent mineral loss from sound enamel blocks is measured. Real plaque and real saliva are present in the mouth to create and modulate the pH fluctuations in both models. The severity of the caries challenge and how it is initiated differs in the two models. The intraoral remineralization/demineralization model relies solely on each panelist's normal diet to create the cariogenic challenge during the clinical phase of the study. In the intraoral demineralization/remineralization model, the caries challenge is increased by using an *ex vivo* 10% sucrose rinse, four times a day, to simulate a high sugar diet and high caries risk situation. These two models, therefore, determine how different dentifrices affect the processes of remineralization and demineralization of enamel under different conditions. Because real dental plaque is retained on the enamel specimens in the demineralization/remineralization model, this model also provides an opportunity to harvest the plaque and determine if specific treatments have resulted in any changes in the plaque. In both intraoral demineralization/remineralization studies, plaque was collected and the metabolic potential of plaque to convert arginine to ammonia was measured. In one study, the ability of plaque to convert sucrose into lactic acid was also measured. The purpose of conducting the plaque metabolism measures was to gain insight into the mechanisms driving observed differences in enamel re- and demineralization between the products with and without arginine.

In the intraoral remineralization/demineralization clinical study, Study 1, two dentifrices containing 1.5% arginine and 1450 ppm fluoride as MFP were compared. These formulations differed in the source of calcium, *i.e.*, one used Dical and the other used calcium carbonate. The intraoral remineralization/demineralization model used in this study has been previously validated and shown to be predictive of the results of conventional caries clinical trials.[33,34] Two Dical dentifrices containing 1450 ppm fluoride and 250 ppm fluoride were used as the positive and negative controls, respectively. As the results clearly show that the positive control was statistically significantly more effective than the negative control in remineralizing demineralized enamel, this study is validated. This is consistent with the known fluoride dose response of this model. Dentifrices with no or low levels of fluoride have previously been shown to result in net demineralization, whereas fluoride products (1000–1450 ppm fluoride), with clinically proven anticaries efficacy, remineralize the enamel. The results of this study showed that the dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride resulted in enhanced remineralization relative to the positive fluoride control. This was true for both calcium variants, which showed no discernable difference in efficacy.

The enhanced remineralization potential of the arginine-containing dentifrices observed in this study is consistent with the findings of six-month coronal and root caries studies, as well as the results of a traditional two-year caries clinical study measuring effects on cavitation. Specifically, three coronal caries studies, using Quantitative Light-induced Fluorescence (QLF) to measure changes in early caries lesions in children, have each shown that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride are significantly more effective in arresting and reversing coronal caries lesions than dentifrices containing 1450 ppm fluoride alone.[40-42] Likewise, two root caries studies in adults have each shown that the new dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride is significantly more effective in arresting and reversing root caries lesions than a dentifrice containing 1450 ppm fluoride alone.[43,44] Finally, a two-year conventional caries clinical study has proven that two dentifrices containing 1.5% arginine and 1450 ppm fluoride in a calcium base, one with di-calcium phosphate and the other with calcium carbonate, are significantly more effective in preventing the formation of cavitated caries lesions than a dentifrice containing 1450 ppm fluoride alone. Three trained and calibrated dentists examined the children at baseline and after one and two years using the National Institute of Dental Research Diagnostic Procedures and Criteria. The number of decayed, missing, and filled teeth (DMFT) and surfaces (DMFS) for the three study groups were very similar at baseline, with no statistically significant differences among groups. After one year, there were no statistically significant differences in caries increments among the three groups. After two years, the two groups using the dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm F had statistically significantly ($p < 0.02$) lower DMFT increments (21.0% and 17.7% reductions, respectively) and DMFS increments (16.5% and 16.5%) compared to the control dentifrice. The differences between the two groups using the new dentifrices were not statistically significant. The results of this pivotal clinical study support the conclusion that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provide superior protection against caries lesion cavitation to dentifrices containing 1450 ppm fluoride alone.[45]

Because plaque metabolic measures were not assessed in the

remineralization/demineralization study (Study 1), it is not possible to determine with certainty what is driving the improved remineralization performance. However, results from separate plaque metabolism studies support that the arginine-containing dentifrice creates a plaque environment that is more favorable for remineralization than a dentifrice with fluoride alone.[46,47] The results of one of these studies are presented in the next article in this Special Issue. In summary, brushing for two weeks with a dentifrice containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride was shown to significantly increase plaque's ability to convert arginine to ammonia relative to brushing with a control dentifrice containing silica and 1450 ppm fluoride as NaF. The group who used the arginine-containing dentifrice also had a significantly higher resting pH than the group using the control product. The resting pH is plaque's natural pH in the absence of a sucrose challenge. It typically ranges from 6.8 to 7. Under resting pH conditions, plaque is supersaturated with respect to enamel and there is a positive driving force favoring remineralization. An increase in resting pH, such as that observed in the arginine-containing dentifrice group in the plaque metabolism study, increases the degree of saturation of the plaque fluid with respect to enamel, and increases the driving force for enamel remineralization.

In Studies 2 and 3, the intraoral demineralization/remineralization model was used to separately assess the ability of two dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride to prevent demineralization of sound enamel specimens. In this model, the enamel specimens undergo a strong cariogenic challenge with the result that they lose mineral during the course of the study. The most effective treatment loses the least amount of mineral. In Study 2, a dentifrice containing 1.5% arginine, Dical, and 1450 ppm fluoride as MFP was compared to matching positive and negative controls with 1450 ppm and 250 ppm fluoride, respectively. Net mineral loss was experienced for both positive and negative controls. The observation that the positive control was statistically significantly more effective in preventing mineral loss than the negative control validates the study. No net mineral loss was experienced following use of the arginine-containing dentifrice; enamel specimens actually showed an increase in hardness after the treatment period. Importantly, the arginine-containing dentifrice was shown to be statistically significantly more effective than the matched positive control dentifrice in preventing demineralization of enamel. This indicates that arginine is playing a significant role in the enhanced efficacy of this product. While the plaque metabolism results of Study 2 did not reach statistical significance, the numeric data indicate that use of the arginine-containing dentifrice increases the ability of plaque to convert arginine into ammonia relative to the fluoride controls.

In Study 3, a dentifrice containing 1.5% arginine, calcium carbonate, and 1000 ppm fluoride as MFP was compared to matching positive and negative controls with 1000 ppm and 0 ppm fluoride, respectively. The results are consistent with the results of Study 2. Specifically, the study was successfully validated by showing that the positive control was statistically significantly more effective than the negative control in preventing demineralization. Furthermore, the arginine-containing dentifrice was statistically significantly more effective than the positive control in preventing demineralization. Use of the arginine-containing dentifrice was shown to result in a statistically significant increase in arginine catabolism to ammonia. In this study, the ability of plaque to convert sucrose into lactic acid was also measured. There was a numerical decrease in lactic acid production for the arginine-containing dentifrice, but the result was not statistically significant. As caries is a dynamic process, differences in acid production at a given time point may be too small to measure, yet they may reduce the driving force for demineralization sufficiently to collectively add up over time to a measurable benefit on enamel. From the perspective of designing a clinical protocol, it is difficult to capture such an effect. With this noted, the plaque metabolism results support the findings of the two demineralization studies. The addition of 1.5% arginine to a dentifrice containing an insoluble calcium compound and fluoride creates a less cariogenic plaque environment which enhances the protective effects of fluoride, and translates into better overall protection against mineral loss.

The results of the three intraoral studies reported in this article provide strong evidence that dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride are significantly more effective than dentifrices with fluoride alone in both promoting remineralization and preventing demineralization. These improved effects on re- and demineralization were observed for both Dical and calcium carbonate dentifrices. In addition, the results on plaque metabolism measures support that both Dical and calcium carbonate dentifrices exhibit the same mode of action. The results support the hypothesis that dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride promote the breakdown of arginine to ammonia by the action of arginolytic bacteria.[29] The ammonia production neutralizes plaque acids to help maintain a pH-neutral environment, creating conditions that favor a healthy plaque community. Cariogenic bacteria utilize analogous mechanisms, in this case of acid production, to create a more acidic plaque that favors their survival at the expense of the nonpathogenic bacteria. The crucial difference between these two processes is the end effect on tooth mineral. Catabolism of arginine helps to create and maintain neutral pH conditions and a high level of supersaturation with respect to enamel that supports a healthy tooth structure and the remineralization process. Metabolism of sugars and production of acids, on the other hand, create conditions of undersaturation, facilitating damage to the teeth.

In summary, dentifrices containing 1.5% arginine, an insoluble calcium compound, and fluoride provide superior caries protection than dentifrices with fluoride alone. Two dentifrices have been evaluated, one with Dical and the other with calcium carbonate as the source of calcium. Both of these variants provided enhanced caries protection. The complex mechanism of action of these dentifrices results from the effects of the arginine on plaque, which are distinct from, yet complementary to, the effects of fluoride on the tooth tissue. This new denti-

frice technology represents a major advancement in caries protection, and a paradigm shift in the approach to improving the efficacy of fluoride dentifrices.

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