JOURNAL OF CAFFEINE RESEARCH
Volume 6, Number 1, 2016

DOI: 10.1089/jcr.2015.0023

Theobromine: A Safe and Effective Alternative for Fluoride in Dentifrices

Tetsuo Nakamoto, DDS, PhD,[1] Alexander U. Falster, MS,[2] and William B. Simmons, Jr., PhD[2]

During the process of studying caffeine's effects on developing teeth, a serendipitous discovery was made. Teeth comprise hydroxylapatite (HAP). Ingestion of caffeine (1,3,7-trimethylxanthine) caused the formation of smaller crystallites of HAP in the developing teeth. This resulted in the increased release of calcium and phosphorus ions from the enamel surface when exposed to acidic solutions *in vitro*. Furthermore, animal study confirmed the hypothesis that smaller HAP crystallites caused the increased incidence of dental caries. In contrast, theobromine (3,7-dimethylxanthine), which is similar to caffeine, caused formation of larger HAP crystallites *in vitro*. The ingestion of theobromine by lactating dams showed a decreased release of calcium and phosphorus ions from the enamel surface in the developing teeth of neonates *in vivo*. The use of fluoride dentifrices is controversial. It is also well documented that young children who brush their teeth often ingest fluoride-containing dentifrices. Based upon our comparative study between fluoride and theobromine, theobromine is a better alternative than fluoride. We believe that theobromine can be used as an ingredient of dentifrices and even if swallowed accidentally, there are no adverse effects.

Introduction

IN MODERN TIMES, people started realizing gradually that what they eat or drink in daily life could be closely related to their health. Well-informed consumers carefully look at the composition of their food and drink before they purchase them. Yet, at the same time, the inclusion of the word, natural, catches consumers' eyes no matter what the product's level of naturalness. Dietary ingredients may play an important role in consumer health. In the laboratory setting, however, determining how these factors might be interrelated is a field still in the developmental stages. Because fetal mass increases hugely during pregnancy and from birth to weaning, the nutritional stress, if any, will be most pronounced during early growth periods. However, because each organ has a different critical period of growth, some organs may be affected, whereas others might not, depending on whether nutritional stress is acting at that particular time of development. However, if affected, even if the diet is corrected later in life, this organ will not recover from the early nutritional stress once the crit- ical growth period has passed.

Caffeine (1,3,7-trimethylxanthine) and fluoride are common ingredients encountered in our daily lives. Caffeine is present in beverages, such as coffee, colas, some teas, and some over-the-counter medications.[1] Fluoride is also contained in most dentifrices and in drinking water in the some parts of the United States.

Although caffeine can be commonly found in most American lifestyles, caffeine's effects on growing offspring during pregnancy and early postnatal period are still debated, even if one considers the amounts of caffeine as not excessive. The half-life of caffeine in neonates is much longer than in adults[2]; therefore, caffeine stays longer in neonate bodies. Fluoride is known to have adverse effects.[3–5]

The neonatal teeth of the suckling pups were affected by protein-energy malnutrition and have shown greater calcium and phosphorus release from the enamel surface compared with the control group when exposed to an acid solution *in vitro*.[6] Originally, this research led us to the study about the possible effects of caffeine-related nutritional stress on the enamel surface of developing teeth.

The presence of caffeine in drinks and medications is surrounding us in our daily life; caffeine's possible

[1]Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, Louisiana.
[2]Maine Mineral and Gem Museum, Bethel, Maine.

effects on developing teeth have not been given adequate attention. Caffeine diffuses in breast milk in humans[7] and rats.[8] The suckling pups took in caffeine through the milk when the maternal diet was supplemented with caffeine. Thus, maternal caffeine supplementation in the diet during the lactation period alters the composition of the developing teeth of the suckling rat pups.[9] The calcium content of the incisor and first molar in the caffeine-supplemented group of the normally nourished group was less than that in the noncaffeine group. We did not know at that time whether this change of calcium content of the whole teeth came from the enamel, dentine, or both. However, we knew that if the enamel surface of the teeth could be impaired by a decrease in calcium resulting from maternal caffeine intake, these animals might develop caries-prone teeth.

How Did the Caffeine Intake by Rat Dams Affect the Developing Teeth of Neonates?

Study of early postnatal period

In a series of experiments, caffeine was added to the maternal diet (2 mg/100 g body weight of dam). The equivalent comparison between caffeine in the rat diet and a human diet is based on metabolic body weight ($kg^{0.75}$) (Metabolic rates are expressed in terms of Kleiber's[10] metabolic body size—i.e., $kg^{0.75}$, the point at which the dependence on different body sizes disappears). The human caffeine intake is comparable with slightly more than two cups of coffee. At the end of weaning postnatal day 22 (birth day counted as day 1), first and second molars were removed. The third molar was in a jelly-like condition (i.e., not fully developed), so we did not study it at that time. The studies were conducted in a similar way as described previously.[6] The calcium, phosphorus, and magnesium amounts released from the enamel surface of the first molars over 80 minutes of *in vitro* study were significantly higher compared with the noncaffeine control group.[11] We did not know why these ions were released from the enamel surface of the first molars.

Because teeth comprise hydroxylapatite (HAP), apparently some physical change might have occurred in the HAP of the enamel surface of the first molars as a result of the exposure to caffeine. A collaboration with Drs. Falster and Simmons at the University of New Orleans, Department of Geology and Geophysics—who were experts on HAP—was begun to determine the cause and effects behind these data.

According to the suggestions, the extracted teeth were pulverized and the sample powder was separated into enamel and dentine.[12] Several pure enamel samples of the untreated control and caffeine groups were run for 4 hours on a 114 mm Gandolfi X-ray powder camera.[13] The data showed that caffeine supplementation in the maternal diet affected the mineralization of enamel and results in smaller crystallites, indicated by peak broadening of the X-ray diffraction peaks compared with the noncaffeine control group.[13] This explains that the smaller crystallites with greater surface area were more easily dissolved when teeth were exposed to acid solutions *in vitro*. Thus, crystallites in acid-susceptible teeth are smaller than those in acid-resistant teeth.[14] Therefore, it would be likely that those teeth exposed to caffeine in the early neonatal period would easily dissolve and result in dental caries-prone teeth in the future.

No such differences are found in the second molars between the caffeine group and the noncaffeine control group within the same experimental conditions.[11] The differing results can be explained by different critical periods of growth of teeth, where the nutritional effects are exerted at a particular time period of growth and development. That is, the first molar was influenced by the caffeine exposure during its critical time period, whereas second molar was not. We did not determine the critical period of second molars.

Study of prenatal period

To study the prenatal effects of caffeine on developing teeth, time-release 100 mg caffeine tablets were subcutaneously implanted in rat dams at day 7 of gestation.[15] The tablet released caffeine into the body at an intake comparable with about one to one and one-half cups of coffee during pregnancy based upon the metabolic body weight ($kg^{0.75}$).[10] At birth, pups were assigned to surrogate dams that were not exposed to caffeine during gestation. Therefore, we determined the caffeine's effects on neonates during only the gestational period. At postnatal day 22, the rat pups were killed and the first and second molars were extracted.

Using the same *in vitro* study method,[11] we measured the release of calcium, phosphorus, and magnesium. Significantly, more calcium and phosphorus were released from the enamel surface of the first molar in the caffeine group compared with the noncaffeine control, but magnesium showed no difference between the groups in the first molar. We found no significant differences in the second molar. Possible explanations for the different data regarding the observed effects of caffeine exposure on developing teeth between the prenatal study[15] and the postnatal study[11] include the slightly smaller caffeine exposure in the prenatal research compared with the postnatal study[11] and differences in the critical developmental period of the first molars. HAP formation between prenatal[15] and postna- tal study[11] might have been slightly different, although both enamel surfaces showed smaller crystallites in the caffeine group compared with the noncaffeine control.[11,15]

Postnatal caffeine exposure and the incidence of dental caries

Subsequently, we hypothesized that caffeine-exposed teeth will develop dental caries more readily than teeth

of noncaffeine controls. To test this hypothesis, we raised rat pups in the same way as described,[11] and then fed them a cariogenic diet from weaning on postnatal day 22 to postnatal day 50.[16] At day 50, the first molars were examined by the established method of caries score.[17] The results clearly demonstrated that caffeine exposure during the neonatal period resulted in signifi- cantly higher caries scores compared with the noncaf- feine control group.[16]

These experiments suggest that even a relatively small amount of caffeine exposure during the early postnatal period and/or pregnancy will influence developing teeth and result in teeth prone to developing dental caries later in life.

How Theobromine's Effect Was Discovered

In vitro *crystal formation study*. Because our series of *in vivo* caffeine experiments took more than several years to complete, we decided to conduct simple *in vitro* experiments to form HAP crystallites in the presence of other xanthine compounds to determine what effects the compounds might have on HAP formation, relative to caffeine. *In vitro* experiments involved growing apatite from dilute solutions of $CaCl_2$ and $Na_3 PO_4$.[18,19] All solutions contained 0.01 M $CaCl_2$ and $Na_3 PO_4$. Several sets of experiments added each of the methylxanthines or uric acid in two concentrations, 50 mg/L and 200 mg/L. The effect of the xanthine compounds was compared with a control solution containing $CaCl_2$ and $Na_3 PO_4$ only.

Solutions were mixed at 25°C and the pH adjusted to 9–9.5 with 0.1 M NaOH and the solutions were left to crystallize for 20 days. The crystalline precipitate was washed five times with distilled water and prepared for X-ray diffraction, which was performed on a SCINTAG XDS 2000™ X-ray diffractometer. The (300) reflection was scanned to investigate crystallinity. The results of this study are given in Table 1.

The experiments that used theobromine or 3-methylxanthine show the most pronounced increases in crystallinity compared with the control group. This result is evident from lower values of the ratios FWHM (full-width–half-maximum peak height) divided by M (maximum peak height) (FWHM/M) compared with the control. Lower ratio values indicate better crystallinity. All of the methylated xanthines, except caffeine, increased the crystallinity of the precipitating apatite. In every case, the crystallinity increased with xanthine concentration. Caffeine and uric acid decreased the crystallinity of the apatite also in a dose-dependent manner.[18,19] We did not expect these results.

Peak broadening of crystallites *in vitro* was measured by X-ray diffraction scans of the (300) reflection of apatite grown *in vitro* without additives (control) and secondary electron images of apatite grown *in vitro* without additives (control) measured to be 0.5 lm.[18,19]

TABLE 1. HYDROXYLAPATITE FORMED *In Vitro* IN THE PRESENCE of VARIOUS XANTHINES

	Amount of additive in mg/L solution	*FWHM/M*
Control	0	0.75
Caffeine	200	1.00
Caffeine	50	0.90
Uric acid	200	0.96
Uric acid	50	0.90
Theobromine	200	0.15
Theobromine	50	0.19
Theophylline	200	0.40
Theophylline	50	0.50
1-Methylxanthine	200	0.60
1-Methylxanthine	50	0.68
3-Methylxanthine	200	0.21
3-Methylxanthine	50	0.39
7-Methylxanthine	200	0.45
7-Methylxanthine	50	0.68

The results in terms of FWHM (full-width–half-maximum peak height) divided by M (maximum peak height) (FWHM/M) and for the (300) reflection are given for the control and the xanthine compounds.

X-ray diffraction scans of the (300) reflection of apatite grown *in vitro* in the presence of 200 mg/L theobromine produced sharper (300) peaks with less peak broadening, and secondary electron images of apatite grown *in vitro* in the presence of 200 mg/L theobromine were measured to be more than 2 lm.[18,19]

Theobromine increased the crystallites approximately four times compared with the control group (without additives) in the *in vitro* study.

These unexpected findings about the increased crystallinity of theobromine were surprising. Why would caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethyxanthine), which are chemically very similar, cause crystallite formation that was entirely opposite: caffeine produced small crystallites, and theobromine produced large crystallites.

Postnatal study of developing teeth by theobromine

Based on the *in vitro* study, we decided to conduct an *in vivo* study with the following hypothesis. If we conducted the same experiment on developing teeth as we had with the caffeine study,[11] but supplemented theobromine in the maternal diet instead of caffeine, we believed that the first molars of the suckling pups whose milk contained theobromine should release fewer minerals from the enamel surface compared with the first molars of the nontheobromine-supplemented group. In the supplemented group, the first molars would be formed with bigger HAP crystallites on the enamel surface. The bigger HAP crystallites were more acid resistant.[14]

The theobromine supplementation of the maternal diet was 1 mg/100 g of the dam's weight. Assuming that the theobromine content of a 1 oz. bar of milk chocolate is

45–105 mg,[20] and that the conversion is based on the metabolic body weight ($kg^{0.75}$),[10] the dosage (1 mg/ 100 g body weight) in rats is equivalent to 129 mg/65 ($kg^{0.75}$). This corresponds approximately to slightly more than one to three bars of 1 oz. milk chocolate for a 65 kg human. During the lactating period, suckling rat pups received theobromine through the maternal milk because theobromine diffuses into milk just as caf- feine does.[2]

On postnatal day 22, first molars from the mandible and maxilla of randomly selected pups were removed. These first molars were mounted with a sticky wax on a small plastic block to study the acid solubility of the enamel surface as was performed previously in the caffeine study.[11] The data showed that significantly less calcium, phosphorus, and magnesium from the enamel surface were released from the theobromine-supplemented group compared with the nonsupplemented group.[18,19] Thus, our hypothesis proved correct as bigger crystalline HAP is more resistant to acid dissolution.[14]

How the crystal structure between control and theobromine differs

It has been reported that the calcium and phosphorus contents of acid-resistant teeth were at least 20% higher than that of the acid-susceptible teeth.[14] Therefore, we further studied how the composition of the increased HAP of the enamel—which was bigger in the theobromine-supplemented group—differs from the nontheobromine control group.

Calcium and phosphorus concentrations were determined in the enamel of first molars extracted from theobromine-exposed rats and control rats by electron microprobe analysis using an ARL-SEMQ electron microprobe. Fluorapatite from Cerro de Mercado (Mexico) was used as a standard. The results obtained are shown in Table 2.

From the data, there is no significant difference in the CaO and P_2O_5 between the two groups. Thus, the previously described results of acid dissolution are related to the crystallite size differences, not to a difference in the chemical composition of the theobromine versus caffeine groups.

TABLE 2. CALCIUM AND PHOSPHORUS CONCENTRATION DETERMINED BY ELECTRON MICROPROBE ANALYSES

Sample No.	*P_2O_5 weight percent*	*CaO weight percent*
Control group		
28	38.11	53.24
30	36.68	53.60
10	36.55	52.20
Average	37.11	53.01
Theobromine group		
29	34.55	52.70
24	37.63	53.17
37	38.53	51.79
Average	36.90	52.55

Does Chocolate Prevent Dental Caries?

First evidence of chocolate and dental caries in humans

Cocoa is a major source of theobromine, and cocoa *per se* has no reported adverse effects that would be injurious to man.[2] Because chocolate contains theobromine, it is interesting to see how the past history reveals the study relationship between chocolate and dental health. In the middle of the 1950s, milk chocolate was provided as part of caries research on patients at the Vipeholm Men- tal Hospital (Sweden).[21] The increase in caries activity during the chocolate-ingestion period was less than expected from the amount of sugar consumed and the sugar clearance time in saliva. These results led to the hypothesis that some kind of caries-inhibiting constituent in chocolate might exist.[21]

Stralfors' study series on cocoa and dental caries

About 50 years ago, using hamsters, Stralfors conducted a series of experiments on the relationship between cocoa powder and the inhibition of dental caries.[22] This research was based upon the study by Gustafson *et al.*,[21] showing that the introduction of milk chocolate led to less caries activity. Whole cocoa powder inhibited caries by 84%, 75%, 60%, and 42%, when the cocoa content of the diet was 20%, 10%, 5%, and 2%, respectively. However, cocoa butter incorporated into a diet in an amount of 15% increased dental caries considerably.[22]

Based upon this initial research,[22] Stralfors concluded that the cariostatic factors are located in the nonfat part of cocoa. There seem to exist at least two cariostatic factors, one insoluble in water at ordinary temperature and another soluble in water.[23] In the following studies, Stralfors speculated that the tannin in cocoa could be a caries-inhibitive constituent and that other caries-inhibitive constituents may be present.[24]

He further studied the purine derivatives, theobromine, caffeine, and xanthine; the phenolic aldehyde vanillin and the tannin-containing material tannic acid—which is a hydrolysable tannin—and mimosa and quebracho extracts.[25] Some of the materials inhibited dental caries, depending upon the concentration added to the diet. When a large amount of the above materials was added, the growth of the animal was inhibited.

Both theobromine and caffeine significantly inhibited dental caries when a higher caffeine concentration was added, but when the concentration of caffeine was less, dental caries was not inhibited.[25] This result is in contrast to our findings in the crystallization study of caffeine.[16]

In a separate study, Stralfors fed milk chocolate to one group and dark chocolate to another group. To his surprise, he found that there was a reduction of caries by 35% for milk chocolate and 73% for dark chocolate.[26]

An earlier article[22] had shown that caries inhibition was caused by fat-free cocoa. Therefore, he explained, the different cocoa content of the two chocolate types (milk chocolate vs. dark chocolate) was likely the main reason for their differing ability to counteract dental caries.

From our studies, the above phenomena can be easily explained.[26] Chocolate liquor is the base substance from which all chocolate products are produced. Cocoa is prepared by pulverizing the material remaining after the fat (cocoa butter) is removed from chocolate liquor.[20] The average percent of theobromine in commercial cocoa is 1.89%, whereas that in commercial milk chocolates is 0.15%.[20] Dark chocolate contains *12 times more theobromine than milk chocolates.

Ooshima *et al.*[27] observed that cacao mass extract possesses some anticariogenic potential, but concluded that its anticaries activity is not strong enough to significantly suppress the cariogenic activity of sucrose. On the other hand, Ito *et al.*[28] reported that the addition of a water-soluble extract of cacao powder significantly reduced caries scores in specific pathogen-free rats infected with *Streptococcus sobrinus* 6715.

Our study finally provided an answer for this old mystery

From the above examples, one can see the speculation that chocolate has some basis to prevent dental caries. However, it was not known until now how and what chemical material(s) might have played the critical role in the prevention of dental caries. Our accidental findings—during the study of caffeine crystallization—that theobromine increases the crystal size of HAP provided the clear answer for the mysterious phenomena of caries reduction related to chocolate consumption.

Theobromine's Effects on the Teeth

Microhardness study by theobromine and fluoride on human teeth

Mineral changes in superficial enamel layers are directly related to the alternation of microhardness. If remineralization occurs, then the increased enamel surface is associated with increased microhardness.[29] The microhardness test using the different concentrations of theobromine on the enamel surface was studied in human teeth as a pilot project. Surface microhardness values showed that 200 mg/L theobromine protected enamel specimens more than 100 mg/L theobromine did. It was concluded that consistent protection of enamel surface was observed in the theobromine group.[30]

We also have conducted detailed microhardness testing using human teeth (Fig. 1).[31] As can be seen, the horizontal line is the logarithm, which indicates that less theobromine was required to produce a much harder enamel surface compared with the amount of fluoride. When the surface is harder, it is resistant to dissolution when exposed to acid solution in *in vitro* studies.[14] Therefore, the teeth will become resistant to dental caries.

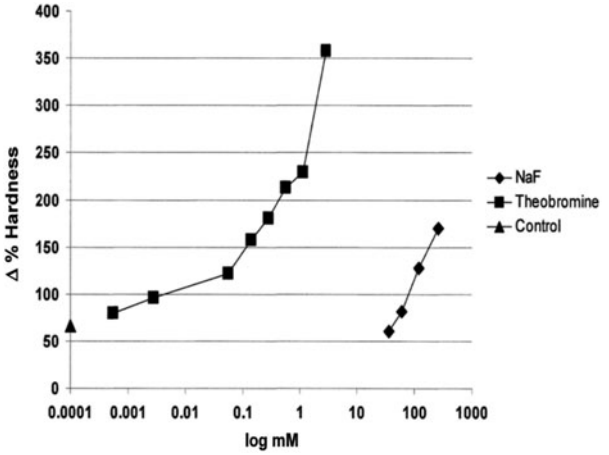

FIG. 1. Changes of hardness of the enamel surface of human teeth by different concentrations of theobromine and fluoride.

In vitro *pH cycling study between theobromine and fluoride*

To prove the points, the following *in vitro* study was conducted. Using human teeth, the study investigated the remineralization potential of theobromine in comparison with a standard NaF dentifrice.[32] Using an established *in vitro* caries pH cycling (demineralization/remineralization) model, it was concluded that theobromine in an apatite-forming medium can enhance the remineralization potential of the medium. Therefore, theobromine could be a viable alternative to fluoride additives in commercial dentifrices.

In this model, theobromine—at a molar level 71 times less than that of fluoride—has a remineralization effect on enamel lesions comparable with that of fluoride.[32] Evidence from the human study,[21] the animal data previously described,[22–26] and a recent *in vitro* study,[32] all point to the possible role of chocolate (via the theo- bromine it contains) in the prevention of dental caries. However, further human clinical studies are needed to exploit the benefit of theobromine on dental caries pre- vention.

Repair of the enamel surface by theobromine

Figure 2A shows the enamel surface, which was scratched using a sharp instrument. Figure 2B shows the result after theobromine solution was applied to the enamel surface. The teeth were bathed in enough solution to cover them for a duration of 30 minutes. The enamel surface repaired smoothly.

The application of theobromine on the enamel sur- face produced a very smooth surface by the process of remineralization.

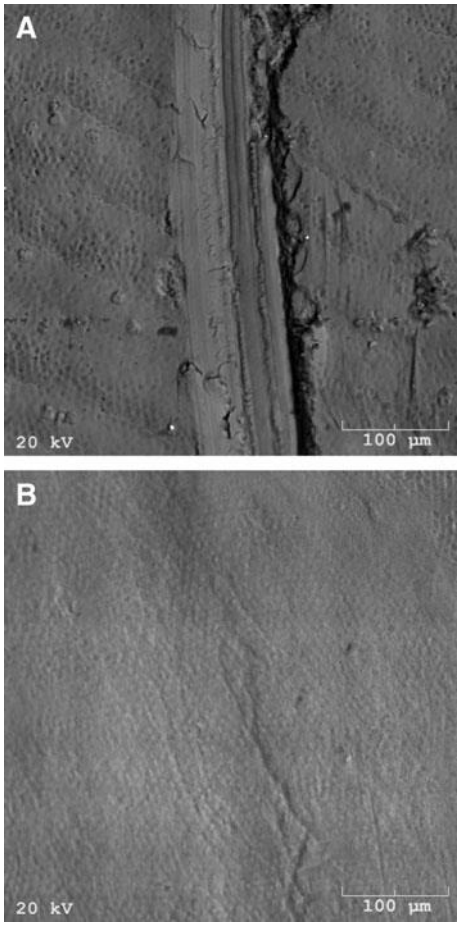

FIG. 2. The scratched enamel surface before (A) and after the theobromine solution was applied (B).

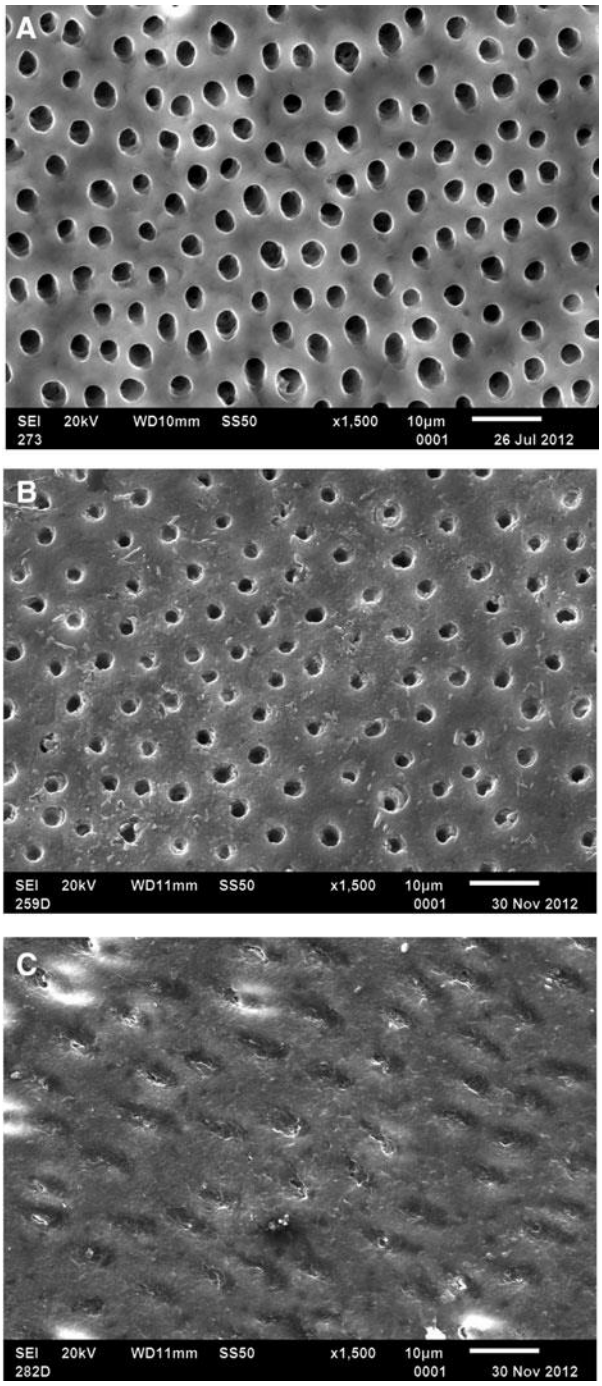

FIG. 3. Tooth surface before brushing (A). Surface after brushing the tooth with fluoride-containing tooth- paste for 1 week (B). Surface after brushing the tooth with theobromine-containing toothpaste for 1 week (C).

Hypersensitivity study of enamel surface of human teeth

Erosion of tooth surfaces can result from consumption of many kinds of soft drinks, fruit juices, and wine. Hypersensitivity from this source is one of the problems often encountered by practicing dentists. It has been estimated that 15–57% of adults suffer from hypersensitivity,[33] and the incidence appears to be increasing.[34]

An 80-person clinical study was conducted recently to determine whether theobromine can alter the hypersensitivity of teeth.[35] The secondary electron microscope images show the results (Fig. 3).

Figure 3A shows an eroded tooth surface before brushing. Note the small dentinal tube openings exposed in the mouth. The more the open tubes are exposed into the oral cavity, the more one will feel pain with cold or hot drinks. One of the treatments for sensitivity is to occlude (close) these tubes.[35]

Results after brushing for 1 week with a regular, commercially available fluoride-containing toothpaste (twice a day, morning and evening) are shown in the middle of Figure 3B. Very little occlusion of the tubes is seen, and most remain open, indicating that the toothpaste is not effective in reducing sensitivity.

Figure 3C shows the results after using toothpaste containing theobromine. Here, all tubes are fully occluded. The detailed study is presented in the original article.[35]

Dentifrices Containing Fluoride Are Associated with Some Reported Problems

Fluoride-based toothpastes have been the standard for many years. In the presence of fluoride, fluoro-HAP crystals are formed. Partially fluoridated crystallites have lower solubility in the acid produced by mouth bacteria more than nonfluoridated HAP and thus protect against tooth decay. Another role of fluoride is to stimulate remineralization of teeth at the early stages of decay.[36] The first fluoridated toothpastes were introduced in 1955.[3]

Note warning with each toothpaste

Although fluoride has been considered the gold standard in oral care, each toothpaste containing fluoride bears the warning ''Keep out of reach of children under 6 years of age. If more than used for brushing is accidentally swallowed, get medical help or contact a Poison Control Center right away.'' In 1994, the American Association of Poison Control Centers recorded 3095 calls about suspected overingestion of fluoridated toothpaste.[37] A report from Poison Control Centers showed 21,513 calls in 2011 concerning fluoridated toothpaste ingestion.[38]

In view of this warning, one wonders what adverse accumulative effects there may be on the general health of children in their later lives, particularly if they are overexposed to fluoride during an early critical growing period. In addition, what effects might we see in adult and elderly populations who may be exposed daily to, or periodically swallow, small amounts of fluoride throughout their lives?

Possible adverse effects of fluoride

Ingestion of excess fluoride is known to be associated with an increased risk of permanent discoloration in developing teeth. More than 90% of toothpaste in the United States is fluoridated, and many children are exposed to fluoride through incidental ingestion of toothpaste.[39] Toothpastes specifically flavored for children have been linked to the use of larger quantities of toothpaste than suggested, increasing the importance of the pathway of excessive fluoride intake.[40]

An increased risk of skeletal fluorosis due to excessive fluoride is reported.[41] Fluoride is also a risk factor for osteosarcoma among boys.[42] However, Douglass and Joshipura[43] warned about the incidence of osteosarcoma, which may require a different interpretation of their finding, because unpublished data contradict the risk of osteosarcoma.[3]

Dentists in the United States are seeing young children with as many as 10 cavities. The American Dental Association recommends using only a pea-sized amount of fluoride toothpaste for brushing, beginning at 2 years of age.[44] Unfortunately, children between the ages of 1 and 3 years ingest 30–75% of the toothpaste on their brushes.[45] It is difficult to train a 2 year old to spit out toothpaste, particularly if it tastes great.[40]

A recent report from China demonstrated an association between fluoride intake and significantly lower IQ scores for children.[4] On the other hand, a more recent report disputes the finding of a relationship between fluoride exposure and IQ.[46] A review by Grandjean and Landrigan[47] suggested that further in-depth studies examine this aspect of fluoride.

It appears that fluoride readily accumulates in the human pineal gland, and a positive correlation between fluoride and calcium content in this gland has been shown.[38] The pineal gland produces melatonin, a hormone related to setting the rhythms and duration of sleep. The degree of calcification has been associated with a decreased secretion of melatonin.[48] Thus, exces- sive fluoride use could result in the disturbance of circa- dian rhythms and sleep patterns.[49]

A possible relationship between fluoride intake and thyroid gland disease has been reported.[50] There are many pro and con arguments as to fluoride's cavity-fighting benefits. In light of the evidence presented above concerning possible adverse effects, it is under- standable that some opposition has developed against daily fluoride use in dentifrices.

Where Are We Going from Here

Since Colorado dentist Dr. Mckay's findings in the early 20th century led to the discovery of fluoride's effect on teeth,[3] fluoride became the most common ingredient in dentifrice and remains so at the present time. Nevertheless, some adverse effects of fluoride have been reported in the present and past.

In a Mayan skull—reported to be *1100 years old—three round jade inlays are clearly embedded in the front teeth.[51] In the ancient time in the Mayan culture, cocoa was used only among the wealthy. What is surprising about the skull is that to embed a jade inlay into each tooth, they had to drill the enamel surface of the tooth. However, drilling the precise hole to embed the jade would have been difficult if not impossible. It seems that somehow after placing the jade into each tooth, they must have had the knowledge to fix the jade within the hole. We speculate that cocoa extract—with the theobromine discussed in this article—extracted from cocoa powder was applied to fill the marginal space around the jade and initiate mineralization. Thus, the jade could be fixed into the enamel surface. This speculation stems from our current study, which is shown in Figure 2, where the impaired tooth surface was filled by HAP with the help of theobromine. Somehow these Mayan elites knew the role of cocoa extract in dental applications more than 1100 years ago.

As Mayan culture already, 1100 years ago, knew cocoa's unique role, it is interesting to imagine that

Natives living in the deep Amazon, for example, may have unique remedies learned from their ancestors. These remedies may be more effective than that created by modern science.

Although dental caries is prevalent within our society, the role of theobromine to prevent the dental caries in the clinical study has yet to be investigated and definitely proven. However, we have every reason to believe that theobromine is 21st century's most important ingredient in future dentifrices in our society. Cocoa has been used for centuries without any ill effects. Our data have convinced us that if fluoride is 20th century's discovery to prevent dental caries, theobromine will play a similar role in the 21st century. Theobromine is superior to and a safer material than fluoride.

Acknowledgments

Just 10 years ago, August, 2005, Hurricane Katrina devastated New Orleans. Many people left New Orleans, including the mentor of graduate student, Arman Sadeghpour, who was planning to study PhD dissertation, although I, Tetsuo Nakamoto, knew him as a high school student. As a result of Hurricane Katrina, I became his mentor for his dissertation. He is a meticulous researcher. Once the comparative study between theobromine and fluoride on hardness using human teeth on his dissertation was done, it became very clear that theobromine is superb in every parameter he studied. Around that time, the authors met Mr. Joseph Fuselier who was organizing a biotechnology group interested in the New Orleans area after the devastation by Hurricane Katrina to revitalize the city. He has a great deal of experience in the industrial aspects of biotechnology. Not long after, Mr. R. Jantzen Hubbard joined the group and became a critical part of the operation the authors started. The authors would like to acknowledge each of these individuals for their selfless participation in this venture and dream. Finally, the authors appreciate Ms. Julie Schiavo, librarian at LSU Health Sciences Center, for her help on various references for the review.

Author Disclosure Statement

No competing financial interests exist.

References

1. Graham DM. Caffeine-its identity, dietary sources, intake and biological effects. Nutr Rev. 1978;36:97–102.
2. Tarka SM Jr. The toxicology of cocoa and methylxanthines: a review of the literature. CRC Crit Rev Toxicol. 1982;9:275–312.
3. Fagin D. Second thoughts about fluoride. Sci Am. 2008;298:74–81.
4. Xiang Q, Liang Y, Chen L, *et al.* Effect of fluoride in drinking water on children's intelligence. Fluoride. 2003;36:84–94.
5. Luke J. Fluoride deposition in the aged human pineal gland. Caries Res. 2001;35:125–128.
6. Aponte-Merced L, Navia JM. Pre-eruptive protein-energy malnutrition and acid solubility of rat molar enamel surfaces. Arch Oral Biol. 1980;25:701–705.
7. Aldridge A, Aranda JV, Neims AM. Caffeine metabolism in the newborn. Clin Pharmacol Ther. 1975;25: 977–981.
8. Aeschbacher HU, Milton H, Pott A, Wurzner HP. Effect of caffeine on rat offspring from treated dams. Toxicol Lett. 1980;7:71–77.
9. Nakamoto T, Shaye R, Mallek HM. Effects of maternal caffeine intake on the growth of tooth germs in protein-energy malnourished neonates. Arch Oral Biol. 1985; 30:105–109.
10. Kleiber M. Body size and metabolic rate. In: *The Fire of Life, an Introduction to Animal Energetics*. New York: Wiley; 1961: pp. 177–216.
11. Hashimoto K, Joseph F Jr, Falster AU, Simmons WB, Nakamoto T. Effects of maternal caffeine intake during lactational period on molar enamel surfaces in newborn rats. Arch Oral Biol. 1992;37:105–109.
12. Manly RS, Hodge HC. Density and refractive index studies of dental hard tissue—I. Method for separation and determination of purity. J Dent Res. 1939;18: 133–141.
13. Falster AU, Hashimoto K, Nakamoto T, Simmons WB. Physical examination of caffeine's effects on the enamel surface of first molars in newborn rats. Arch Oral Biol. 1992;37:111–118.
14. Besic FC, Bayard M, Weimann MR Jr, Burrell KH. Comparison and structure of dental enamel: elemental composition and crystalline structure of dental enamel as they relate to its solubility. J Am Dent Assoc. 1975;91:594–601.
15. Falster AU, Yoshino S, Hashimoto K, Joseph F Jr, Simmons WB, Nakamoto T. The effect of prenatal caffeine exposure on the enamel surface of the first molars of newborn rats. Arch Oral Biol. 1993;38:441–447.
16. Nakamoto T, Cheuk SL, Yoshino S, Falster AU, Simmons WB. Cariogenic effect of caffeine intake during lactation on first molars of newborn rats. Arch Oral Biol. 1993;38:919–922.
17. Keyes PH. Dental caries in the molar teeth of rats. II. A method for diagnosis and scoring several types of lesions simultaneously. J Dent Res. 1958;37:1088–1099.
18. Nakamoto T, Simmons WB Jr, Falster AU. Products of apatite-forming-systems. Patent No. 5, 919, 426; July 6, 1999.
19. Nakamoto T, Simmons WB Jr, Falster AU. Apatite-forming-systems: methods and products. Patent No. US 6,183,711B1; February 6, 2001.
20. Zoumas BL, Kreiser WR, Martin RA. Theobromine and caffeine content of chocolate products. J Food Sci. 1980;45:314–316.
21. Gustafson BE, Quensel CE, Swenander-Lank I, *et al.* The effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years. The Vipeholm dental caries study. Acta Odontol Scand. 1954;11:232–273.
22. Stralfors A. Inhibition of hamster caries by cocoa. The effect of whole and defatted cocoa, and the absence of activity in cocoa fat. Arch Oral Biol. 1966;11:149–161.

23. Stralfors A. Inhibition of hamster caries by cocoa. Caries inhibition of water and alcohol extracts of cocoa. Arch Oral Biol. 1966;11:323–328.
24. Stralfors A. Effect on hamster caries by dialyzed, detanned or carbon-treated water-extract of cocoa. Arch Oral Biol. 1966;11:609–615.
25. Stralfors A. Effect on hamster caries by purine derivatives, vanillin and some tannin-containing materials. Arch Oral Biol. 1967;12:321–332.
26. Stralfors A. Inhibition of hamster caries by substances in chocolate. Arch Oral Biol. 1967;12:959–962.
27. Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumoto M. Cariostatic activity of cacao mass extract. Arch Oral Biol. 2000;45:805–808.
28. Ito K, Nakamura Y, Tokunaga T, Iijima D, Fukushima K. Anti-cariogenic properties of a water-soluble extract from cacao. Biosci Biotechnol Biochem. 2003;67: 2567–2573.
29. ten Cade JM, Arends J. Remineralization of artificial enamel lesions in vitro. II. Determination of activation energy and reaction order. Caries Res. 1978;12:213–222.
30. Kargul B, Ozcan M, Peker S, Nakamoto T, Simmons WB, Falster AU. Evaluation of human enamel surfaces treated with theobromine: a pilot study. Oral Health Prev Dent. 2012;10:275–282.
31. Sadeghpour A, Nakamoto T. Methods and compositions to improve mechanical resistance of teeth. International Patent application No. PCT/US2011/024734; 2011. Available at http://patentscope.wipo.int/search/en/WO2011100671
32. Amaechi BT, Porteous N, Ramalingam K, *et al.* Remineralization of artificial enamel lesions by theobromine. Caries Res. 2013;47:399–405.
33. Cummins D. Dentin hypersensitivity: from diagnosis to a breakthrough therapy for everyday sensitivity relief. J Clin Dent. 2009;20:1–9.
34. Pray WS, Pray GE. Dentinal hypersensitivity. US Pharm. 2011;36:12–15.
35. Amaechi BT, Mathews SM, Mensinkai PK. Effect of theobromine-containing toothpaste on dentin tubule occlusion in situ. Clin Oral Investig. 2015;19:109–116.
36. ten Cate JM. Contemporary perspective on the use of fluoride products in caries prevention. Br Dent J. 2013; 214:161–167.
37. Schulman JD, Wells LM. Acute fluoride toxicity from ingesting home-use dental products in children, birth to 6 years of age. J Public Health Dent. 1997;57:150– 158.
38. Bronstein AC, Spyker DA, Cantilena LR, Rumack B, Dart RC. 2011 Annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 29th annual report. Clin Toxicol. 2012; 50:911–1164.
39. Erdal S, Buchanan S. A quantitative look at fluorosis, fluoride exposure, and intake in children using a health risk assessment approach. Environ Health Perspect. 2005;113:111–117.
40. Levy SM. Review of fluoride exposures and ingestion. Community Dent Oral Epidemiol. 1994;22:173–180.
41. Heifetz S, Horowitz HS. Amounts of fluoride in self-administered dental products: safety considerations for children. Pediatrics. 1986;7:876–882.
42. Bassin EB, Wypij D, Davis RB, Mittleman MA. Age-specific fluoride exposure in drinking water and osteosarcoma. Cancer Causes Control. 2006;17:421–428.
43. Douglass CW, Joshipura K. Caution needed in fluoride and osteosarcoma study. Cancer Causes Control. 2006;17:481–482.
44. American Dental Association Council on Scientific Affairs. Fluoride toothpastes use for young children. J Am Dent Assoc. 2014;145:190–191.
45. Zero DT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. BMC Oral Health. 2006; 6(Suppl 1):S9.
46. Broadbent JM, Thomson WM, Ramrakha S, *et al.* Community water fluoridation and intelligence: prospective study in New Zealand. Am J Public Health. 2015;105: 72–76.
47. Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006;368:2167–2178.
48. Kunz D, Schmitz S, Mahlberg R, *et al.* Anew concept for melatonin deficit: on pineal calcification and melatonin excretion. Neuropsychopharmacology. 1999;21:765–772.
49. Kunz D, Bes F, Schlattmann P, Herrmann WM. On pineal calcification and its relation to subjective sleep perception: a hypothesis-driven pilot study. Psychiatry Res. 1998;82:187–191.
50. Susheela AK, Bhatnagar M, Vig K, Mondald NK. Excess fluoride ingestion and thyroid hormone derangements in children living in DeIhi, India. Fluoride. 2005;38:98–108.
51. Sadeghpour A. Chocolate and dental health. In: *Chocolate and Health: Chemistry, Nutrition and Therapy.* P.K. Wilson and W.J. Hurst (Eds). London: Royal Society of Chemistry; 2015: pp. 196–210.

Address correspondence to:
Tetsuo Nakamoto, DDS, PhD
1901 Perdido Street
Department of Physiology
Louisiana State University Health Sciences Center
New Orleans, LA 70112

E-mail: tnakam@lsuhsc.edu