

# **ORIGINAL ARTICLE**

# Milk Reverts the Effects of an Enamel Erosive but Healthy Diet

Alexandre Rezende Vieira<sup>1</sup>, Christine Chung<sup>1</sup>, Sarah K. Raffensperger<sup>1</sup>, Pallavi Muluk<sup>1</sup>

<sup>1</sup>Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, United States.

Author to whom correspondence should be addressed: Alexandre R. Vieira, Dept. Oral Biology, 412 Salk Pavilion, University of Pittsburgh School of Dental Medicine, Pittsburgh, PA, USA 15261. Email: arv11@pitt.edu.

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# Abstract

**Objective:** To determine the erosive effects of popular fruits and vegetables on enamel, and the potential counter effects of milks. Material and Methods: Ten popular fruits and vegetables were individually blended in a juicer to form a fruit or vegetable juice. The labial surfaces (three for each treatment group) were then immersed for thirty minutes in a fresh juice of one of ten products: strawberry, spinach, pineapple, grapefruit, green pepper, pickle, orange, apple, tomato, and carrot. In a second experiment, human enamel was treated with Coca-Cola for 30 minutes, followed by a variety of milks: Skim, 2%, Whole, Chocolate, Lactaid, Almond, and Silk Soy. Three teeth were used for each treatments and enamel microhardness of each sample was measured at baseline, after erosive exposure, and after the 30-minute variable treatment. Results: All fruits and vegetables showed differences between the pre- and posttreatment microhardness values. For the second experiment, tested milks result in significant increases (p<0.05) in enamel microhardness. Paired-t test was used for all comparisons. A thirty-minute exposure to the juices of all fruits and vegetables examined in the study significantly changed the microhardness of enamel, with grapefruit and pickle having potentially the most erosive effect on enamel. Diets that are high in fruits and vegetables can predispose to enamel erosion. For the second experiment, filtered water, ACT fluoride rinse, and varying milks all result in remineralization of enamel. Conclusion: Eroded enamel exposed to milk demonstrated a significant greater gain of enamel microhardness as compared to filtered water.

Keywords: Hardness Tests; Dental Enamel; Fruit and Vegetable Juices.



#### Introduction

The availability of high energetic foods has led to an increase in weight gain of certain populations, particularly in North America. As a reaction to these trends of increased prevalence of obesity, a push for eating healthier has been proposed as a sensible public health approach. Fruits and vegetables have been suggested as the ideal substitutes for foods rich in carbohydrates.

The growing market of healthier foods has coincided with the perception that the prevalence of dental enamel erosive lesions is increasing [1]. Dental erosion is a progressive loss of dental hard tissue that is not mediated by bacteria but chemically etched away from the tooth surface by acid and/or chelation [2]. The acids responsible for erosion are originated from the diet or stomach contents and are not products of the intraoral flora. Reports from around the world show a prevalence of dental erosion varying between 6% and 52% in children 2 to 5 years of age (primary dentition) [3,4], and between 3% and 95% in permanent teeth of adolescents [5].

This perceived increase in prevalence of dental erosion and a healthier diet that can be more acidic has come to light more recently and investigators have suggested utilizing fluoride to counteract the effects of acids from the diet on the dental enamel [6]. A diet rich in fruits and vegetables will impact risks of cardiovascular diseases, certain types of cancer, diabetes, gastrointestinal system issues, and aging-related eye diseases [7-26]. Hence, it is important to generate evidence that can support preconizing a diet that is rich and fruits and vegetables and at the same time has less emphasis on carbohydrates and sugars and minimizes the potential for erosive wear of the dentition.

Human studies and clinical trials are not only very expensive, but also will unlikely provide the insight we need because the outcomes for both the dentition and the overall health are difficult to measure, require long term follow ups, and are confounded by a number of demographic, social, environmental, and biological factors. To measure the impact of a diet rich in fruits and vegetables on the enamel surface we designed a series of ex vivo experiments, in which we used human dental enamel samples in the laboratory under conditions that would mimic the oral cavity. We tested the hypothesis that fruits and vegetables have distinct erosive effects on enamel. We also tested the hypothesis that using a fluoridated toothpaste, xylitol solution, or drinking milk or green tea can minimize the effects of a diet that can potentially cause dental erosion.

## **Material and Methods**

Labial surfaces of 75 anterior human teeth were sanded and polished prior to exposure of treatment agents. The treatment agents studied included juices from fruits and vegetables, milk, tea, carbonized soda, fluoridated toothpaste solution, and green tea.

Enamel samples were obtained from 75 human incisors selected. Roots of the teeth were sawed off using the Isomet<sup>®</sup> Low Speed Saw (Buehler, Lake Bluff, IL, USA) followed by the mounting of each tooth on its cross-sectioned surface with double sided tape and wax. The facial, lingual, and incisal edges were parallel to the short end of the microscope slide. Each tooth was then cut in half in the mesial-distal direction, discarding the lingual half. The facial half was then remounted on the slide in a similar manner, with the facial surface facing upwards. The facial surface was then cut in half in the incisal-gingival direction. Each enamel sample was mounted on a teflon disc with the facial surface facing down. Once again, double sided tape and wax was used for the mounting. The teflon disc was then attached to the MiniMet® 1000 Grinder Polisher (Buehler, Lake Bluff, IL, USA) and deionized water was added to completely submerge the tooth specimen. Tooth was subsequently sanded down using a 320 grit abrasive paper for 2 minutes (speed 20 meters/second, force 1 Newton). Following completion, each tooth was remounted with the newly sanded, flat surface face down on the teflon disc. Deionized water was added to completely submerge the tooth specimen, and each tooth was sanded down using a 400 grit abrasive paper for 2 minutes (speed 20 meters/second, force 1 Newton), followed by a 600 grit abrasive paper for 2 minutes (speed 20 meters/second, force 1 Newton). The procedure was completed for each of the 12 specimens in this fashion. The 320 grit abrasive paper was not used in this step so as to retain the enamel.

Upon conclusion of the sanding, the teeth were then polished using the MiniMet<sup>®</sup> 1000 Grinder Polisher. Polishing cloth was first wet with a small amount of distilled water, and 5 squirts of polycrystalline diamond suspension of 6µm were added. Specimen was polished for 8 minutes (speed 25 meters/second, force 6 Newtons gradually increasing from 1 Newton). Procedure was then repeated with polycrystalline diamond suspension of 1µm using the same settings. Finally, specimen was polished using the polycrystalline diamond suspension of 0.25µm for 5 minutes (speed 20 meters/second, force 3 Newtons). This procedure was repeated for each of the 75 specimens. The samples were then sonicated for 3 minutes and examined under the microscope, confirming there were no visible scratches or particles on the sample. If scratches were present on a given sample, the sample was then polished again using the Polycrystalline Diamond Suspension of 0.25µm for 5 minutes at (speed 20 meters/second, force 3 Newtons).

Using a Buehler Microhardness Instrument (Buehler, Lake Bluff, IL, USA), each of the 75 samples was submitted for baseline measurements, which were done in triplicate. Samples were marked and recorded so each subsequent measurement would be taken in the same vicinity on the tooth.

After preparation of teeth samples were completed, each was treated to its own treatment agent, in a series of independent experiments. First we developed a dental erosive model by exposing the enamel samples to a Coca-Cola treatment for 30 minutes. We and others have shown that carbonated soft drinks have the potential to precipitate dental erosion [27,28]. In the first experiment, ten popular fruits and vegetables (strawberry, spinach, pineapple, grapefruit, green pepper, pickle, orange, apple, tomato, and carrot; Table 1) were individually blended in a juicer to form a fruit or vegetable juice. Biotene saliva substitute was used to aid the juicing process of spinach, which did not have enough liquid to create a solution. The labial surfaces (three for each treatment group) were then immersed for 30 minutes in a fresh juice of one of ten products. We used artificial saliva as a negative control (we did not expect to see erosive effects) and Coca-Cola as

positive control (we expected to see marked erosive effects) to aid in the interpretation of the results. Microhardness assessments of the enamel were taken pre-treatment and post-treatment.

For the second experiment, we tested the "reminerilizing" effects of filtered drinking water, artificial saliva, two fluoridated solutions (fluoridated solution and fluoridated toothpaste), seven types of milk, xylitol (Spry Rain Oral Mist), and green tea. Three teeth samples were used for each treatment (Table 2). First, all samples were treated for 30 minutes with Coca-Cola to cause erosion. Then, samples were immersed for 30 minutes with one of the agents described above. Samples were placed in a 37oC incubator with Spry Rain® Oral Mist (Xlear, American Fork, UT, USA) to simulate the human oral cavity between treatments.

Enamel microhardness of each sample was measured at baseline pre-treatments and after treatments. For each sample each time, measurements were taken at the incisal, middle, and gingival thirds, providing nine measurements total because three samples were subject to each treatment. Student's t test was used to compare baseline values with post-treatment assessments with alpha of 0.05.

#### Results

All fruits and vegetables showed differences between the pre- and post-treatment enamel microhardness values: strawberry (p=0.02), pineapple (p=0.01), grapefruit (p=0.00001), green pepper (p=0.03), pickle (p=0.00001), orange (p=0.04), apple (p=0.04), tomato (p=0.03), spinach (p=0.05), and carrot (p=0.05). We calculated the percentage of increased enamel softness as measured by enamel microhardness (Table 1).

microhardness) after 30-minute exposure to fruits and vegetables juices.		
Solution	Approximate Percentage of Increased Enamel	
	Microhardness Softness after Treatment	
Artificial Saliva (negative control)	2%	
Coca-Cola (positive control)	52%	
Strawberry	10.7%	
Pineapple	40.8%	
Grapefruit	61.3%	
Green Pepper	2.5%	
Pickle	27%	
Orange	32.5%	
Apple	29.3%	
Tomato	35.1%	
Spinach	30.4%	
Carrot	21%	

 Table 1. Percentage of increased enamel softness (assessed through knoop enamel microhardness) after 30-minute exposure to fruits and vegetables juices.

When we tested the ability of certain solutions to reestablish the dental enamel after it has been exposed to an erosive attack, whole milk and soy milk had the most robust effects, bringing enamel microhardness levels close to baseline (Table 2). Conversely, we can see the negative effects on enamel microhardness of green tea and Spry Rain Mist. A solution of fluoridated toothpaste also had little impact in imporivng enamel microhardness after acidic exposure.

		Densentana of Francish Mismahanduran	
and subsequent treatments with water, fluorides, milk, xylitol, and green tea.			
	dental enamel softness of 50% softness (assessed through knoop enamel microhardness)		
	Table 2. Percentage of enamel microhardness	gain after erosive attack and increased	

Solution	Percentage of Enamel Microhardness
	Gain after Erosive Attack
Filtered Water	8%
Fluoride Rinse	72%
Fluoridated Toothpaste	12%
Xylitol	-46%
Green Tea	-55%
Skim Milk (30% Calcium, 25% vitamin D)	50%
2% Milk (30% Calcium, 25% vitamin D)	78%
Whole Milk (30% Calcium, 25% vitamin D)	94%
Chocolate Milk (30% Calcium, 25% vitamin D)	42%
Lactose-free Milk (50% Calcium, 25% vitamin D)	62%
Almond Milk (45% Calcium, 25% vitamin D)	40%
Soy Milk (45% Calcium, 30% vitamin D)	90%

## Discussion

Our data suggest that a healthy diet rich in fruits and vegetables may predispose some individuals to dental erosive wear. To counteract these effects, it appears that subsequently drinking milk dramatically reduces the potential erosive effects on the teeth of any diet.

Dental erosion has been reported in high frequencies in the Americas, Africa, Asia, Arabic countries, and in Europe [3-5]. Acidic diet, low socioeconomic status, and poor oral hygiene are reported to be risk factors for dental erosion [5]. An acidic diet can be quite healthy, rich in fruits and vegetables. Previous experiences such as fluoridation of drinking water and the prevalence of fluorosis show that despite the benefits, public health interventions or guidelines may suffer from criticism and strong lobbying against. No one wants to see practices that help lower risks for cardiovascular diseases, certain types of cancer, diabetes, gastrointestinal system issues, and aging-related eye diseases [7-26] be demonized due to potential risks for the dentition. Recent reports are already suggesting that the overall increase in the prevalence of dental erosion will require large-scale dental treatments for many young adults seeking to improve their appearances [29]. In order to limit the amount of treatment needed for patients in the future due to a healthy diet, it is imperative to stress and educate patients on how to minimize these risks.

Designing clinical trials to demonstrate the effects of milk in the consumption of acidic diets would be likely impossible due to costs and the amount of time required to measure outcomes. Our ex vivo study is likely the best possible surrogate to provide the necessary insight needed to implement a preventive concept that includes lowering the risks for cardiovascular diseases, certain types of cancer, diabetes, gastrointestinal system issues, and aging-related eye diseases at the same time that protects vulnerable individual dentitions to erosive wear. We suggest that milk should be consumed after every meal that includes acidic drinks, fruits, and vegetables. Soy milk and whole milk provided the strongest protective effects to the dental enamel after being exposed to an acidic beverage, although all milks had some protective effect. As seen from the results simulating a person's oral cavity, saliva alone is not enough to remineralize the tooth enamel. Green tea, a product originating in China and widespread to many countries, also had further erosive effects on the dental enamel. It is common now for people to sip on tea throughout the day, which is further eroding away the tooth structure. The use of fluoridated toothpaste also appears to have little effect in reestablishing the loss of enamel minerals after the consumption of an acidic beverage. It is important to make note that despite fluoride itself helping remineralize enamel, the act of brushing the toothpaste on when enamel is already compromised from an acidic meal is counteracting the effects of the fluoride.

Similar to other public health initiatives such as the fortification of foods with folic acid to impact risks of having children with neural tube defects, it is not possible to know who the individuals are that will benefit the most (or the least) to a diet reach in acidic fruits and vegetables. At the same time, we have evidence that individual susceptibility to dental enamel erosion differs among individuals, even if they are at very high risk such as wine tasters [30]. However, the same rationale that led to the decision of suggesting food fortification with folic acid can be applied here and the concept of drinking milk after meals will provide additional protection to the dentition of individuals at higher risk of dental enamel erosive wear.

## Conclusion

Consistently drinking milk along with a diet rich in fruits and vegetables will likely minimize any erosive effects.

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#### References

1. Mulic A, Vidnes-Kopperdu S, Skaare AB, Tveit AB, Young A. Opinions on dental erosive lesions, knowledge of diagnosis and treatment strategies among Norwegian dentists – A questionnaire survey. Int J Dent 2012; 2012:716396. doi: 10.1155/2012/716396.

2. ten Cate JM, Imfeld T. Dental erosion. Summary. Eur J Oral Sci 1996; 104:241-4.

3. Kreulen CM, va't Spijker A, Rodriguez JM, Bronkhorst EM, Creugers NH, Bartlett DW. Systematic review of the prevalence of tooth wear in children and adolescents. Caries Res 2010; 44(2):151-9. doi: 10.1159/000308567.

4. Corica A, Caprioglio A. Meta-analysis of the prevalence of tooth wear in primary dentition. Eur J Paediatr Dent 2014; 15(4):385-8.

5. Salas MMS, Nascimento GG, Huysmans MC, Demarco FF. Estimated prevalence of erosive tooth wear in permanent teeth of children and adolescents: An epidemiological systematic review and meta-regression analysis. J Dent 2015; 43(1):42-50. doi: 10.1016/j.jdent.2014.10.012.

6. Hove LH, Stenhagen KR, Mulic A, Holme B, Tveit AB. May caries-preventive fluoride regimes have an effect on dental erosive wear? An in situ study. Acta Odontol Scand 2015; 73(2):114-20. doi: 10.3109/00016357.2014.

7. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. New Engl J Med 1997; 336(16):1117-24. doi: 10.1056/NEJM199704173361601.

8. Aldoori WH, Giovannucci EL, Rockett HR, Sampson L, Rimm EB, Willett WC. A prospective study of dietary fiber types and asymptomatic diverticular disease in men. J Nutr 1998; 128(4):714-19.

9. Brown L, Rimm EB, Seddon JM, Giovannucci EL, Chasan-Taber L, Spiegelman D, Willett WC, Hankinson SE. A prospective study of carotenoid intake and risk of cataract extraction in US men. Am J Clin Nutrition 1999; 70:517-524.

10. Lembo A, Camilleri M. Chronic constipation. New Engl J Med 2003; 349(14):1360-68. doi: 10.1056/NEJMra020995.

11. Cho E, Seddon JM, Rosner B, Willett WC, Hankinson SE. Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. Arch Ophthalmol 2004; 122(6):883-92. doi: 10.1001/archopht.122.6.883.

12. Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, Colditz GA, Rosner B, Spiegelman D, Willett WC. Fruit and vegetable intake and risk of major chronic diseases. J Nat Cancer Inst 2004; 96(21):1577-84. doi: 10.1093/jnci/djh296.

13. Moeller SM, Taylor A, Tucker KL, McCullough ML, Chylack LT Jr, Hankinson SE, Willett WC, Jacques PF. Overall adherence to the dietary guidelines for Americans is associated with reduced prevalence of early age-related nuclear lens opacities in women. J Nutr 2004; 134(7):1812-19.

14. Appel LJ, Sacks FM, Carey VJ, Obarzanek E, Swain JF, Miller ER 3rd, Conlin PR, Erlinger TP, Rosner BA, Laranjo NM, Charleston J, McCarron P, Bishop LM; OmniHeart Collaborative Research Group. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. J Am Med Assoc 2005; 294(19): 2455-64. doi: 10.1001/jama.294.19.2455.

15. Christen WG, Liu S, Schaumberg DA, Buring JE. Fruit and vegetable intake and the risk of cataract in women. Am J Clin Nutr 2005; 81(6):1417-22.

16. He FJ, Nowson CA, MacGregor CA. Fruit and vegetable consumption and stroke: Meta-analysis of cohort studies. Lancet 2006; 367(9507):320-6. doi: 10.1016/S0140-6736(06)68069-0.

17. Giovannucci E, Liu Y, Platz EA, Stampfer MJ, Willett WC. Risk factors for prostate cancer incidence and progression in the health professionals follow-up study. Int J Cancer 2007; 121(7):1571-78. doi: 10.1002/ijc.22788.

18. He FJ, Nowson CA, Lucas M, MacGregor GA. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Hum Hypertension 2007; 21(9):717-28. doi: 10.1038/sj.jhh.1002212.

19. Kavanaugh CJ, Trumbo PR, Ellwood KC. The U.S. Food and Drug Administration's evidence-based review for qualified health claims: tomatoes, lycopene, and cancer. J Nat Cancer Inst 2007; 99(14):1074-85. doi: 10.1093/jnci/djm037.

20. Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. Diabetes Care 2008; 31(7):1311-7. doi: 10.2337/dc08-0080.

Christen WG, Liu S, Glynn RJ, Gaziano JM, Buring JE. Dietary carotenoids, vitamins C and E, and risk of cataract in women: a prospective study. Arch Ophthalmol 2008; 126(1):102-9. doi: 10.1001/archopht.126.1.102.
 Wiserman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: A global perspective. Proc Nutrition Soc 2008; 67(3):253-6. doi: 10.1017/S002966510800712X.

23. Muraki I, Imamura F, Manson JE, Hu FB, Willett WC, van Dam RM, Sun Q. Fruit consumption and risk of type 2 diabetes: Results from three prospective longitudinal cohort studies. Br Med J 2013; 347:f5001. doi: 10.1136/bmj.f5001.

24. Pastor-Valero M. Fruit and vegetable intake and vitamins C and E are associated with a reduced prevalence of cataract in a Spanish Mediterranean population. BMC Ophtalmol 2013; 13:52. doi: 10.1186/1471-2415-13-52.

25. Mursu J, Virtanen JK, Tuomainen TP, Nurmi T, Voutilainen S. Intake of fruit, berries, and vegetables and risk of type 2 diabetes in Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr 2014; 99(2):328-33. doi: 10.3945/ajcn.113.069641.

26. Yokoyama Y, Nishimura K, Barnard ND, Takegami M, Watanabe M, Sekikawa A, Okamura T, Miyamoto Y. Vegetarian diets and blood pressure: A meta-analysis. J Am Med Assoc Internal Med 2014; 174(4):577-87. doi: 10.1001/jamainternmed.2013.14547.

27. Chung C, Deeley K, Vieira AR. Dental bleaching associated with fluoride application and its impact on enamel loss. Forum Dent Student Res Innov 2013; 1:22-31.

28. Gravelle BL, Hagen II TW, Mayhew SL, Crumpton B, Sanders T, Horne V. Soft drinks and in vitro dental erosion. Gen Dent 2015; 63(4):33-8.

29. Klink A, Huettig F. The challenge of erosion and minimally invasive rehabilitation of dentitions with BEWE grade 4. Quintessence Int 2016; 47(5):365-72. doi: 10.3290/j.qi.a35262.

30. Mulic A, Tveir AB, Hove LH, Skaare AB. Dental erosive wear among Norwegian wine tasters. Acta Odontol Scand 2011; 69(1):21-6. doi: 10.3109/00016357.2010.517554.

