Effect of a Common Diet and Regular Beverage on Enamel Erosion in Various Temperatures: An In-Vitro Study

Zahra Khamverdi¹, Mohammad Vahedi²*, Shermin Abdollahzadeh³, Mohammad Hosein Ghambari⁴

¹Associate Professor, Operative Dentistry Department, Dental Research Center, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
²Associate Professor, Oral Medicine Department, Dental Research Center, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
³Assistant Professor, Oral Medicine Department, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.
⁴Dentist, Private practice

* Corresponding author: M. Vahedi, Department of Oral Medicine, Dental Research Center, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
vahedi_md@yahoo.com

Received: 14 March 2013
Accepted: 26 May 2013

Abstract

Objective: This study compared diet and regular Coca-Cola on enamel erosion in cold and room temperatures.

Materials and Methods: Seventy five enamel specimens were prepared and divided into 5 equal groups (N=15) as follows: Group 1: regular beverage at room temperature, Group 2: regular beverage at refrigerator temperature, Group 3: diet beverage at room and Group 4: diet beverage at refrigerator temperature. The specimens were immersed in the regular or diet beverage (Coca-Cola, trade mark regd. Khoshgover Co., Tehran, Iran) at room (20°C) or refrigerator (2°C) temperatures for 20 minutes, 3 times per day for 7 days. Specimens in the control subjects (group 5) were placed in synthetic saliva at room temperature for 7 days. The hardness of specimens was tested using Vickers test under 500 gr loads for 5 seconds. The data were analyzed using two-way ANOVA and Tukey tests.

Results: The mean and standard deviations of micro-hardness values of the studied groups were as follow: G1: 304.26±29.71, G2: 285.53±42.14, G3: 279.06±39.52, G4: 266.80±23.98 and G5: 319±30.79. There was a significant difference in the beverage type as the main factor (p<0.05), but temperature factor and their interaction effect on enamel hardness showed no significant difference (p>0.05). Tukey tests showed that there were significant differences between control and diet groups as well as regular and diet groups.

Conclusion: Diet Coca-Cola is more erosive than the regular type and the temperature of the beverages used had no significant influence on enamel erosion.

Key Words: Beverages; Cola; Tooth Erosions; Enamel

INTRODUCTION

Nowadays, a remarkable increase has been reported in the incidence of non-curious lesions in the general population [1]. Dental erosion is one of these lesions. Factors such as bulimia, gastric acid reflux and frequent acidic diet exposure play an important role in the development of dental erosion [2-5].
There is a global increase in the ingestion of acidic beverages, such as soft drinks and fruit juices especially by young children [6,7].

A high drinking rate of non-alcoholic beverages in Iran is one of the main causes of dental erosion. It was reported that the annual drinking of beverages in Iran is 48 litres per person per year that is equal to 144 bottles per person which is relatively a high rate [8]. Attempts were performed to decrease the side effects of these beverages and to introduce types of light and regular drinking. The ability of acidic soft drinks in producing dental erosion is related to factors such as acid type containing, titratable acid, buffered capacity and the temperature of the drink [9].

The solubility of solutes depends on the temperature. It appears that this factor may influence the loss of hard tissues of the tooth at the cervical third [10]. There are various evaluation techniques, such as profilometry, atom force microscopy, scanning electron microscopy, micro-radiography, iodide permeability and the micro-hardness test for measuring the loss of dental hard structures and softening of tooth tissues by erosive processes, [11-14]. The micro-hardness technique is often used for measuring surface hardness of dental hard tissues. Early stages of dental hard tissue dissolution that result in tooth surface weakening can be well determined by hardness measurement techniques [15].

Since there is no definitive information about the effects of varied temperatures of beverages in dental erosion; therefore, the present study was designed to evaluate the effect of common diet and regular Coca-Cola on enamel erosion in cold and room temperatures.

MATERIALS AND METHODS
Thirty eight intact human maxillary premolars that were extracted during the previous two months were collected from dental centers and private offices. The teeth were cleaned with pumice-water slurry using bristle brushes in a low-speed handpiece. The teeth were stored in 0.5% Chloramine-T solution at room temperature to prevent bacterial growth until the next step. Then, they were examined with a stereomicroscope to discard those with cracks, fractures or structural abnormalities that could interfere with the results. The crown of each tooth was removed at the cement-enamel junction with the water-cooled diamond saw of a precision sectioning machine (Miniton; Struers A/S, Copenhagen, Denmark). Subsequently, the crowns of the teeth were sectioned into two labial and palatal parts. The prepared specimens were fixed in self-cured acrylic resin (Acropars, Kaveh, Tehran, Iran) so that the complete tooth was embedded in acrylic resin except for the enamel area leaving a circular enamel area (3 mm in diameter) on the flattest region. To prevent dehydration, the teeth were kept in de-ionized water until the beginning of the tests. The mounted species were divided into 5 equal groups randomly. Four groups were considered as experimental and the remaining one group as control. The specimens in groups 1 and 2 were immersed in the regular beverage (Coca-Cola trademark regd., Khoshgovar co., Tehran, Iran) at room (20° C) and refrigerator (2° C) temperatures, respectively for 20 minutes, 3 times per day for 7 days. The interval time between each immersion cycle was 1 hour. Enamel surfaces were kept in synthetic saliva (Potassium: 20 meq/lit, Chloride: 27.4 meq/lit, Sodium: 0.22 mg, Magnesium: 1.5 mg, Calcium: 0.6 mg, Fluoride: 0.5 mg, Phosphorous: 0.21 mg, Hydroxy propyl methyl cellulose 3%) between each immersion cycle as well as the complete resting time. In groups 3 and 4, the specimens were immersed in diet beverage (Diet coke Trademark regd., Khoshgovar Co., Tehran, Iran) in room and refrigerator temperatures, respectively similar to groups 1 and 2. Specimens in control subjects (group 5) were placed in synthetic saliva at room temperature for 7 days. In this study, Vickers test was applied for at least 3 points with 500 gr force for 5 seconds by micro-hardness tester (Micrometer
1, Buehler, Lake Bluff, IL, USA). The mean of micro-hardness values for points were considered for the micro-hardness value of each sample. The PH value of regular and diet beverages were measured with a digital pH meter (Accumet 925 pH/ion meter, Fisher Scientific, Pittsburgh, PA) and acid titration for both beverages was performed. SPSS software (version 13) was used to categorize the data. Data were analyzed by using two-way ANOVA and Tukey tests. The level of confidence was set at 95% (α=0.05).

**RESULTS**
The mean and standard deviations of micro-hardness values of the studied groups are summarized in Table 1. Group 2 showed the highest microhardness values and the lowest ones were seen in group 4.

Two-way ANOVA for the studied groups showed a significant difference in the beverage type as the main factor (p=0.017), but temperature factor (p=0.089) and its interaction effect on enamel hardness showed no significant difference (p=0.719). Tukey test showed that there were significant differences between control and diet groups as well as regular and diet groups (p<0.05). There were no significant differences between control and regular groups (p>0.05).

**DISCUSSION**
In the present study, the effect of diet and regular Coca Cola beverages on enamel micro-hardness in room and cold temperature was evaluated.

| Group | N   | Micro-Hardness (Mean ± SD) |
|-------|-----|----------------------------|
| 1. (room temp., regular) | 15 | 304.26± 29.71<sup>a</sup> |
| 2. (cold temp., regular) | 15 | 285.53±42.14<sup>a</sup> |
| 3. (room temp., diet) | 15 | 279.06±39.52<sup>b</sup> |
| 4. (cold temp., diet) | 15 | 266.80±23.98<sup>b</sup> |
| 5. (control) | 15 | 319± 30.79<sup>a</sup> |

Superscripts indicate statistically significant differences between values a and b.

| Beverage   | pH  | Phosphoric Acid (mg/ml) | Citric Acid (mg/ml) |
|------------|-----|-------------------------|---------------------|
| Regular Coca-Cola | 2.75 | 0.51 | 0 |
| Diet Coca-Cola | 2.98 | 0.36 | 0.25 |
The micro-hardness test, a valid test in the previous studies [16-18], was used to measure enamel loss. Enamel and dentin dissolution and surface softening in early stages may be determined by this test. The main reason for choosing this technique was the availability and accessibility. The micro-hardness method has been previously used in several studies to determine enamel erosion [1, 16, 19]. In this study, Vickers test was designed using a diamond tip indentation and known geometrical dimensions for a special load and duration [14]. Micro-indentations for determining erosive variations in the surfaces have been performed with different forces. Because of the capability of the loading machine used, 500 gram force was selected for testing in the present study while different forces have been used in other studies [1, 19, 20]. Due to presence of slight variations in the nature of substra, micro-hardness was measured from 3 to 5 points [21]. Regarding immersion and rinsing cycles, Torres immersed the specimens for 5 minutes in 75 ml of the beverage, 3 times a day with 4-h intervals between the immersion cycles, during a 60-day experimental period [1]. In another study, the specimens were placed in several containers of beverages for 14 days continuously [9]. Van Eygen immersed the enamel surfaces in the groups in Coca-Cola at different frequencies: 1, 2, or 3 times per day for 20 minutes each for 7 days [19]. Three times soaking increases the possibility to obtain a measurable result. The specimens in the experimental groups in this study were immersed in diet and regular Coca-Cola for 7 days. The frequency was 3 times per day for 20 minutes each time. This is the approximate total exposure time in the mouth per each year for everyone except those who sip soft drinks continuously [9]. The findings of the present study showed that both studied beverages can potentially erode the enamel surfaces after immersion cycles. Regular beverage did not show any significant difference in comparison with the control group, but the diet type showed a considerable significant difference in terms of enamel softening. These results are in opposition with the study performed by Rios et al. who indicated diet cola promoted less enamel wear than regular cola [22]. It can be explained that erosion might be related to some of its characteristics, such as its lower pH, buffering capacity and titratable acidity [21,23]. PH measurement of both beverages showed that there were no considerable differences between this value. Although regular beverages contain more phosphoric acid, the erosive potential of diet coke is more pronounced due to the presence of citric acid. Citric acid acts as a chelator that binds to the minerals of the hydroxyapatite such as calcium [24,25]. It seems that in the oral environment, the greater the titratable capacity of the beverage, the longer it causes saliva to neutralize the acid [21]. It is predicted that erosion is more severe at high temperatures and reduced at low temperatures, because the solubility of solutes depends on temperature and heat is required to break the bonds holding the molecules in the solid together [26]. Banan and Hedge observed that frozen and refrigerated acidic juices caused more decrease in plaque and salivary pH than juices in room temperature [27]. Ferreira concluded that with a decrease in the temperature of dairy beverages, their pH is reduced [28]. Eisenburger and Addy also confirmed the results of the study conducted by Ferreira and Pozzobon [29]. Temperature had no considerable effect on hardness in all experimental groups of the present study. It seems that under the conditions of this study, temperature did not alter pH beverages significantly. arbour, also showed there were no significant differences between enamel softening and erosion caused by soft drinks at a range of temperatures [30]. In the present study, a cycling model for simulation of physiological conditions was used. On the other hand, complete simulation of oral condition is difficult since pellicle on tooth surfaces affects the erosive destruction in-
duced by acidic solutions [31]. Furthermore; some factors such as tooth brushing or tongue movement in the oral cavity may have an additive abrasive influence on erosively altered dental hard tissues [32]. Therefore, it should be noted that the findings observed in the present study may not reflect the in vivo situation, which was a limit for this study. Nevertheless, it is possible to make certain projections. In the present study, the cycling model was compatible with the study performed by Van Eygen et al. [19]. In order to simulate the oral cavity conditions, future in vivo studies are recommended. In addition, the exposure time was limited; therefore, we recommend other studies with long term exposure times in order to obtain more accurate findings.

**CONCLUSION**

Within the limits of this study, it may be concluded that:

1. The diet Coca-Cola is more erosive than the regular type.
2. Temperature of the used beverages had no significant influence on enamel erosion.

**REFERENCES**

1- Torres CP, Chinelatti MA, Gomes-Silva JM, Rizoli FA, Oliveira MA, Palma-Dibb RG et al. Surface and subsurface erosion of primary enamel by acid beverages over time. Braz Dent J. 2010;21(4):337-45.
2- Machado C, Lacefield W, Catledge A. Human enamel nanohardness, elastic modulus and surface integrity after beverage contact. Braz Dent J. 2008;19(1):68-72.
3- Ulusoy C, Müjdeci A, Gökay O. The effect of herbal teas on the shear bond strength of orthodontic brackets. Eur J Orthod. 2009 Aug;31(4):385-9.
4- Mandel L. Dental erosion due to wine consumption. J Am Dent Assoc. 2005 Jan;136(1):71-5.
5- Borjian A, Ferrari CC, Anouf A, Touyz LZ. Pop-cola acids and tooth erosion: an in vitro, in vivo, electron-microscopic, and clinical report. Int J Dent. 2010;2010:957842.
6- Wang X, Megert B, Hellwig E, Neuhaus KW, Lussi A. Preventing erosion with novel agents. J Dent. 2011 Feb;39(2):163-70.
7- Nirmala SV, Subba Reddy VV. A comparative study of pH modulation and trace elements of various fruit juices on enamel erosion: an in vitro study. J Indian Soc Pedod Prev Dent. 2011 Jul-Sep;29(3):205-15.
8- Dolatshahi Sh, Malakootian M, Akbari H. Acidity rate and fluoride content of consumed beverages in Kerman/Iran. J Res Health Sci. 2009 Dec 28;9(2):41-7.
9- Von Fraunhofer JA, Rogers MM. Dissolution of dental enamel in soft drinks. Gen Dent. 2004 Jul-Aug;52(4):308-12.
10- Erickson PR, Alevizos DL, Rindelaub DJ. soft drinks: hard on teeth. NorthWest Dent. 2001 Mar-Apr;80(2):15-9.
11- Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. Monogr Oral Sci. 2006;20:66-76.
12- Addy M, Mostafa P. Dentine hypersensitivity and effects produced by the uptake in vitro of toothpastes onto dentine. J Oral Rehabil. 1989 Jan;16(1):35-48.
13- Irwin CR, McCusker P. Prevalence of dentine hypersensitivity in general population. J Ir Dent Assoc 1997;43(1):7-9.
14- Bakhos Y, Brudevold F, Aasenden R. In-vivo estimation of the permeability of surface human enamel. Arch Oral Biol. 1977;22(10-11):599-603.
15- Herkströter FM, Witjes M, Ruben J, Arends J. Time dependency of microhardness indentations in human and bovine dentine compared with human enamel. Caries Res. 1989;23(5):342-4.
16- Lussi A, Kohler N, Zero D, Schaffner M, Megert B. A comparison of the erosive potential of different beverages in primary and per-
manent teeth using an in vitro model. Eur J Oral Sci. 2000 Apr;108(2):110-4.
17- Attin T, Buchalla W, Gollmer N, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. Caries Res. 2000 Jan-Feb;34(1):48-52.
18- Collys K, Cleymaert R, Coomans D, Michotte Y, Slop D. Rehardening of surface softened and surface etched enamel in vitro and by intraoral exposure. Caries Res. 1993;27(1):15-20.
19- Van Eygen I, Vannet BV, Wehrbein H. Influence of soft drink with low pH on enamel surfaces: an in vitro study. Am J Orthod Dentofacial Orthop. 2005 Sep;128(3):372-7.
20- Seow WK, Thong KM. Role erosive effects of common beverages on extracted premolar teeth. Aust Dent J. 2005 Sep;50(3):173-8.
21- Featherstone JD, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res. 1983;17(5):385-91.
22- Rios D, Santos FC, Honório HM, Magalhães AC, Wang L, de Andrade Moreira Machado MA et al. An in situ/ex vivo comparison of the ability of regular and light colas to induce enamel wear when erosion is combined with abrasion. Quintessence Int. 2011 Mar;42(3):e44-50.
23- Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res. 2004;38 Suppl 1:34-44.
24- Lodi CS, Sassaki KT, Fraiz FC, Delbem AC, Martinhon CC. Evaluation of some properties of fermented milk beverages that affect the demineralization of dental enamel. Braz Oral Res. 2010 Jan-Mar;24(1):95-101.
25- Attin T, Meyer K, Hellwig E, Buchalla W, Lennon AM. Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol. 2003 Nov;48(11):753-9.
26- Pinto SC, Batitucci RG, Pinheiro MC, Zandim DL, Spin-Neto R, Sampaio JE. Effect of an acid diet allied to sonic tooth brushing on root dentin permeability: An in vitro study. Braz Dent J. 2010;21(5):390-95.
27- Banan LK, Hegde AM. Plaque and salivary pH changes after consumption of fresh fruit juices. J Clin Pediatr Dent. 2005 Fall;30(1):9-13.
28- Ferreira FV, Pozzobon RT. Processed dairy beverages pH evaluation: consequences of temperature variation. J Clin Pediatr Dent. 2009 Summer;33(4):319-23.
29- Eisenburger M, Addy M. Influence of liquid temperature and flow rate on enamel erosion and surface softening. J Oral Rehabil. 2003;30:1076-80.
30- Barbour ME, Finke M, Parker DM, Hughes JA, Allen GC, Addy M.. The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures. J Dent. 2006 Mar;34(3):207-13.
31- Hannig M, Balz M. Protective properties of salivary pellicles from two different intraoral sites on enamel erosion. Caries Res. 2001 Mar-Apr;35(2):142-8.
32- Attin T, Siegel S, Buchalla W, Lennon AM, Hannig C, Becker K. Brushing abrasion of softened and remineralised dentin: an in situ study. Caries Res. 2004 Jan-Feb;38(1):62-6.

www.jdt.tums.ac.ir September 2013; Vol. 10, No. 5