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Evaluation of the Erosive Potential of Selected Isotonic Drinks: *In Vitro* Studies

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Isotonic drinks are an important component of the diet of athletes. Sports drinks cause the body to maintain proper hydration and supplement minerals which are lost in sweat during excessive exercising. Aside from the benefits of isotonic drinks, it is important to be aware of the harmful effects of citric acid within the products, which could cause enamel erosion.

Objectives. The aim of the study was to evaluate the erosive potential of sports drinks using confocal scanning laser microscopy (CLSM).

Material and Methods. The studies measured the change of surface roughness of the dental enamel after etching using Isostar, Powerade and Gatorade drinks, and Fortuna orange juice. Measurements were repeated after 1, 2 and 3 h of exposure to the selected liquid. The evaluation of calcium compound contents was carried out using the complexometric method.

Results. The surface roughness measurements of dental enamel showed that the lowest values of the parameters Ra and Rz were obtained for Isostar and orange juice. The research of the calcium content in the selected beverages showed the highest value in Isostar (320.0 mg/L) and the lowest in Powerade (40.0 mg/L) and Gatorade (21.0 mg/L).

Conclusions. Our study confirms that Isostar is the safest sports drink, among the analyzed beverages, for athletes, because it causes the least erosive changes in dental enamel. It is recommended to supplement beverages to reduce their potential for erosion using calcium compounds (*Adv Clin Exp Med* 2016, 25, 6, 1313–1319).

Key words: dental erosion, athletes, soft drinks, scanning laser confocal microscopy.

Isotonic drinks are an important component of the diet of athletes. They are often confused with “energy drinks”, which have a significantly different composition. Energy drinks contain stimulants such as caffeine or guarana extract, which stimulate the central nervous system. Isotonic drinks, on the other hand, provide the human body with proper hydration, supplements and minerals which are lost through sweating during physical activity. For athletes, the guidelines for drinking isotonic sports drinks are clearly stated. It is recommended to drink small amounts of the beverage before, during and after training [1]. As the osmotic pressure of isotonic drinks is similar to the osmotic pressure of body fluids, proper absorption of fluid is

assured, resulting in a rapid quenching of thirst without burdening the stomach. Hence, these beverages can be drunk safely during exercise. Apart from the benefits of isotonic drinks, there are many disadvantages associated with them, such as the erosive effect of one of the ingredients (citric acid) on tooth enamel [2–4].

Dental erosion is a pathological, chronic, localized loss of dental hard tissue due to chemical processes. The acids responsible for erosion are not products of the metabolism of the bacterial flora of the mouth, but they originate from diet or intrinsic factors [5–7]. The etiology of erosion lesions is complex. According to Lussi [8], in addition to general health status, health awareness or educa-

tion, there are other biological, behavioral, and chemical groups of factors, among others, which determine the erosive potential of the product.

The term “erosive potential” has been introduced to determine the chemical properties of food. High erosive food consumption may have a significant impact on the occurrence of erosive lesions of teeth. In addition to the pH and titrimetric acidity of the food products, the content of calcium is also an important factor. Furthermore, dietary habits, such as frequency and manner of ingestion of acidic foods, and a healthy lifestyle involving a combination of regular consumption of sports drinks with intense exercise have a strong influence on the etiology of erosion [9–12]. Sports carry a lot of benefits to the whole body, but it should be noted that a significant loss of fluids occurs during increased physical activity, as well as a consequential reduction in the amount of saliva, which is an important buffer of acids in the mouth [13, 14]. Hence, athletes can be regarded as a group particularly prone to erosion lesions of teeth. Thus, the aim of the study was to evaluate the erosive potential of selected isotonic sports drinks with the use of a laser scanning confocal microscope.

Material and Methods

Sample Preparation

The study comprised 16 intact premolars, extracted for orthodontic reasons from young people. The teeth were stored in a saturated thymol solution for a maximum period of 3 months. The tooth crowns were sectioned longitudinally in a buccal-lingual direction (B and L in Fig. 1). To obtain the largest possible measurement surface, the enamel samples were obtained from the

Fig. 1. Longitudinal section of tooth sample; p – vestibular surface of the tooth, j – lingual surface of the tooth; x – tested area of tooth enamel

vestibular site of greatest convexity of the tooth crown (Fig. 1). The samples were embedded in epoxy resin, ground and polished to obtain a smooth measuring surface. The surfaces were polished using a sequence of abrasive papers: 320, 600 and 1200 (Presi, France). The final polishing was performed using a Reflex LDM 3 μ m diamond suspension (Presi, France). All samples were evaluated under the optical microscope to assess the quality of the measuring surface.

Experiment Methodology

The erosive effect of 3 isotonic drinks (Powerrade – PA, Gatorade – GA, and Isostar – IS) and orange juice (OJ, reference beverage) was evaluated. The enamel samples were placed respectively in 100 mL of testing fluids. A roughness measurement was carried out in 4 places on the tooth enamel. Thus, 64 measuring points were obtained from 16 samples of the enamel. After 1 h, changes in the roughness of the enamel were assessed under the confocal microscope. Each drink was then replaced with a new one. The samples were examined in the same way in the microscope after 2 and 3 hours of exposure to the liquids.

Confocal Scanning Laser Microscopy

The study of the impact of isotonic drinks on human enamel was performed using a confocal laser scanning microscope (CLSM). The base of the microscope is a versatile MA200 Nikon microscope with inverted optics, equipped with the latest C1 confocal system. The resolution of the captured images was 512×512 pixels. The C1 system allows the observation and recording of optical sections of testing samples, which were used in this case to analyze the surface topography. To scan the surface of the samples, an argon laser with a wavelength $\lambda = 488$ NM was used. Images were recorded using the Software EZ-C1 Free Viewer. A detailed analysis of the data obtained through confocal microscopy was performed using Mountains Map Premium software (Digital Surf, France). To present the effect of isotonic drinks on the change in the surface geometry (SGS), Ra and Rz parameters were selected [15]. Ra is the arithmetic mean of the absolute departures of the roughness profile from the main line while Rz is the mean value of the maximum peak to valley height of the profile within the sampling lengths.

Drink Testing

The evaluation of calcium compound contents was carried out using the complexonometric method. The known volume of the sample was diluted in a volumetric flask with a volume of 100 cm³ to the mark and mixed thoroughly. Then the analyzed solution was pipetted into a conical flask of 25 cm³ and 10 ml of NaOH, and 0.5 g sodium 1-amino-4-[(3-carboxyphenyl) amino] -9,10-dioxo-9,10-dihydro-2-anthracene sulfonate was added. The whole was titrated with EDTA solution until the color changed from red-purple to blue. Titration was repeated at least twice.

The calcium content was calculated from the formula:

$$mCa^{2+} = C_{EDT} \cdot V_{EDTA} \cdot M_{CA} \cdot \text{in [g]}$$

C_{EDT} – EDTA solution concentration [Mol/L]

V_{EDTA} – volume used per EDTA titration in the presence of sodium 1-amino-4-[(3-carboxyphenyl) amino] -9,10-dioxo-9,10-dihydro-2-anthracene sulfonate [L]

M_{CA} – molar mass 40.08 g/Mol

In – the matching flask with a pipette

Statistical Analysis

A two-way analysis of variance, with repeat observations on a single factor to compare the “erosive effect” of isotonic drinks, was used to evaluate the results. Repeated observations of the selected parameters were measured at several subsequent time points. The isotonic drinks determined the categories of the randomized factor and the time points were categories of the nonrandomized fac-

tor. The analysis was complemented by an assessment of the simple effects.

A linear regression model, weighted by the inverse of SD in time points, was used to evaluate the relationships between time and the values of the roughness parameters Ra and Rz.

For all statistical tests, the accepted level of significance was $\alpha = 0.05$.

Results

The results are presented in 2 tables which illustrate the dynamic process of etching the enamel after exposure to orange juice and isotonic drinks. Table 1 shows the results of the parameter Ra.

As shown in Table 1, the mean Ra value of orange juice did not differ from that of Isostar after an hour of exposure to the enamel samples. However, both values were significantly lower than the corresponding values for Gatorade and Powerade; both of which also had very similar values. Due to the large variation between the standard deviations, it was impossible to indicate any clear difference between the Ra values after 2 h of enamel exposure. Further, although the average values for orange juice and Isostar did not differ from each other at 0 and 1 h, they were significantly lower than the total average after 3 h. There are no other statistically significant differences. The average Ra in hour 3 for Gatorade and Powerade were significantly higher than average at times 0 and 1h, while their average values were similar in hours 2 and 3. The Rz roughness parameter is shown in Table 2.

As shown in Table 2, the average values for orange juice and Isostar were similar and signif-

Table 1. Ra parameter value for tested beverages depending on time exposure

Beverage (randomizable factor)	Time elapsed before measurement (hours) (nonrandomizable factor)				P-value for evaluating simple effects
	0	1	2	3	
1. Orange juice	0.0010 ^a (0.0007)	0.0071 ^a (0.0044)	0.0737 (0.0851)	0.0875 (0.0583)	0.004
2. Isostar	0.0011 ^a (0.0005)	0.0099 ^a (0.0136)	0.0329 (0.0395)	0.0901 (0.0948)	0.003
3. Gatorade	0.0011 ^b (0.0008)	0.1410 (0.1183)	0.2774 (0.5279)	0.2808 ^c (0.1765)	< 0.0005
4. Powerade	0.0016 ^b (0.0035)	0.1229 (0.0814)	0.2628 (0.1229)	0.3343 ^c (0.1116)	< 0.0005

The average values and standard deviations in brackets; ^a the average in hour 0 and 1 not differing from each other, however these averages are significantly lower than average for hour 3; no other differences; ^b average in hour 0 is significantly lower than any other; ^c the average in hour 3 is significantly higher than the average hours 0 and 1; averages in hours 2 and 3 do not differ from each other.

Table 2. Rz parameter value for tested beverages depending on time exposure

Beverage (randomizable factor)	Time elapsed before measurement (hours) (nonrandomizable factor)				P-value for evaluating simple effects
	0	1	2	3	
1. Orange juice	0.0877 ^a (0.0684)	0.4555 ^a (0.2545)	1.4104 (0.7255)	1.6987 (0.4569)	< 0.0005
2. Isostar	0.0879 ^b (0.0390)	0.4036 ^b (0.4067)	0.7968 (0.6506)	1.2487 ^c (0.8535)	< 0.0005
3. Gatorade	0.0054 ^d (0.0087)	1.4488 (0.9889)	1.5784 (0.9604)	2.2425 (1.2329)	< 0.0005
4. Powerade	0.0839 ^d (0.0826)	1.7893 ^e (0.5606)	2.4165 (0.6887)	2.7359 (0.5622)	< 0.0005

Average values with standard deviations in brackets; ^a the average in hour 0 and 1 do not differ from each other, however these averages are significantly lower than averages in hour 2 and 3; averages in hours 2 and 3 do not differ between each other; ^b the average in hour 0 and 1, not differing from each other, are significantly lower than averages in hours 2 and 3; ^c the average in hour 3 is significantly higher than the average in other points in time; ^d the average in hour 0 is less than the average in other points in time; ^e the average in hour 1 is less than the average in hours 2 and 3; averages in hours 2 and 3 do not differ between each other.

icantly lower than those of the other two (Gatorade and Powerade), which were also very similar to each other after one hour of enamel exposure. The average values for the orange juice were significantly lower than the ones for Powerade after 2 and 3 h. The mean Rz values for Isostar after 2 and 3 h were significantly lower than the average

values for Gatorade and Powerade. After 2 h, the average value for Powerade was significantly higher than the average for Gatorade.

Figures 2 and 3 represent the relationships between time and the values of the roughness parameters (Ra and Rz, respectively) for each of the drinks. Table 3 shows that Gatorade had the lowest

Fig. 2. Changes in Ra parameter as a function of erosion time for selected drinks

Fig. 3. Changes in Rz parameter as a function of erosion time for selected drinks

Table 3. Calcium concentration in selected beverages

Beverage	Ca ²⁺ mg/L
Powerade	40.0
Isostar	320.0
Gatorade	21.0
Orange juice	109.0

content of calcium and Isostar had the highest. It is worth taking into consideration that the presented values of the Ra and Rz parameters highly correlate with the Ca content of the selected drinks. Orange juice and Isostar drinks, with Ca content of 109 mg/L and 320 mg/L, respectively, showed the lowest loss of enamel while Gatorade and Powerade, with a low level of calcium (21 mg/L and 40 mg/L), were more aggressive and caused more erosive loss of dental hard tissues. These results are represented graphically in Fig. 4.

Discussion

According to the literature, the greatest impact on the occurrence of enamel erosion lesions is connected to dietary factors [7, 8]. Currently, it

is believed that not the quantity, but the frequency of acidic food consumption is a crucial factor in the etiology of dental erosion. Prolonged contact of acidic food with dental hard tissues results in demineralization. The erosive properties of food products are derived from their acid content, which is mainly citric acid (pH 1.8). Meurman et al. attribute the strong decalcifying properties of isotonic drinks to the citric acid within them [16].

The erosive potential of isotonic drinks is not generally perceived by consumers as well as by dentists. Jarvinien et al. [2] in 1991, studying the dietary habits of adults, noted that drinking an isotonic sports drink once a week or more is associated with a risk of erosion lesions. Sirimaharaj et al. [3] also emphasized in his study that athletes who drink isotonic drinks are in a high-risk group regarding enamel erosion. A study by Ostrowska and Piatowska [4] confirmed the statistical relationship between the consumption of isotonic drinks and the occurrence of erosive defects in athletes. However, these drinks are not only purchased by persons engaging in professional sport. Aggressively marketed as a healthy and necessary food supplement, they are especially popular with young people whose enamel is more sensitive to acids and can rapidly be subjected to destruction under their influence [17].

Fig. 4. CLSM topography images for different erosion times and beverages: a) Isostar, b) orange juice, c) Gatorade, and d) Powerade

As our research has shown, the safest drink for dental enamel is Isostar, which proved to be the most minor etchant for the tested surfaces. However, Gatorade and Powerade resulted in major changes in enamel erosion. This is consistent with the results of Owens et al. [18] who, after testing samples of teeth in the SEM, indicated Gatorade as being more erosive than Coca-Cola. Also, the study by Hooper et al. [19], comparing Isostar and Gatorade in a profilometric study, showed that the changes of erosion caused by Gatorade were significantly greater than those caused by Isostar.

Of particular note is a study by Davis et al. [20] concerning the application of 100% orange juice either enriched with calcium or without. A polarizing microscope was used to investigate changes in enamel and root cement samples after exposure to the selected juice. The results confirm that the calcium content protects the teeth from erosion. Our results related to the erosive potential may also be explained by the calcium content in the tested fluids, which demonstrated that Pow-

erade and Gatorade are the most erosive. In the literature, there are other reports of beverages modified by added calcium which similarly demonstrate a reduced erosive potential [21–23]. Weghaupt et al. [24] measured the erosive wear of enamel using surface profilometry after contact with orange juice modified with calcium effervescent tablets. The erosive changes of enamel were significantly reduced by a modification of orange juice with calcium dietary supplements. Moreover, Cochrane et al. [25] acknowledged that sports drinks with higher calcium content (Sukkie and Endura) compared with the other drinks had a significantly lower erosive potential. These results, as well as the results of our work, confirm the validity of adding calcium to isotonic drinks. Our study confirms that Isostar, among the analyzed beverages, is the safest isotonic drink for dental enamel, because it caused the minimal erosive changes in enamel. It is recommended to supplement beverages to reduce their potential for erosion using calcium compounds.

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