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Does Pre-Workout Supplement Consumption Affect Enamel Microhardness?

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Abstract

The aim of this study was to compare *in vitro* the micro hardness of enamel exposed to the erosive effects of three pre-workout drinks. Forty blocks (4mmx4mm) of bovine enamel were randomly selected and divided among the following pre-workout drinks (n=10): G1: Universal Shock Therapy®, G2: 1.M.R Vortex™, G3: Jack3Dmicro™, G4: Control (distilled water). The enamel microhardness was evaluated before (T1) and after (T2) the *in vitro* erosive test (5 min/3 times/5 days) using a Vickers digital microduremeter (load 100g/penetration 15s). The data was analyzed using descriptive statistics (mean, ±sd) and for comparison testing, using the Kruskall Wallis test and the t Student test ($p \leq 0.05$). All groups showed a reduction in micro hardness after the erosive test ($p = 0.001$).1.M.R Vortex™ drink showed a large decrease in micro hardness (%SMH:34,92%). Pre-workout drinks significantly decreased the superficial micro hardness of enamel

Keywords: Dietary Supplements, Tooth Enamel, Dental Erosion, Sports

Introduction

Dental erosion is pathological tooth surface loss or tooth wear [1-3], which is characterized by the irreversible gradual dissolution of hard dental tissue as a result of non-carious causes. It represents the extent and areas of unacceptable dental hard tissue loss, and usually requires treatment. The erosive lesions are caused several factors, such as the type, concentration and temperature of the acid involved and its time in contact with the tooth surfaces. Other factors, such as pH (potential of hydrogen), titratable acidity, ion concentrations and frequency and method of exposure, also affect the erosive potential [1-3]

The critical pH at which enamel becomes susceptible to erosion is estimated to be 5.5. Hence, when acidic material whose pH is at or below this level comes into contact with enamel frequently and for a prolonged period of time, enamel erosion occurs. Dentine is more susceptible to erosion than enamel, and can be eroded at a relatively high pH (6.0) [2, 4].

The etiological factor of erosive drink beverages are those which have a low pH and higher buffer capacity, in other words, to resist changes in pH or highly acidic, low pH carbonated soft drinks (which usually contain two acids: citric and phosphoric acid) such as energy drinks, fruit juices, alcohol based drinks, fruit flavored alcoholic drinks and recently, pre-workout (PW) beverages [4, 5].

Dental erosion has been recently diagnosed with higher prevalence in people who follow a strict diet consisting of foods and supplements that have an acidic pH, in conjunction with a continuous workout routine, mainly in athletes [5, 6]. This acidic pH produces an unfavorable effect on the teeth, provoking enamel demineralization, which is caused by the radical changes of pH (the measure of acidity or alkalinity of a solution) in the oral cavity. Erosive lesions can cause dental hypersensitivity, loss of dental tissue and even pain, impairing the athlete's physical performance [2,4].

Pre-workout supplementation is a nutritional strategy that involves consuming a mixture of bioactive compounds and dietary ingredients prior to a bout of exercise for ergogenic purposes. For example, PW supplements often contain beta-alanine, caffeine, citrulline, tyrosine, taurine, creatine, arginine and carnitine, as well as numerous vitamins and minerals, and often have a pH below 5 [5,7]. The new drinks are pre-workout ones that are currently sold without regulation, some in markets or pharmacies and in different socioeconomic sectors, which increases their consumption by athletes. These pre-workout beverages are produced with the idea of achieving maximum development and physical potential during sporting activities, increasing physical performance, and even compensating for muscle degradation to avoid catabolism. Mainly, this type of beverages is suitable for high-intensity exercises and provides lots of hyperactivity and strength, as well as quicker recovery. On the other hand, young people and physically active people are the most frequent consumers of these beverages, often exceeding the recommended levels of consumption without knowing the risk that this presents to their health [4,6,7].

Therefore, the aim of this in vitro study was to compare the erosive effect of three pre-workout beverages: 1.M.R Vortex™, Jack3D-micro™, and Universal Shock Therapy® on bovine enamel microhardness.

Materials and Methods

This study was experimental in vitro. The brand, composition, weight/liquid, pH, and batch of pre workout beverages used in this study are shown in Figure 1. The sample size was 40 blocks of completely healthy bovine enamel (software Stata-v 12.0®, power 80%, 95%confidence level). This study was approved by the Institutional Ethics Committee No. CEI 001-01-16 EX.

Nro.	Pre work supplement	Brand	Composition	Weight/liquid	pH	Batch
1	Vortex 1.M.R™	BPI Sports	1 scoop(3g): . Niacin (20mg, DV:100%), FULL SPEED VORTEX™ (1,705mg, DV:*) *Glycerol as monostearate, Indigofera pulchra (aerial), White leadwort (root), Securienga (sffruticosa: leaf and root), Yohimbe (Pausinystalia yohimbe) (bark). **Other Ingredients:** Maltodextrin, Citric Acid, Natural And Artificial Flavors, Sucralose, Malic Acid, Acesulfame K, and FD&C Blue No. 1.	6 g/6onz	2.58	1503092
2	Universal Shock Therapy®	Universal Nutrition	1 scoop (20g): . Calories (20), Total Carbohydrates (5g, DV:2%), Sugars (0g DV:**), Niacin (10mg, DV:50%), Vitamin B6 as Pyridoxine HCL (10mg, DV:500%), Vitamin B12 as Cyanocobalamin, Methylcobalamin (10mcg, DV: 167%), Calcium as Carbonate (25mg, DV: 3%), Magnesium as Oxide (25mg, DV:6%), Sodium (as Bicarbonate, Trisodium Phosphate) (120mg, DV:5%), Potassium as Phosphate (60mg, DV:2%). **Other Ingredients:** Natural And Artificial Flavors (Sulfites), Citric Acid, Malic Acid, Sucralose, Acesulfame Potassium made In A GMP Facility On Equipment That Processes Milk, Soy, Egg, Peanuts, Tree Nuts, Fish, Shellfish, And Wheat.	40 g/6onz	3.83	V4128DA
3	Jack3Dmicro™	Usp labs	1 scoop (3.6g): . ENOS Super Performance System ™ (2247mg), L-Citrulline, Arginine Nitrate, Agmatine Sulfate, Grape Seed (95% Proanthocyanidins) Extract, CNS Contractile Stimulant System ™ (182mg), Caffeine, Norcoclaurine HCL (Norcoline™), 3,4-Dihydroxycinnamic Acid, Vitamin C (Ascorbic Acid- 50mg, DV: 84%). **Other Ingredients:** Citric Acid, Natural & Artificial Flavors, Silicon Dioxide, Acesulfame-K, Sucralose, Vegetable Stearate.	7.26 g/6onz	2.77	189660

Figure 1: Composition and description of each pre workout beverages used in this study

Preparation of Enamel Blocks

Forty recently extracted bovine teeth (permanent central incisor) were maintained in distilled water at 4°C-8C. The teeth were cleaned and randomly divided into 4 groups (n=10)- G1: Universal Shock Therapy ®(UST)- Universal Nutrition, G2:75 1.M.R Vortex™ (1MRV)- BPI Sports, G3: Jack3Dmicro™ (J3Dm)-Usp Labs, G4: Distilled Water (DW)-control group.

Enamel blocks measuring 4×4 mm were cut, starting from the vestibular surface using a double diamond disc Isomet cutting machine (on low speed and with cooling). Thereafter, the specimens were embedded in circular acrylic resin blocks to polish the enamel surface (Sandpaper 600/1 min to 1200/1 min) and the final polishing was performed with a polishing machine (Struers, Birmensdorf, Switzerland /diamond paste (1μm alumina) / 3 minutes), to obtain a uniform and highly polished surface that resembles the smoothness of human enamel surfaces. All of them were preserved in distilled water at 37 ° C±5°/24 hours. In the initial phase of the study, a random pre-selection of enamel specimens was performed, confirming the initial microhardness values of the enamel at 347.2-347.3 HVN.

Selection and Preparation of Pre-Workout Beverages

The pH of 3 different brands of PW beverages was measured using a pH meter (HANNA Instruments pH 210, microprocessor pH meter). Then they were selected: 1.M.R Vortex™ (pH 2.58), Jack3Dmicro™ (pH 2.77) and Universal Shock Therapy® (pH 3.83). Each PW beverage was prepared according to the manufacturer's specifications. The standard solution was prepared in a "shaker" (Smartshake™, Original Series, 20oz/ 600ml) which was mixed solute with solvent (water) and stirred for one minute. The dosage for the PW drinks was: Universal Shock Therapy® (40 g / 6oz), 1.M.R Vortex™ (6 g / 6oz) and Jack3Dmicro™ (7 26 gr / 6 oz).

Erosive Challenge

The methodology of this study was validated in vitro protocol to assess the effect of beverages on dental erosion [8]. All specimens were eroded with 300 ml PW beverage solution under controlled conditions (5min/3 times a day/5days) All samples were in a container with distilled water at room temperature.

Microhardness Measurement

Surface microhardness was evaluated using a Vickers microhardness tip with a load of 100 g for 15 s (High Quality Microhardner BUEHLER®) at two points in time: 1. with sound enamel immediately after the preparation of the samples SMH1, 2. after the in vitro erosive test- SMH2. Surface microhardness (SMH2) was measured with the same static load and time as SMH1. The specimens were divided into quadrants. Indentations were made in the center of each quadrant. The fifth reading was performed 100 µm from the first indentation in quadrant 1 to ensure that the same area was not measured. The final value was obtained by using the average of the 5 indentations to obtain the mean of each specimen. The change (ΔSMH) was determined as follows: ΔSMH1 = SMH2 – SMH1, with negative values representing a decrease on microhardness. The percentage of superficial microhardness change (%SMH) was calculated (%SMH=[SMH2-SMH1]/SMH1] *100). The response variable was the depth of enamel surface wear (µm). Percentage of superficial microhardness change (%SMH) was also employed as a complemental response variable. The percentage of surface microhardness loss (%SMH) represents the amount of enamel softened and lost. The %SMHS values were 26.63% for Universal Shock Therapy®, 34,92% for 1.M.R Vortex™ and 23.56% for Jack3Dmicro™, Table 1.

	SMH1(±s.d)	SMH2(±s.d)	ΔSMH	%SMH
1.M.R Vortex™	347.3 ±20.91	226 ±22.25*	-121.3**a	34.93%
Universal Shock Therapy®	347.3 ±17.46	254.8 ±23.04*	-92.5**b	26.63%
Jack3Dmicro™	347.2 ±17.46	265.4 ±14.85*	-81.8**b	23.56%
Distilled Water	347.8 ±18.08	347.2 ±18.22*	-0.6	0.17%

*T-Student test, (SMH2-SMH1), p=0.01

**Kruskall Wallis test (ΔSMH/ 3 pre-workout beverages), p ≥0.05

Bonferroni correction test- Lowercase letters indicate significant differences between treatments (p < 0.05).

Table 1: Comparison of enamel microhardness (mean ±sd, ΔSMH and %SMH) eroded by pre workout beverages

The assumptions for the equality of variances and normal distribution of errors were checked for the tested response variables. The data was analyzed using the univariate analysis: mean ±deviation standard and for bivariate analysis, Student's t-test and Kruskal Wallis Test (α=0.05), and Bonferroni correction test was applied to pairwise comparisons of treatment.

Results

Statistically significant differences in mean surface microhardness values were found in the experimental groups performed at different evaluation times (pre and post erosive test, p<0.001), Table 1.

Discussion

The aim of this study was to compare in vitro the microhardness of enamel exposed to erosive effects of three pre-workout beverages. According to the results, under an experimental in vitro study, the three PW beverages evaluated caused the diminution of microhardness of enamel, in comparison to the control group.

Despite the different literature that currently exists in relation to erosive beverages (acid drinks), few studies containing an in vitro or in vivo studies of PW beverages have been found [5,9,11]. However, there are studies reporting the effects of their components on other areas of the body, related to cardiovascular, cognitive, calcium deposits in the bones and hyperglycemia [5,7,9,10]. It is well known that the first point of entry of these beverages is through the oral cavity, therefore, it is important to know the effect it could have on this system [5, 11].

In this study, the pH values of the PW beverages were below the critical pH value (5.5) for enamel demineralization and ranged from 2.58 to 3.83. In general, the erosive potential of the acidic drinks may be predicted based on the type of acid content, pH value, titratable acidity, and ion concentration which caused a loss of the hardness to their structure: enamel or dentine, making them susceptible to caries or erosion lesions [4, 5]. There is also a strong association with the chelant properties of the acid in respect of calcium, which is more evident for citric acid. As the values are below neutral pH, these drinks can be critical not necessarily for the pH, but due to their frequency of consumption and type of composition. As such, having an acidic pH will not necessarily lead to a major enamel loss, because in its composition there are other elements or minerals that can interact, also influencing the buffer capacity of saliva and the oral environment [12,13]. However, according to our results, the 1.M.R VortexTM drink caused a higher percentage of superficial microhardness change (%SMH), coincidently also having a lower pH (2.58) than the others and containing ingredients such as acid and maleic acid.

Our results reflected the findings in scientific literature about the structural alteration of dental tissues in erosive challenge. The assessment of the enamel exposed to the three PW beverages showed a decrease in surface microhardness, with a similar result to other studies [8,12, 14,15].

These PW beverages usually consumed during endurance training may have a greater capacity in terms of dissolution of enamel surface (demineralization) depending on their physicochemical proprieties associated with pH and titratable acidity such as citric and malic acid present in their composition. Hydrogen in these acids interacts with the enamel by capturing calcium phosphates, producing the initial softening, i.e. demineralization. The protein part tries to repair this situation by neutralizing the acid attack to maintain a balance in the oral cavity, a process known as remineralization, by means of the buffer effect (directly related to the pH level and the erosive effect). Therefore, if demineralization takes place very frequently, an imbalance will occur and as a result erosion will take place, since the longer the acid is exposed to the enamel, the more harmful it will be [4,5,7, 13-15].

The composition of the PW beverages (minerals, acids, sweeteners,etc) has an important effect not only on the oral cavity directly, also could have harmful consequences on the body, compromising the athletes physical performance. For example, Norcoclaurina HCL (Higemina) present in its adrenergic antagonist composition such as Beta1 and Beta 2. The latter is used to treat asthma and other lung disorders, being one of the main components of Salbutamol, which has been found in literature to produce a lower saliva rate and a dried mouth and is related to dental erosion [4]. Also, some PW beverages contains arginine as nitrate, which can contribute to pH homeostasis and ecological balance of biofilms, thus reducing the risk of the development of carious lesions. On the other hand, others have in its composition potassium acesulfame, which, having interacted with the artificial sweeteners, promotes caries and dental erosion [12]. Also, they contain magnesium as oxide, which is an essential element and has an important role as a co-factor in enzymatic reactions involving energy metabolism. Thus, these metallic ions could be candidates for participation in the process of de- and remineralization under conditions of erosive challenges. The pre workout beverages contains ingredients as calcium as carbonate that could increase or maintain pH, promote remineralization and the detection of caries lesions [4,9].

Within the limitations of experimental study, the consumption of citric fruit, juices, and industrialized beverages, especially soft or isotonic drinks, and pre workout beverages has been associated with an increase in the prevalence of dental erosion. The uncontrolled high consumption of the acidic drinks in athletes or athletes of high and low performance sports could cause oral diseases as caries and erosion lesions, as well as their components affecting other organs of the body, which could result in irreversible effects, decreasing sport performance as well as their quality of life.

Conclusion

All three pre-workout beverages tested were found to be erosive, they caused a high decrease in the microhardness of enamel. The composition of these pre workout beverages plays a key role in the prevalence of dental erosion.

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