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Enamel Dissolution Associated with Regular and Diet Energy Drinks

A Thesis

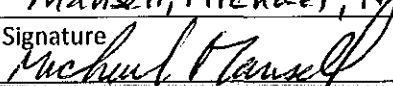
Presented to the Faculty of the Advanced Education in General Dentistry, Two-Year Program,  
United States Army Dental Activity, Fort Hood, Texas  
And the Uniformed Services University of the Health Sciences – Post Graduate Dental College  
In Partial Fulfillment of the Requirements for the Degree of  
Master of Science in Oral Biology

By

Evan J. Endsley, CPT, USA, DC

May 2017

**Uniformed Services University of the Health Sciences  
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A handwritten signature in black ink, appearing to read 'E. Endsley', with a long horizontal line extending to the right.

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9 MAY 2017

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11. Is this research project related to an active research project of the advisor identified in Section B? If yes, complete this Part 13; if no, proceed to Part 14. ☐ Yes ☒ No

Project Number: \_\_\_\_\_

Project Title: \_\_\_\_\_

Project Start Date: \_\_\_\_\_

Project End Date: \_\_\_\_\_

Anticipated Period of

12. Performance: Project Start Date: 1 MAY 2016 Project End Date: 1 JUN 2017

13. List all performance sites and indicate percentage of the work being performed at each site:

Performance Site (Should not exceed 100%)

% of  
Work

USUHS (Postgraduate Dental School and/or on-campus space)

Other off-site location(s): Billy Johnson Dental Clinic, US Army DENTAC, Ft Hood, TX

100%

14. Does this project involve any classified information? (Contact the USUHS Security Office for guidance) ☐ Yes ☒ No

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16. What is the funding source?

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☒ Federal (specify):

Ft. Hood DENTAC

☐ No Funding

☐ Other External Agency (Specify) \_\_\_\_\_

17. If "Federal" or "Other External Agency" is marked, does the Sponsor allow indirect cost? ☐ Yes ☒ No

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19. List budget breakdown below: (May not include non-mission essential travel, secretarial/administrative support, or scientific conferences)

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Enamel Dissolution Associated with Regular and Diet Energy Drinks

A REPORT ON

Research project investigating the correlation between human tooth enamel lost following prolonged exposure to both regular and diet formulations of commonly used energy drinks

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May 2017

## ABSTRACT

**Purpose:** The purpose of this study is to compare the difference in enamel dissolution between the regular and diet formulations of 3 brands of popular energy drinks.

**Methods:** Sixty intact, unerupted third molars were used for testing. Initial weights were recorded for each tooth to 0.001g. All teeth were then painted with 2 layers of acrylic nail polish up to the cemento-enamel junction. The teeth were weighed again to determine the weight of the acrylic and placed into 1 of the 6 test beverages: Red Bull, Red Bull Sugar Free, Monster, Monster Absolutely Zero, Rip It Tribute, and Rip It Tribute Sugar Free. Tooth specimens were submerged randomly in 50 mL of freshly opened energy drink daily, for a period of 5 days at room temperature. The teeth were dried and weighed after each day. The initial coated weights were subtracted from the coated weights after each day to determine the percent of tooth weight lost. This difference is attributed to enamel erosion. The test beverages were also assessed for pH and titratable acidity.

**Results:** Monster Absolutely Zero was significantly more erosive than Rip It Tribute ( $p = 0.0106$ ), with all other energy drinks being statistically similar. There was no difference in erosion between the regular and diet drinks ( $p > 0.05$ ). A strong positive linear correlation was observed between titratable acidity and percent of tooth weight lost ( $r = 0.9584$ ;  $p = 0.0026$ ).

**Conclusions:** Based on the results of this in-vitro study testing 3 most commonly utilized energy drinks in the Army, it can be concluded that there is no difference in the erosive potential between the pooled regular and diet formulations. Rip It Tribute caused the lowest mean percentage of tooth weight loss, but only significantly less than Monster Absolutely Zero. Also, the results of this study support earlier research in this area by suggesting that titratable acidity of beverages is more closely related to erosion by energy drinks than initial pH.

## ACKNOWLEDGMENTS

The author would like to thank the following:

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## Disclaimer

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## INTRODUCTION

Energy drinks are beverages that often times contain calories and caffeine, in addition to any number of “presumed energy-enhancing ingredients such as taurine, herbal extracts, and B vitamins.”<sup>1</sup> Together with sports drinks and others that are consumed specifically for a perceived benefit, energy drinks make up the group known as functional beverages. Yet, despite growing scrutiny of the sector due to health concerns, energy drink popularity is on the rise. Sales of energy drinks in the US are projected to eclipse \$21 billion by 2017, up from \$12.5 billion in 2012.<sup>2</sup> In the Army, energy drinks are ubiquitous. They have become the top-selling cold beverages in the Army and Air Force Exchange Service (AAFES) worldwide.<sup>3</sup>

Research has indicated that most soldiers imbibe energy drinks for an “energy boost” and increased “mental alertness,”<sup>4</sup> and according to data from 2010, nearly 45% of servicemembers drank at least one energy drink per day while deployed in Afghanistan, with over 13% drinking 3 or more.<sup>5</sup> Decades of research on caffeine indicate that if used properly, caffeine is effective.<sup>6</sup> However, the same data from deployed soldiers, as well as several civilian studies, reveals that caffeine consumption, especially an excessive amount, is associated with daytime somnolence.<sup>5, 7, 8</sup>

This widespread usage observed in soldiers may partly be explained by easy access to these drinks in the military, as some brands are available to soldiers for free while deployed, and targeted marketing by the manufacturers. Also, studies have shown that increased consumption is directly related to masculine norms and, more broadly, risk-taking behaviors.<sup>9, 10</sup> Clearly, these are prevalent traits throughout the military ranks, as they have been evaluated numerous

times.<sup>11, 12</sup> As such, the individuals involved in military service are a perfect cohort for such targeted marketing.

The elevated rate at which soldiers are consuming energy drinks can have significant dental consequences. As recent as 2014, the Consultant to the Surgeon General for Dental Public Health noted that, “Army dentists are all too familiar with the rampant decay that results when a Soldier sips on sugary drinks throughout the day.”<sup>13</sup> Indeed, dentists struggle to control the resultant disease stemming from soldiers’ frequent snacking and drinking habits, to include energy drinks. Nearly all Active Duty dentists have anecdotal evidence of the destruction that can be caused, in part, by energy drinks.

Some of this destruction is a result of dental erosion, a chronic loss of dental hard tissue which is chemically removed by acid and/or chelation without the involvement of microorganisms.<sup>14</sup> It is irreversible and usually painless. The incidence of dental erosion is on the rise, and recent data shows a more pronounced trend in younger age groups.<sup>15</sup> This can be viewed as perhaps a direct effect of the increasing popularity of acidic beverages, like energy drinks. Erosion can be deemed intrinsic, extrinsic or a combination of both. Intrinsic erosion occurs through tooth contact with gastric, or hydrochloric, acid. Extrinsic erosion is a result of dietary acids, such as phosphoric, citric, carbonic and ascorbic acids.<sup>16</sup> Often, energy drinks contain these acids and others.

Yet, while the systemic safety concerns related to energy drinks and young adults have been well-documented,<sup>17-19</sup> quantitative data relating energy drinks to tooth damage is needed. It is evident that policy makers and healthcare practitioners throughout the military are aware of the many general health concerns stemming from energy drink consumption; such as increased

blood pressure, panic attacks, heart palpitations, anxiety, and dehydration,<sup>20</sup> as well as certain military-centered concerns, like disturbed sleep leading to difficulty staying awake during briefings or guard duty.<sup>5</sup> Yet, the trend is still toward increased usage.<sup>20</sup> Knowing this, it would seem prudent as dental professionals to act preventatively, and offer advice as to which drink, if any, may be a better alternative.

Dentists across the globe can no doubt recall the idea of “critical pH” from their undergraduate studies. Fosdick and Starke were the first to reference the idea of a critical pH in the oral cavity that results in demineralization of tooth structure.<sup>21</sup> Since that time, this pH has been established at 5.5.<sup>22</sup> Below this pH, it is understood that the hydroxyapatite crystals will dissociate into their component ions: calcium, phosphate and hydroxyl. According to Le Chatelier’s Principle, the reaction of enamel into its associated ions is in dynamic equilibrium, and the pH at which the equation favors dissolution is highly dependent on the surrounding concentration of enamel ions, as well as the temperature, and to a lesser extent, pressure and volume. All other parameters being equal, a person with a higher salivary or plaque concentration of calcium and phosphate should require a lower “critical pH” than someone with those ions in lower concentrations. Taking this idea a step further, research has already investigated whether or not beverages with increased levels of these tooth minerals limits erosion.<sup>23</sup>

Furthermore, many patients commonly believe diet drinks to be less harmful to their teeth. Indeed, some energy drinks, such as the Rockstar Punched Guava, have as much as 78g of sugar per can. According to the American Heart Association, that equates to more than double the recommended daily allowance for most American men.<sup>24</sup> This has pushed many health-minded consumers towards sugar free varieties of their favorite drink. Yet, recent research has

shown diet or sugar free formulations may be just as, or more, erosive than the regular alternatives.<sup>19</sup>

To date, abundant research has been dedicated to different beverage categories and their erosive potential. However, little investigation has been done solely assessing the destructive capacity of energy drinks, let alone those used most frequently in the military and their diet formulations. In view of the rising consumption of energy drinks by soldiers, this study was undertaken to quantitatively assess the enamel erosion caused by some of the most popular energy drinks used throughout the Armed Forces.

## PURPOSE

The purpose of this study is to compare the difference in enamel dissolution between 1) the regular formulations of 3 flavors from popular brands of energy drinks (Red Bull, Monster, Rip It Tribute). 2) The diet formulations of 3 flavors from popular brands of energy drinks (Red Bull Sugar Free, Monster Absolutely Zero, Rip It Tribute Sugar Free). 3) The pooled regular and diet formulations. It has also been designed to reveal any correlation between titratable acidity and erosive potential.

## HYPOTHESES

Research questions: Will there be a significant difference in the percentage of enamel lost through dissolution when teeth are exposed separately to Monster, Monster Absolutely Zero, Red Bull, Red Bull Sugar Free, Rip It Tribute, or Rip It Tribute Sugar Free? Will there be a

significant difference in the amount of enamel lost through dissolution when comparing the pooled data from the regular and diet energy drink groups? Is there a correlation between titratable acidity and erosive potential?

Null hypothesis #1: There will be no difference in the percentage of enamel lost between the energy drinks.

Null hypothesis #2: There will be no difference in the percentage of enamel lost between the pooled regular and diet energy drinks.

Null hypothesis #3: There is no correlation between the percentage of enamel lost and the titratable acidity of the energy drinks.

## MATERIALS AND METHODS

### *Specimen Collection and Preparation*

Testing was undertaken at the Billy Johnson Dental Clinic located at Ft. Hood, TX. The study protocol was approved by the Brooke Army Medical Center, Human Research Protection Office (Ref. C.2016.099n). Sixty unerupted and intact third molars that were treatment planned for removal were collected and stored according to Collection of Extracted Teeth Standard Operating Procedures (SOP) at Fort Hood Advanced Education in General Dentistry (AEGD-2) Residency. Specimens were obtained from both the Billy Johnson Oral Surgery Clinic and the Oral and Maxillofacial Surgery Clinic at the Carl R. Darnall Army Medical Center, Ft. Hood, TX. Being unerupted, the surface enamel was assured to be unaffected by both normal and cariogenic oral bacterial flora. Patients were consented for the use of extracted teeth for

educational purposes and surgeons and auxiliary staff were trained on proper collection of desired specimens for testing.

After extraction, teeth were stored in 0.2% sodium azide solution prior to sterilization via autoclave. Following sterilization, teeth were rehydrated in sterile 0.9% saline (Baxter Healthcare Corporation) and any residual soft tissue was removed from the root surfaces using a stiff nylon bristled brush. Initial weights of all teeth were recorded to 0.001g using an enclosed analytical balance (Torbal, AGZN100, Scientific Industries, Inc.) (Figure 1). After hand drying with 2x2 inch cotton gauze, all specimens were allowed to dry under room air (23°C) for 24 hours. The root surfaces were then painted with quick-dry acrylic nail polish (Sally Hansen). Root surfaces were submerged in nail polish to the cemento-enamel junction and allowed to dry. A second coat was applied in the same manner to ensure full coverage of cementum. This was done in order to allow only enamel to be exposed to the test beverages and better simulate the area usually in contact with the energy drinks during normal consumption. All teeth were then rehydrated in 0.9% saline while awaiting testing.

#### *Beverages and Properties*

Specimens were submerged in 50 mL of one of the following freshly opened carbonated energy drinks: Red Bull (Red Bull GmbH), Red Bull Sugar Free (Red Bull GmbH), Monster (Monster Beverage Corporation), Monster Absolutely Zero (Monster Beverage Corporation), Rip It Tribute (National Beverage Corporation), Rip It Tribute Sugar Free (National Beverage Corporation).

Prior to experimentation, 3 cans of each energy drink were tested for pH and titratable acidity at room temperature (23°C). Fifty milliliters of each can were poured into a beaker

immediately after opening and pH was measured with a benchtop pH probe (HI 5221, Hanna Instruments). The probe was calibrated to test acidic liquids as directed by the manufacturer. After this, the test beverage was stirred utilizing a magnetic stir plate (3368001, IKA) and bar while a quantity of 1M NaOH (Ricca Chemical Company) was added until a pH of 7 was obtained.

### Procedure

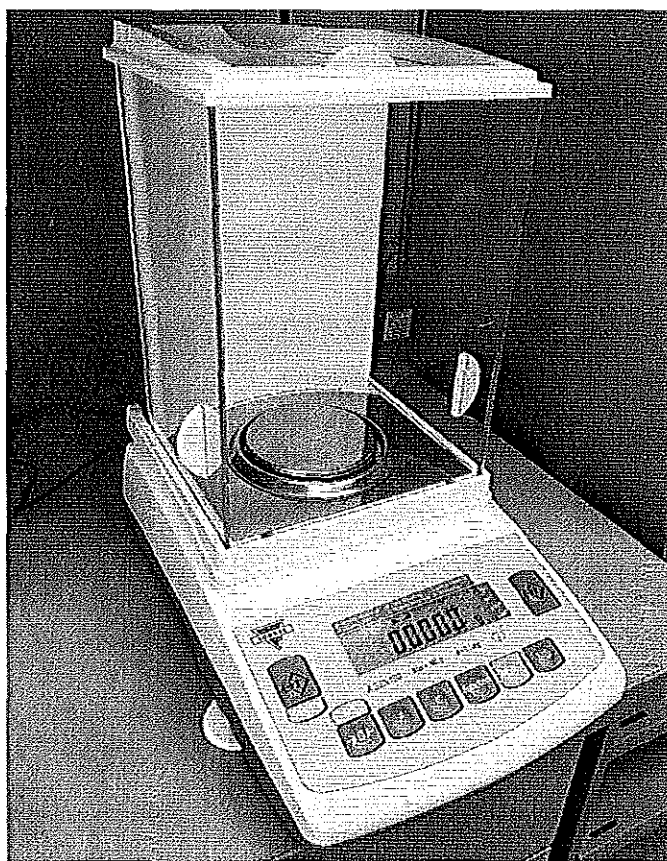
Following initial preparation and rehydration, initial weights were recorded (Figure 2). The 60 molars were then randomly placed into graduated beakers containing 50 mL of one of the test beverages (Figure 3). All testing was completed in the same location over the same time period at room temperature (23°C). Specimens were tested for a period of 5 days with the energy drink being refreshed after each day to retain some of the carbonation. After each day, the specimens were removed and dried with 2x2 inch cotton gauze to remove all visible liquid. The teeth were then dried with oil- and water-free pressurized air for 3 seconds. Each tooth weight was then immediately recorded.

After the final weight was recorded on the fifth day, the nail polish was removed from all teeth with acetone-based nail polish remover (Arcadia Beauty Labs). Care was taken to not disturb or remove the softened enamel. The teeth were then rehydrated in 0.9% saline for 1 hour prior to the final weight being recorded. This was done to compare the initial uncoated tooth weight to the final corresponding weight and determine a final weight lost through erosion of enamel. This could then be expressed as a percentage of total tooth weight.

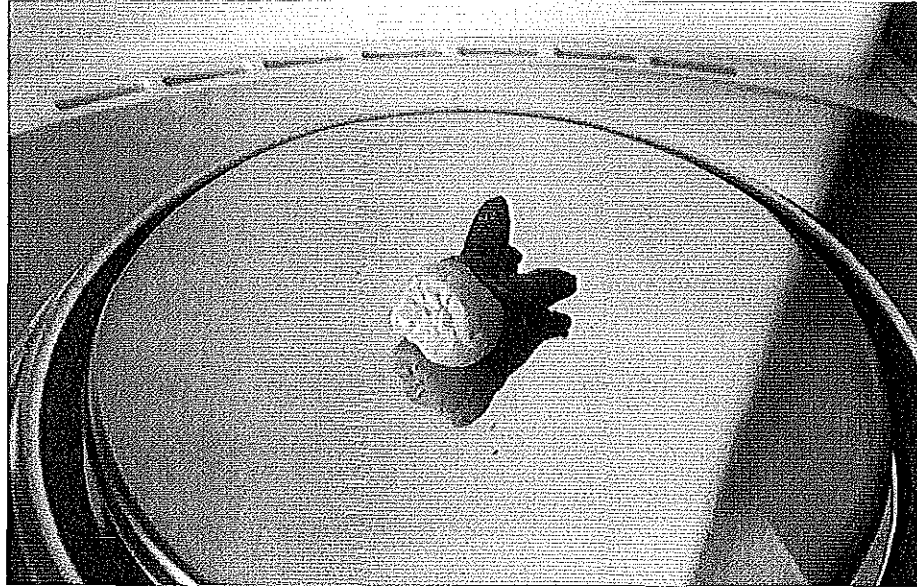
### *Statistical Analyses*

In this study, the independent variables are energy drink or treatment (Red Bull, Red Bull Sugar Free, Monster, Monster Absolutely Zero, Rip It Tribute, Rip It Tribute Sugar Free) and time (0-5 days). The dependent variable is weight loss. The appropriate test is a two-factor analysis of variance (ANOVA) (treatment, time) with repeated measures on one factor (time). Tukey's HSD post-hoc testing will be utilized to determine significant differences between treatments.

Linear regressions will also be completed and modeled to obtain Pearson correlations and evaluate linear dependence of weight loss (enamel dissolution) on titratable acidity and pH.



**Figure 1** The Torbal AGZN100 analytical balance



**Figure 2** Prior to testing, initial weights were recorded of the prepared teeth



**Figure 3** Testing Rip It Tribute Sugar Free (front) and Monster Absolutely Zero

## RESULTS

Red Bull contained the highest amount of titratable acid, requiring an average of 5.05 mL of 1M NaOH prior to reaching a neutral pH of 7. Conversely, Rip It Tribute displayed the lowest amount of titratable acid by requiring only 3.01 mL of base (Table 1). A strong positive linear correlation was observed between titratable acidity and percentage of tooth weight lost.

( $r = 0.9584$ ;  $p = 0.0026$ ) (Figure 4).

Rip It Tribute was shown to be the most acidic energy drink tested, with an average initial pH of 3.12. Monster Absolutely Zero had the highest pH of the tested beverages at 3.40 (Table 2). A scatterplot of energy drink pH and weight loss values is supplied in Figure 6.

Average percent weight loss data for each drink after 5 days is shown below (Table 3; Figure 5). After the results of the two-factor ANOVA were completed, a significant difference between type of energy drink was observed ( $p = 0.0106$ ). Tukey's HSD post-hoc testing showed that Rip It Tribute caused significantly less weight loss than Monster Absolutely Zero, with all other drinks being statistically similar.

ANOVA also showed that there was no difference in the percentage of weight lost when considering pooled data for the regular and diet energy drinks ( $p = 0.6138$ ).

|                   | <u>Trial 1</u> | <u>Trial 2</u> | <u>Trial 3</u> | <u>Average</u> | <u>Std. Dev.</u> |
|-------------------|----------------|----------------|----------------|----------------|------------------|
| Red Bull          | 4.80           | 4.95           | 5.40           | <i>5.05</i>    | 0.312            |
| Red Bull SF       | 4.56           | 4.47           | 4.71           | <i>4.58</i>    | 0.121            |
| Monster           | 5.04           | 4.74           | 4.86           | <i>4.88</i>    | 0.151            |
| Monster AZ        | 4.92           | 4.65           | 5.10           | <i>4.89</i>    | 0.226            |
| Rip It Tribute    | 2.94           | 2.97           | 3.12           | <i>3.01</i>    | 0.096            |
| Rip It Tribute SF | 3.24           | 3.30           | 2.88           | <i>3.14</i>    | 0.227            |

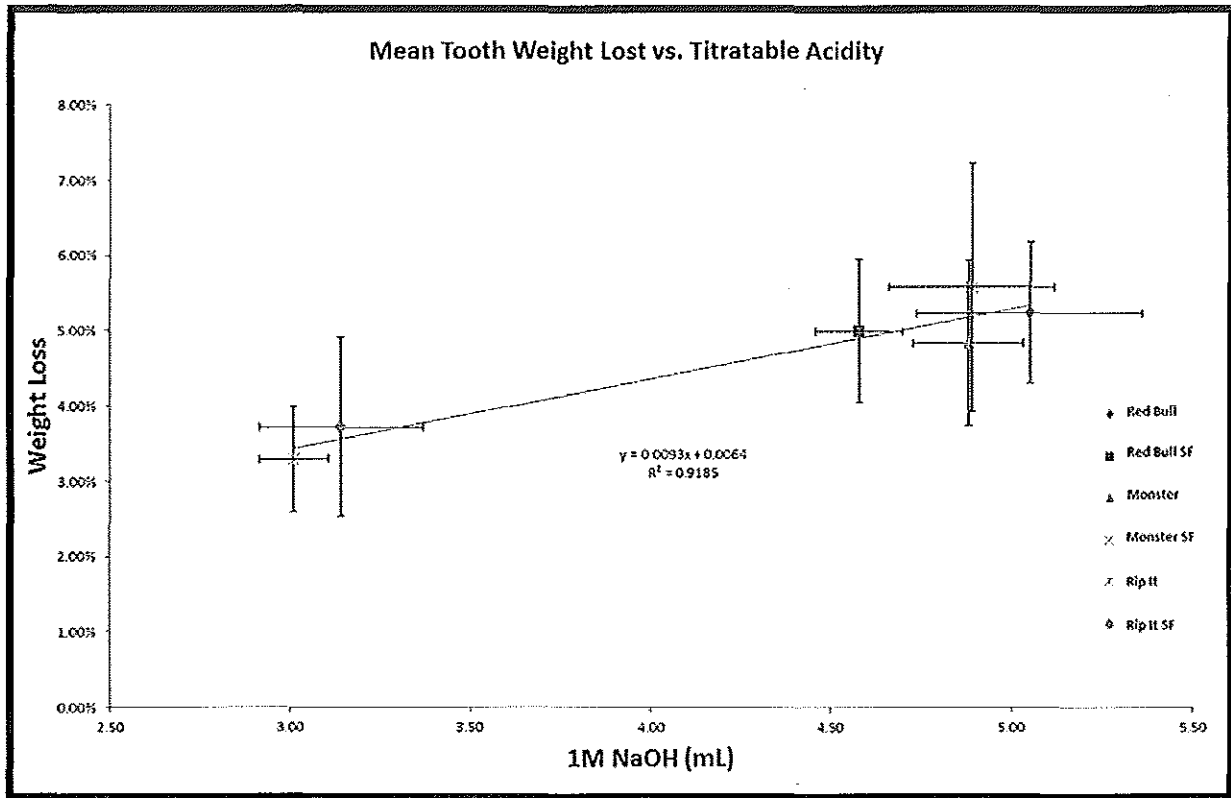
**Table 1** Titratable Acidity (mL)

|                   | <u>Trial 1</u> | <u>Trial 2</u> | <u>Trial 3</u> | <u>Average</u> | <u>Std. Dev.</u> |
|-------------------|----------------|----------------|----------------|----------------|------------------|
| Red Bull          | 3.28           | 3.29           | 3.28           | <i>3.28</i>    | 0.006            |
| Red Bull SF       | 3.30           | 3.31           | 3.30           | <i>3.30</i>    | 0.006            |
| Monster           | 3.30           | 3.34           | 3.33           | <i>3.32</i>    | 0.021            |
| Monster AZ        | 3.37           | 3.42           | 3.40           | <i>3.40</i>    | 0.025            |
| Rip It Tribute    | 3.11           | 3.14           | 3.12           | <i>3.12</i>    | 0.015            |
| Rip It Tribute SF | 3.21           | 3.24           | 3.23           | <i>3.23</i>    | 0.015            |

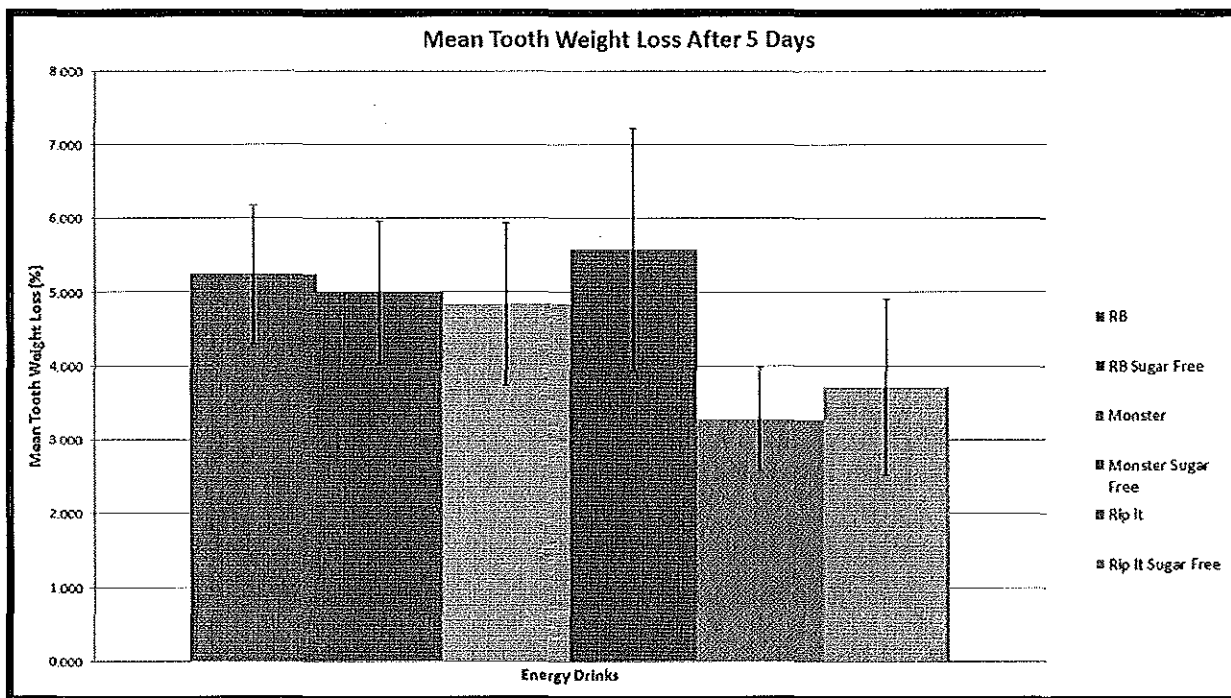
**Table 2.** Initial pH

|                   | <u>Weight Loss</u> | <u>Std. Dev.</u> |
|-------------------|--------------------|------------------|
| Red Bull          | <i>5.2</i>         | 0.9392           |
| Red Bull SF       | <i>5.0</i>         | 0.9540           |
| Monster           | <i>4.8</i>         | 1.0968           |
| Monster AZ        | <i>5.6</i>         | 1.6447           |
| Rip It Tribute    | <i>3.3</i>         | 0.6982           |
| Rip It Tribute SF | <i>3.7</i>         | 1.1926           |

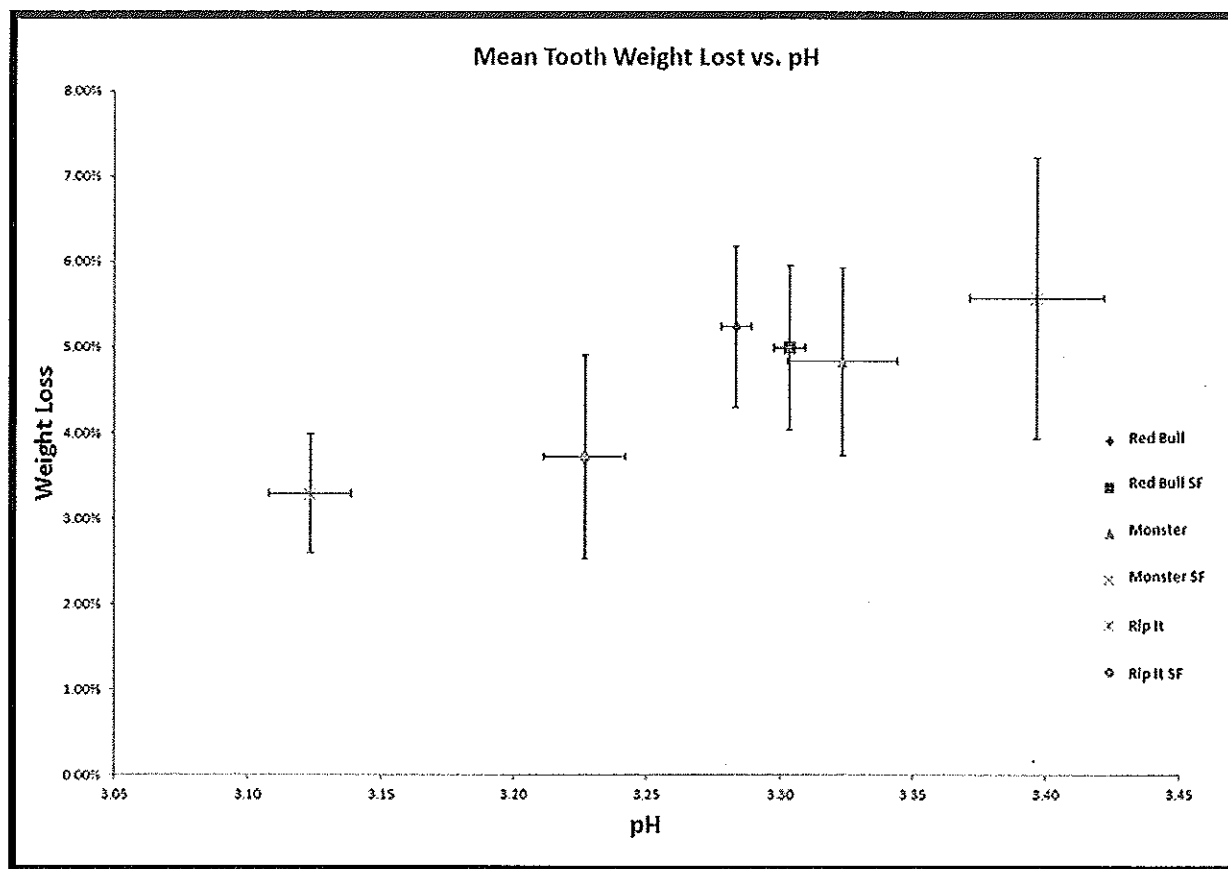
**Table 3.** Mean weight loss after 5 days (%)



**Figure 4**



**Figure 5**



**Figure 6**

## DISCUSSION

### *Experimental Considerations*

The testing period of 5 days was chosen to simulate a real-life exposure time of 3.7 years, which approximates a typical active duty enlistment contract. If it is assumed that a soldier drinks 16 ounces of energy drink per day with an average contact time of 20 seconds before salivary clearance,<sup>25</sup> an average soldier would have 320 seconds of contact per day and 116,800 sec/yr. This study amounted to 432,000 seconds of exposure over the 5 testing days, or 3.7 years of simulated exposure for a typical soldier.

Various issues were encountered during experimentation, though none were assumed to affect the outcome of testing. The acrylic polish did not adhere particularly well to cementum and had a tendency to flake away near the cemento-enamel junction (Figure 7). This seemed to be exacerbated by the continual drying and re-wetting of specimens. A fragment of acrylic was weighed using the analytical balance and did not register, so this minor chipping was viewed to have limited direct effect on measurements. However, it is possible that the resulting exposed cementum and dentin dissolved at an increased rate as compared to enamel, though research suggests that at pH similar to energy drinks (~3.2), the rate of dissolution of enamel and dentin is nearly identical.<sup>26</sup>

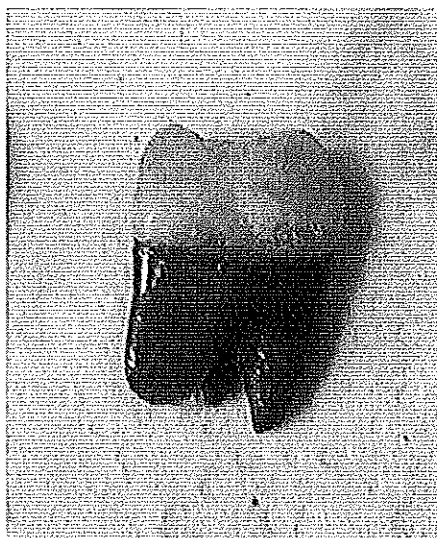
The drinks used in this study contained different concentrations of citric acid as a main ingredient. Indeed, all of the tested beverages listed citric acid as a top four ingredient, with Monster Absolutely Zero and Red Bull Sugar Free listing citric acid as the second ingredient behind carbonated water. Citric acid is a naturally occurring compound commonly added to candy and beverages to impart a tangy flavor. It is also used as a preservative.<sup>27</sup> Research

indicates that citric acid is weakly destructive when compared with phosphoric acid, another common food additive, but that its erosive potential is strongly dependent on temperature.<sup>28</sup>

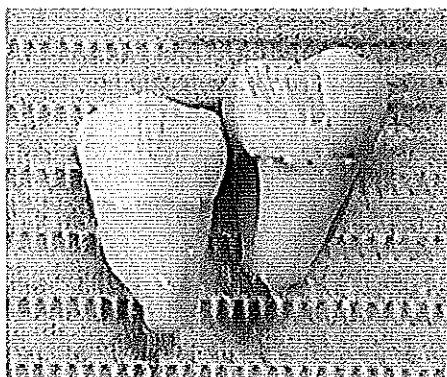
Again, this is intuitive after considering Le Chatelier's principle, as previously mentioned. Changing the conditions of a reaction, in this case the dissolution of enamel, by adding heat, will drive the reaction towards equilibrium faster. In this instance, one would see more tooth weight lost under warmer conditions. In fact, a linear relationship has been found between tooth material lost and temperature of acidic beverages.<sup>29</sup> For this study however, testing was completed at room temperature (23°C) for practical reasons, but also to better simulate real-world conditions. Anecdotally, soldiers have communicated that they frequently consume energy drinks at warmer than usual temperatures in the barracks, in the field and on deployment. In any event, some authors have suggested that temperature may be immaterial when compared to other more relevant factors, such as frequency and duration of consumption,<sup>28</sup> not to mention individual biological factors.

Though this study did not seek to quantify softness of enamel as many studies have previously,<sup>29,30</sup> qualitative observations were able to be made following testing. After 5 days, the enamel of the specimens that were submerged in Monster Absolutely Zero was markedly softer than those that were exposed to Rip It Tribute (Figure 8), and was removed with ease, comparatively. Also, it was observed that all teeth were considerably stained with the color of their respective beverage (Figure 9). Although several publications have described the complicated process of extrinsic staining in the absence of pellicle and its relation to the molecular structure of the outermost layers of enamel,<sup>31,32</sup> this is beyond the scope of this thesis. It should be noted, however, that research indicates that more acidic substances were found to have increased staining potential, regardless of pigment.<sup>33</sup> This may run counter to the commonly

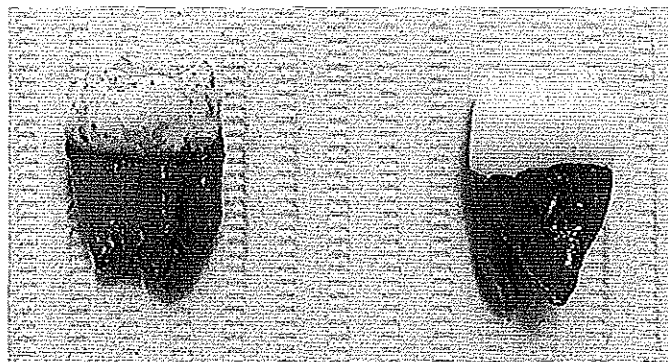
held belief that drinks such as wine and cola are, by the nature of their colors, prone to stain. Again, though staining is a complicated process involving chromogens and their interactions with individual variations of pellicle and enamel, it appears closely related to acidity as well.



**Figure 7.** Chipping of acrylic near CEJ



**Figure 8.** Teeth following light scraping of enamel with explorer after submersion; Rip It Tribute (L), Monster Absolutely Zero (R)



**Figure 9.** Staining was evident following testing. Monster Absolutely Zero (L); Rip It Tribute (R)

### *Saliva*

This study was undertaken *in vitro* due to obvious practical limitations. As mentioned earlier, saliva plays an integral protective role in the potential demineralization of enamel by acidic beverages into its constituent ions calcium and phosphate. Though seemingly logical, a brief review of saliva is required to understand how the concentrations of these ions, either in saliva or in energy drinks, may not be crucial to significant alterations of the critical pH.

Human saliva is a unique biological fluid composed of, among other things, electrolytes, proteins and nitrogenous products. It serves to modulate pH and demineralization and aid in digestion and immune function, among a host of other important roles.<sup>34</sup> As with many salivary components, the concentration of inorganic phosphate is highly dependent on flow rate, and phosphate has an inverse relationship with flow. Therefore, phosphate is viewed as having a minor influence during stimulated flow, such as immediately prior or during food or beverage consumption, but does play a larger role during resting, or unstimulated, flow.

Free, ionized calcium is also important for dental hard tissue maintenance during rest, since it is this calcium that plays a role in the equilibrium of hydroxyapatite and the surrounding

fluid. However, the amount of calcium needed to maintain equilibrium during an acid attack, either through ingestion of an acidic beverage or at the plaque-enamel interface, is many times greater than the concentration present in even stimulated saliva.<sup>35</sup> But, when these two components form calcium phosphate salts, human saliva is known to be saturated, or even supersaturated, and thereby has mechanisms to stabilize the salts and inhibit precipitate formation.<sup>36</sup> It is clear that saliva already contains the maximum concentration of both of these ions, and whatever concentration is contained in a beverage would have very minimal effect on any potential remineralization. The strengths and types of acids in a beverage are of much more consequence than any small amount of mineral contained therein. The influence of minerals is chiefly exerted during homeostatic conditions and not during an acid challenge.

Buffering capacity and flow rate can be seen as much more important salivary roles in regard to protecting tooth structure from erosion. Average flow can cover a large range, from nearly zero during sleep to over 7 mL/min when stimulated.<sup>37</sup> Average unstimulated flow is around 3 mL/min, with anything under 1 mL/min being considered hypofunction.<sup>38</sup> As previously mentioned, tooth mineral components contained in saliva are dependent on flow, but so is bicarbonate buffer. Accordingly, studies have shown a possible link between low salivary flow and dental erosion.<sup>39</sup> Without proper flow, conditions favoring demineralization are allowed to persist for extended periods. Salivary flow is integral to prompt clearance of acidic byproducts, food debris and bacteria, and is vitally important to hard tissue maintenance, as well as continued dilution of acidic conditions. However, it has been observed that an inordinate and physiologically impossible amount of saliva is required to completely offset enamel dissolution during an acid challenge.<sup>40</sup>

The normal pH of saliva is slightly acidic, between 6 and 7.<sup>34</sup> However, it is easier to view saliva in regard to its buffering capacity than as a direct actor, as it serves to modify extremely acidic substances in the mouth, such as dental plaque or dietary acids. As mentioned above, salivary bicarbonate is increased during periods of increased flow. As bicarbonate is the chief means of buffering the acidic oral environment, a person who utilizes energy drinks after a period of exertion or immediately in the morning when dehydrated, will be doubly impacted by both reduced buffering capacity and salivary clearance. Accordingly, soldiers who utilize these beverages as part of their morning routine or as a work out supplement, which are their most commonly reported uses, would be at increased risk for damage to dental hard tissues.

All of this is to make the point that erosion caused by any particular food or beverage is largely dependent on individual variables. Some of these include the beverage's chemical and physical properties. Indeed, research has shown that some carbonated beverages have the ability to be retained in enamel,<sup>25</sup> thereby increasing the drink's acidogenicity. Salivary factors (flow, buffering capacity, pellicle formation, etc.), and behavioral factors such as frequency and duration of exposure are also variable.<sup>41</sup> Yet, in vitro studies of enamel erosion following immersion, such as this one, are still considered predictive of actual erosive potential.<sup>42</sup>

#### *Titrateable Acidity and pH*

At this point, it should be clear that although Le Chatelier's Equilibrium Law posits that energy drinks with more calcium and phosphate should cause less enamel dissolution, due to the aforementioned salivary factors and other individual variables, this has not been observed.<sup>23</sup> Even using pH as the sole predictor of enamel erosion has smartly been abandoned in favor of

also looking at buffering capacity or titratable acidity of individual beverages, which has been observed as significantly associated with enamel dissolution.<sup>43-46</sup>

Importantly, pH indicates the initial hydrogen ion concentration, while titratable acidity gives a measure of all undissociated ions, or the total acid content of a beverage. So, titratable acidity may give a better representation of all possible hydrogen ions that are available for interaction with the enamel surface. This is why, as mentioned, it is seen as a better means of predicting erosive potential. Some current literature still suggests that pH is a more useful predictor of erosive potential because of the perceived limited contact time of the beverage on the dentition and the limited ability for the acids to be “titrated,”<sup>27</sup> yet it stands to reason that residual liquid remains in the mouth and in contact with tooth surfaces after swallowing. Titratable acidity plays a crucial role here in the drink’s ability to resist salivary buffering. Notably, this is only when considering beverages in a similar pH range. Of course, a drink of pH 3 will be more erosive than one with a pH of 6. However, when differences in pH are minimal, as is the case when comparing energy drinks, a more useful value is titratable acidity.

Indeed, the results of the present study show that there was a direct relationship shown between pH and weight loss. Yet, unlike the data for titratable acidity, the author chose not to represent this relationship with a linear correlation coefficient due to the potential for confusion. The data revealed the opposite of what may be commonly assumed; that is, that drinks with a lower pH will contribute to more tooth weight lost through enamel erosion. The results showed that Monster Absolutely Zero (pH 3.40) caused the most weight loss, while Rip It Tribute (pH 3.12) caused the least. Again, this is counterintuitive and goes against commonly supplied information to consumers and patients.

The data from this study showed a strong linear relationship between titratable acidity of a beverage and percent of tooth weight lost. This is in agreement with prior research in this area, which suggests that erosion over time for enamel is linear, while dentin erosion is a non-linear process.<sup>26, 47</sup> This is because as the dissolution front continues towards the center of the tooth through dentin, a layer of demineralized collagenous matrix is left behind. This impedes the exchange of ions, which can now only occur through pores in the matrix, and dissolution is slowed. This is not the case in enamel, which is known to be 96% hydroxyapatite, while dentin is only around 70% inorganic material. The period of submersion utilized in the current study was adequate to model the linear nature of the dissolution that occurred.

While *in vitro* experimentation is no substitute for clinical investigations, important information can be gathered in order to provide a basis for further research. Though effects tend to be exaggerated from these sorts of studies, this investigation has shown that energy drinks commonly utilized by military personnel have a distinct capacity for tooth erosion.

## CONCLUSIONS

This study failed to show a significant difference between the tested regular and diet energy drinks commonly utilized in the military. Significantly more weight was lost though enamel dissolution with Monster Absolutely Zero when compared to Rip It Tribute. The null hypothesis that there would be no difference in the percentage of enamel lost between the energy drinks was rejected. However, the null hypothesis stating that there would be no difference in the percentage of enamel lost between the pooled regular and diet energy drinks was accepted. Also, because a strong, positive linear correlation was observed between titratable acidity and weight loss through enamel erosion, the null hypothesis that there would be no correlation was rejected as well.

This study also reinforced an abundance of current literature that suggests that titratable acidity is a more reliable predictor of erosive potential than pH, displaying a direct linear relationship. Information obtained from this study will further enable dentists and other oral health care providers to counsel patients appropriately regarding nutritional and lifestyle choices, particularly those in the Armed Forces. While this study clearly displays that none of the tested beverages displayed positive or even neutral effects on tooth enamel, it appears as though there are real differences between their erosive potentials.

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