Association Between Ketogenic, Vegan, and Paleo Diets and Dental Biofilm Formation In Vitro

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ABSTRACT

The purpose of this study was to investigate the relationship between three lifestyle diets (ketogenic, vegan, and paleo) and dental biofilm formation. The study was set up in glass test tubes containing hydroxyapatite discs, an artificial saliva and 5 percent human saliva combination (Streptococcus mutans, and Lactobacillus acidophilus) Food items from three meals (breakfast, lunch, and dinner) were selected for each diet, blended to form a consistent mixture, and added to 25 percent final volume in the test tubes. Biofilms were grown for 48, 60, and 72 hours. Biofilm density was measured quantitatively using a crystal violet assay while titer was determined using a vortex-sonication-vortex method, followed by plating. Results showed that the ketogenic-based culture formed a biofilm consisting mainly of S. mutans while the vegan-based culture formed a biofilm comprised mainly of L. acidophilus. The paleo-based culture formed a biofilm with a mixture of both S. mutans and L. acidophilus. The abundance of L. acidophilus grown with the vegan diet suggests this diet is most likely to contribute to the formation of dental caries.

INTRODUCTION

Many people go on lifestyle diets typically to lose weight or to improve their systemic health. However, very few consider how that specific diet could be affecting their oral health. Consuming foods that contain high sugar or starch content put teeth at risk for developing tooth decay. When left untreated, dental caries can cause pain and infection, and ultimately lead to tooth loss (1). With about 92 percent of Americans ages 20 to 64 having had dental cavities in their permanent teeth at some point in their life and 36 percent of Americans admitting to following a specific diet or eating pattern, it is important to understand the correlation between diet and tooth decay (2).

METHODS

- For each diet, complete meals for breakfast, lunch, and dinner were selected based on the diet’s guidelines.
- Ingredients for each meal were blended to form a consistent mixture.
- In a glass test tube: hydroxyapatite disc, 1425 µL artificial saliva, with sucrose, and trace amounts of ascorbic acid and/or yeast extract, 75 µL filtered human saliva, 500 µL diet mixture, and 20 µL each of S. mutans and L. acidophilus (approximately 10^9 colony forming units of each) (4).
- Test tubes were incubated at 37°C for 24 hours, then incubated in a 37-degree CO_2 incubator for an additional 24, 36, or 48 hours.
- Discs were rinsed with water and submerged in 0.1% crystal violet for staining for 10 minutes.
- Discs were rinsed with water, and placed in test tubes containing 5 mL of acetic acid for 10 more minutes to solubilize the stain.
- The solution was added to a Wasserman tube and the absorbance was read at 595 nm in a spectrophotometer (5).
- Discs were rinsed with water and placed in test tubes of 5 mL of sterile saline.
- Test tubes were vortexed, sonicated, and vortexed, for 1, 3, and 1 minute, respectively.
- Solutions were diluted twice, plated in triplicate, and incubated for 48 hours in CO_2 incubator. Then the number of colonies was counted (6).

RESULTS

- **Ketogenic Diet**: The titer of S. mutans remained high, decreasing only after 72 hours. The titer of L. acidophilus remained low, with the culture’s lowest pH reaching 5.0. The majority of the diet was fat (54%), followed by protein (37%). Carbohydrates were very low in this diet (9%).
- **Vegan Diet**: The titer of L. acidophilus was significantly elevated, while the titer of S. mutans was considerably lower, and the pH, which reached a minimum of 3.0, became too acidic to support S. mutans growth. The diet contained mostly carbohydrates (54%), with 36% fat and 10% protein.
- **Paleo Diet**: The titer of L. acidophilus was relatively high and the titer of S. mutans was moderately lower with a minimum pH of 3.5. The diet had a more even distribution of carbohydrates, proteins, and fats with percentages being 21, 46, and 33 percent, respectively.
- **Control**: The titer of S. mutans was initially high because of the inoculum, but decreased rapidly without nutrients to support growth. Very few L. acidophilus were counted. The pH remained constant at 6.8, reflecting the inability of the bacteria to grow or carry out fermentation.

DISCUSSION

- The high amount of carbohydrates in the vegan diet led to increased fermentation and a dramatic decrease in pH. The only species able to survive in such an acidic environment was L. acidophilus, explaining why they grew extensively.
- The pH range tolerated of S. mutans is demonstrated by the noticeable drop in the number of S. mutans in the paleo diet once the pH reached 3.5.
- The control reveals the nutrients in the diet contributed to growth of the organisms because the control titer decreases greatly with time.

CONCLUSION

- The vegan diet may contribute to increased risks of tooth decay.
- Dentists can educate their patients about the risks of high carbohydrate diets, and suggest regular brushing and flossing after eating to prevent the colonization of cariogenic bacteria.

REFERENCES

2. Dental Caries (Tooth Decay) in Adults (Age 20 to 64), 2018. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6306989/

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