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Changes in the oral ecosystem induced by the use of 8% arginine toothpaste

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ABSTRACT

Objective: Bacterial metabolism of arginine in the oral cavity has a pH-raising and thus, potential anti-caries effect. However, the influence of arginine on the oral microbial ecosystem remains largely unresolved.
Design: In this pilot study, nine healthy individuals used toothpaste containing 8% arginine for eight weeks. Saliva was collected to determine arginolytic potential and sucrose metabolic activity at the Baseline, Week 4, Week 8 and after a two weeks Wash-out period. To follow the effects on microbial ecology, 16S rDNA sequencing on saliva and plaque samples at Baseline and Week 8 and metagenome sequencing on selected saliva samples of the same time-points was performed.
Results: During the study period, the arginolytic potential of saliva increased, while the sucrose metabolism in saliva decreased. These effects were reversed during the Wash-out period. Although a few operational taxonomic units (OTUs) in plaque changed in abundance during the study period, there was no real shift in the plaque microbiome. In the saliva microbiome there was a significant compositional shift, specifically the genus *Veillonella* had increased significantly in abundance at Week 8.
Conclusion: Indeed, the presence of arginine in toothpaste affects the arginolytic capacity of saliva and reduces its sucrose metabolic activity. Additionally, it leads to a shift in the salivary microbiome composition towards a healthy ecology from a caries point of view. Therefore, arginine can be regarded as a genuine oral prebiotic.

1. Introduction

Caries is an old and still common problem among humans (Grine, Gwinnett, & Oaks, 1990; Listl, Galloway, Mossey, & Marcenes, 2015; WHO, 2012). Severe caries can lead to excruciating pain and tooth loss, which in turn can lead to an inability to chew or even eat. Logically, treatment and prevention of caries have been a priority of the dental community for quite some decades. Therefore it is now recognized that the lowered pH, as a result of the acids formed by bacterial fermentation of sugars, leads to demineralization of the enamel (Fejerskov, 1997; Peterson, Snesrud, Schork, & Bretz, 2011; ten Cate, 2013). In contrast, the bacterial metabolism of arginine has a pH-raising effect (Huang, Exterkate, & ten Cate, 2012).

The human oral cavity is an ecosystem, and like all ecosystems, the oral ecosystem is a combination of many different components (e.g. bacteria, fungi, metabolic compounds, host cells, salivary constituents) and in one way or another, this system retains a balance. The introduction of a specific compound might shift the balance of an ecosystem towards a different microbiological composition and functional potential. For instance, the frequent intake of sucrose facilitates a favorable environment for fermentative bacteria, leading to a cariogenic environment (Bradshaw & Lynch, 2013; Raner et al., 2014). In contrast, the bacterial metabolism of arginine elevates the pH in the oral cavity. The pH-raising effect of arginine is, amongst others, facilitated by the bacterial arginine deiminase system (ADS). This ADS metabolizes arginine, forming ammonia in the process and the subsequent protonation of ammonia into ammonium causes the pH to rise

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(Burne & Marquis, 2000; Casiano-Colon & Marquis, 1988; Marquis, Bender, Murray, & Wong, 1987).

Compounds that have a positive effect on human health through the selective stimulation of growth or activity of specific microbes are called prebiotics (Mugambi, Young, & Blaauw, 2014). Some research has been done on the effect of prebiotics on the ecosystems of the human gastro-intestinal tract and skin (Nole, Yim, & Keri, 2014; Orel & Kamhi Trop, 2014; Sanders et al., 2014), while knowledge on the use of prebiotics in the oral cavity is very limited.

Arginine naturally occurs in a variety of foods and has recently been added to oral care products, for its pH-raising effect and anti-caries potential (Burne & Marquis, 2000; Kraivaphan et al., 2013; Nascimento et al., 2013; Sharif, Iram, & Brunton, 2013; Wu et al., 2009). Yet, how arginine affects the total oral ecosystem remains largely unknown.

Therefore, the aim was to enhance knowledge on the effect of arginine on the human oral ecosystem and to shed light on the question if this potential prebiotic is active on a functional as well as compositional level.

2. Materials & methods

2.1. Pilot study design and sampling

Nine healthy volunteers with no overt caries, who had given written informed consent, participated in this pilot study. The study was approved by the review board of the local Medical Ethical Committee (VU University Medical Center, reference number 2010/147).

In the two weeks prior to the first of a total of four sampling moments (Fig. 1), the participants used a control toothpaste (1.45 mg g^{-1} fluoride, Prodent, Sara Lee Household & Bodycare, Exton, PA, USA). Sampling moments consisted of two consecutive days. Samples were taken on each day, and the data of these duplicate samples were averaged. After the first samples were taken (Baseline), the subjects were instructed to brush their teeth using toothpaste containing 8% arginine (1.45 mg g^{-1} fluoride, Colgate-Palmolive, New York, NY, USA). Subsequent samples were taken four weeks (Week 4) and eight weeks (Week 8) after the Baseline. After Week 8, use of the 8% arginine toothpaste was stopped and the control toothpaste was reintroduced. The last

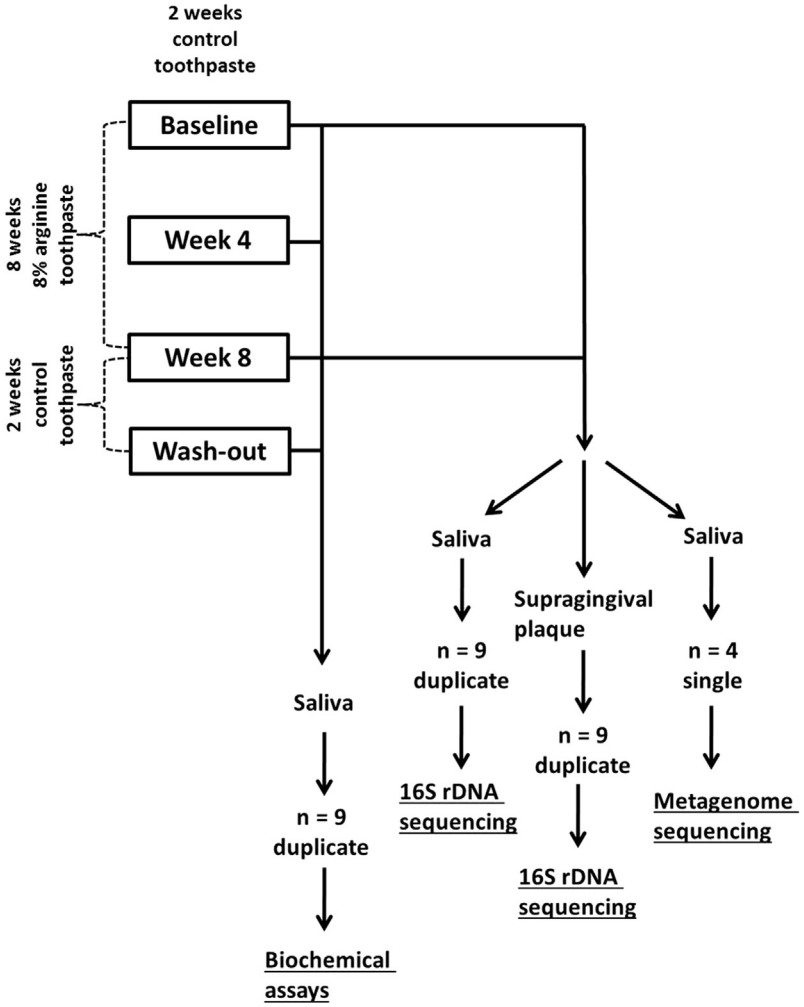

Fig. 1. Sampling scheme of the study. The n represents the number of subjects contributing to the samples from different visits. Duplicate indicates that the samples were taken on two consecutive days. The data from these samples were averaged for subsequent statistical analysis. Single indicates that only one of the samples taken at the two consecutive days was used for further analysis. Saliva samples for the biochemical assays were taken before the participants started using the 8% arginine toothpaste (Baseline), four weeks (Week 4) and eight weeks (Week 8) after the Baseline, and after a two week wash-out period that followed the finish of the 8% arginine toothpaste use. The saliva and plaque samples to be used for DNA analysis were taken at the Baseline visit and Week 8.