

# Interproximal *in situ* Plaque pH after a Sugar Challenge in Relation to Caries in Adults before and after Short-Term Use of 1.5% Arginine Toothpaste

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## Abstract

**Background:** The evidence of the anti-caries effect of arginine toothpaste has been questioned and randomised trials independent of commercial interests have been required.

**Aim:** To examine, in relation to caries, the pH response in interproximal plaque *in situ* to an acidic challenge and the pH and buffer capacity of stimulated saliva following a six-week period of using a toothpaste containing 1.5% arginine/1450 ppm fluoride or no arginine/1450 ppm fluoride.

**Methods:** Thirty-three healthy adults with no visible caries lesions or  $\geq 1$  current manifest lesion participated in the study. Acid plaque responses were examined by interproximal pH-measurements using pH-indicator strips. Two weeks after professional cleaning, pH-measurements were performed before and up to 15-min after a 1-min rinse with 10 ml of a 10% sucrose solution. This procedure was repeated after a 6-week test period of using randomly selected toothpaste with arginine and fluoride or with fluoride alone. The stimulated salivary-pH and buffer capacity were also measured.

**Results:** In the caries group, the use of toothpaste with arginine and fluoride resulted in increased pH-values in the four sites tested. No statistically significant increased plaque pH-values were found in the no-caries group. In addition, saliva buffer capacity and pH was increased in the caries group.

**Conclusion:** Using fluoride toothpaste with 1.5% arginine affects the oral environment positively in adults with caries, in the form of increased supragingival plaque pH and increased salivary pH and buffer capacity.

**Keywords:** Acidic challenge; Arginine; Caries; Plaque pH; Saliva pH

**Abbreviations:** AA-After Using fluoride Toothpaste with 1.5 % Arginine; AB-After Using fluoride Toothpaste; AUC-Area Under the Curve (pH units multiple time); BA-Before Using Fluoride Toothpaste with 1.5 % Arginine; BB-Before Using Fluoride Toothpaste; DMFS-Decayed, Missed and Filled Tooth Surface; PTC-Professional Tooth Cleaning PTC

## Background

Despite a global decline in dental caries the end of the last century it still remains a significant public health concern [1]. Dental caries is considered a multifactorial disease with a complex aetiology [2]. Dynamic interactions between the microbial community of the dental biofilm, and e.g. dietary components, oral hygiene, and genetic and environmental factors will affect the biofilm-pH [3]. A low pH biofilm environment is related to the activity of acidogenic/aciduric microorganisms, which can lead to development of a caries lesion. The alkaligenic potential of the dental biofilm can, however, counteract acid production and the demineralization process, and thereby control and prevent dental caries [4]. Clinical studies have revealed a positive correlation between alkali production and caries resistance in different age groups [5,6].

It has been reported that metabolic substrates in dental plaque ecology i.e. the amino acid arginine and urea may modulate the alkali-generating potential of the dental biofilm, and it was suggested that arginine could be applied to dental plaque in complement with fluoride for caries management [7,8]. In a study on the combination of arginine and fluoride on the oral bacteria *in vitro* it was found that the presence of arginine provided a substrate source for arginolytic bacteria that could increase the alkali production and resist the growth of acidogenic and aciduric species [9]. There are also several clinical studies reporting a superior anti-caries effect of toothpaste containing arginine together with fluoride compared to toothpaste with fluoride only [10] whereas an *in situ* study did not show an additional anti-caries effect from arginine [11]. Furthermore, it was recently reported that the use of a fluoride toothpaste containing

zinc-oxide together with arginine resulted in reduced bacteria on both hard and soft oral surfaces [12]. It was suggested that such toothpaste could be used for whole mouth anti-bacterial protection resulting in improved plaque control and protection against dental diseases [13]. However, systematic reviews acknowledged that the evidence of the anti-caries effect of arginine toothpaste is inadequate [14,15]. Few studies only fulfilled the inclusion criteria, and these were supported by the manufacturer, which increased the risk of bias. It was concluded that rigorous and high-qualitative trials with less or no commercial interests were required.

In this study, which was conducted with no commercial interests, it was hypothesized that, compared to fluoride toothpaste without arginine, a toothpaste containing arginine alongside with fluoride would increase the alkali activity and pH in the supragingival dental plaque as well as the salivary buffer capacity and pH. The primary aim of the study was to investigate *in situ* the pH response to an acidic challenge in interproximal supragingival plaque following a six-week period of using toothpaste with 1.5% arginine and 1450 ppm fluoride or with 1450 ppm fluoride alone, in relation to caries status. Another aim was to compare the effect on saliva pH and buffer capacity before and after the test periods.

## Methods

### Study groups

Healthy adults were randomly selected among patients, staff and students from the Middlesex University in London, UK. Verbal and written information of the study was given to the invited participants and a detailed email about the study design and project protocol was sent to those interested in participating. A dental surgeon (HH) performed a dental examination to determine the individual caries index DMFS. A no-caries group was defined as having no visible caries lesions with DMFS (Decayed, Missed and Filled Tooth Surface, manifest caries)=0, and a caries group with DMFS  $\geq 1$ . The inclusion criteria were healthy adult  $\geq 18$  years, normal stimulated salivary secretion rate ( $\geq 1.0$  ml/min), no smoking and regular attendance to a dental clinic. The exclusion criteria were not suffering from systemic disease,  $<18$  years of age, had received antibiotics within the last 3 months prior to the study, smoker and irregular attendance to a dental practice. A total of 33 individuals (21 females and 12 males) with a mean age of  $25 \pm 10$  yrs (range 19-58 yrs) who met the inclusion and exclusion criteria were included in the study. 14 of them had no visible caries (DMFS=0) and 19 had manifest caries (DMFS= $3.4 \pm 2.7$ ). The study was approved by the Ethics Committee at the Middlesex University (NSESC 1570). Written informed consent was obtained from the participants prior to the start of the study.

### Study design

The study was designed as a double-blinded (from examiner and participant's perspective), randomized, controlled, two-leg crossover trial. The total duration of the study was 16 weeks, including two washout periods (two weeks each) and two test periods (six weeks each). All participants came to the laboratory (Hatchfort Lab, Natural Science, Middlesex University, London, UK) five times (Figure 1). At the first visit, medical and dental history was obtained, detailed information about the study was given, a dental/oral examination was taken, a saliva secretion test was performed, and the consent form was signed. At the end of each visit Professional Tooth Cleaning (PTC) was performed by the examiner (HH) using prophylactic paste (Classic oil-based prophylactic paste, UnoDent, Essex, UK).

At the end of the first and third visits, all participants received non-arginine, fluoride toothpaste to be used during the 2-week wash-out periods. At the end of the second visit proceeding the first 6-week test period, the participants were randomly assigned to continue using this toothpaste or to use a fluoride toothpaste containing also arginine. During the second 6-week test period, the non-arginine toothpaste was used by the former arginine users and *vice versa*.

During the whole study period, the participants were asked to keep their regular dietary and oral hygiene habits as well as to avoid using any mouthwash product. Three days prior to visits II, III, IV and V, the participants received a text message to remind them about their upcoming visit and to refrain from any oral hygiene procedure during the last two days before the visit.

Another text message was sent to the participants on the same day as the visit reminding them to avoid eating and drinking 2 hours prior to the visit. All visits were performed in the morning.

At visits II, III, IV and V interproximal plaque pH was measured at four sites before and up to 15 minutes after a 1-min rinsing with 10 ml of a 10% sucrose solution. Finally, a stimulated saliva sample was obtained.

### Toothpaste regimen

Two commercial toothpastes, which according to the manufacturer (Colgate-Palmolive®, New York, USA,) were designed especially for caries protection, were used. One contained 1.5% arginine and 1450 ppm fluoride (Colgate Maximum Cavity Protection plus Sugar Acid Neutraliser™-toothpaste A) and one fluoride toothpaste with 1450 ppm fluoride without arginine (Colgate Cavity Protection®, Colgate-Palmolive®, New York, USA-toothpaste B). The composition of the toothpastes was not identical. Toothpaste A contained 1450 ppm Na-monofluorophosphate whereas toothpaste B contained 450 ppm NaF and 1000 ppm Na-monofluorophosphate. Toothpaste A further contained calcium carbonate and sodium bicarbonate for abrasive effect, and Na-hydroxide as product pH regulator. Toothpaste B contained dicalcium phosphate dihydrate as abrasive.

The non-arginine fluoride toothpaste B was also used during the wash-out periods. All participants received a standard manual toothbrush (Colgate-Palmolive®, New York, USA), which they were instructed to use by adding 2 cm toothpaste and to brush twice daily for two minutes in the morning after breakfast and in the evening before bedtime, during the complete study period. They were further told to refrain from eating or drinking two hours after tooth brushing and not to rinse with water directly after brushing.

### Interproximal supragingival plaque pH

Interproximal, supragingival plaque pH was measured using the pH Strip method as previously described [16]. Measurements were performed at four sites: between the upper left lateral incisor and canine (site 22/23), between the upper left second premolar and first molar (site 25/26), between the lower canine and right lateral incisor (43/42), and between the lower first molar and right second premolar (site 46/45). pH was measured before (baseline, 0 min) and at 2, 5, 10, and 15 min after a 1-min rinse with 10% sucrose solution. A strip (Spezialindikator, Merck, Darmstadt, Germany) measuring pH 4.0-7.0 was inserted into the interproximal sites for 10 s before the pH was determined from a scale according to the manufacturer.

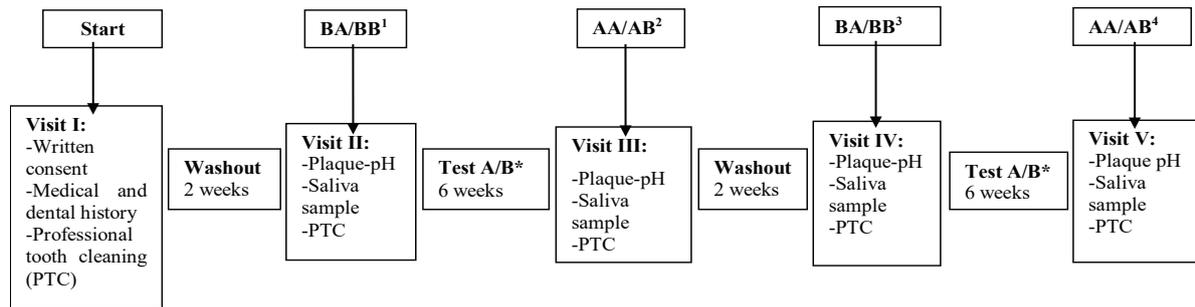


Figure 1: Study design.

**Samplings and pH measurements performed:**

- <sup>1</sup>Before start of the first test period brushing with toothpaste A (BA) or toothpaste B (BB)
- <sup>2</sup>After the 6-weeks test period brushing with toothpaste A (AA) or toothpaste B (AB) and before the 2-weeks washout period using a tooth paste without arginine
- <sup>3</sup>Before the second six-week test period brushing with toothpaste A (BA) or toothpaste B (BB)
- <sup>4</sup>After the second 6-weeks test period brushing with toothpaste A (AA) or toothpaste B (AB)
- \*Randomly selected toothpaste A or B (A=toothpaste with 1.5% arginine and 1450 ppm fluoride B=toothpaste with 1450 ppm fluoride).

**Stimulated whole saliva**

Stimulated saliva was collected by chewing on a piece of paraffin for 5 min and continuously spitting the obtained saliva into an ice-chilled tube. The stimulated salivary flow rate was determined and the pH and buffer capacity was measured by using a chair-side saliva kit (Saliva-Check, GC, Japan) as previously described [17].

**Statistical analyses**

Statistical descriptive analyses were performed using Minitab ± Statistics (version 16.2.2, Windows software, 2010). The mean value (± SD) of the interproximal plaque pH at the four sites was calculated for each time point and individual. The maximum pH fall, final pH reached and the minimum pH after the rinse were also calculated. Changes in plaque pH during 15 min were determined from the area of the curve below the critical pH of enamel (pH 5.7; AUC5.7) and of dentin (pH 6.2; AUC 6.2) using a computer based program [18]. Student's two sample, paired t-test was used to analyse the significance of differences for the individual interproximal plaque pH-values and variables, stimulated whole saliva secretion, pH and buffer capacity before and after the 6-week test periods. Differences in these variables between the groups were analysed using student's two-sample, unpaired t-test p<0.05 was considered statistically significant.

**Results**

**Supragingival plaque pH before and after using arginine toothpaste**

The pH varied between sites and followed an overall pattern with statistically significant higher values in the lower front (site 43/42) and molar regions (site 46/45) compared with the upper front (site 22/23) (Figure 2, Table 1). Compared with before the test period of using arginine toothpaste, the sugar challenge resulted in statistically significant higher pH values after arginine use in the caries group only (Table 1). The baseline pH before the challenge (time point 0) was in this group significantly increased at the molar sites 25/26 and 46/45 after using arginine toothpaste (Table 1). After the test period, minimum pH was significantly increased at site 22/23 and the final pH was numerically increased at all sites and significantly at site 25/26.

In accordance with these findings, using arginine toothpaste resulted in significantly decreased mean AUC-values at all sites apart from 46/45 (Table 1). The areas under the curve below pH 5.7 and 6.2 were generally smaller in the lower jaw regions compared with the upper front. For the no-caries group numerical, but not statistically significant, differences were seen for some of the supragingival plaque pH variables when comparing pH before and after 6-week use of arginine toothpaste (Table 1).

**Supragingival plaque pH before and after using non-arginine fluoride toothpaste**

There were no statistically significant differences within the no-caries and the caries group before and after 6-week use of non-arginine fluoride toothpaste, for any of the pH-variables at any of the sites tested (data not shown).

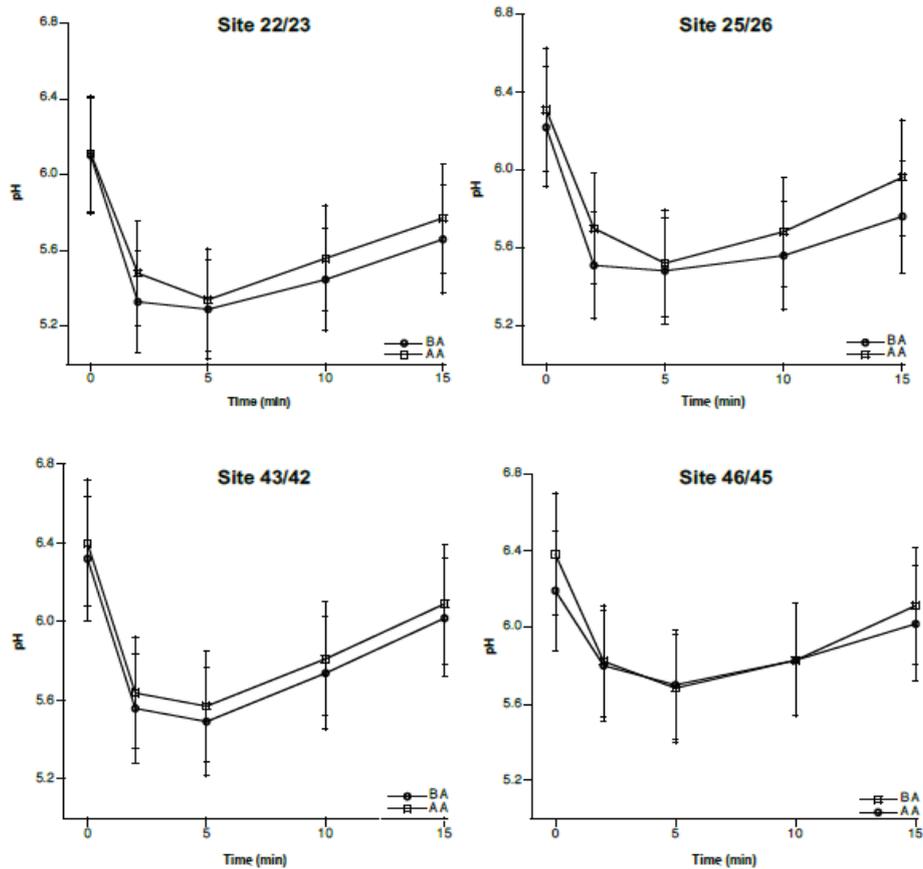
**Stimulated whole saliva**

Using arginine toothpaste resulted in significantly increased stimulated saliva buffer capacity and pH in the caries group (Table 2). There was no significant difference in the secretion rate, buffer capacity and pH between the groups neither before nor after the usage of toothpaste with or without arginine, respectively.

**Discussion**

This randomised, controlled, cross-over study focused on the effect of 6-week use of fluoride toothpaste with and without arginine, on interproximal supragingival plaque pH *in situ* in individuals with and without manifest caries respectively. Significant effects of using arginine toothpaste was only seen for the individuals with caries where the interproximal supragingival plaque pH as well as the stimulated saliva pH and buffer capacity were increased.

The compositions of the toothpastes used were not identical. The type of fluoride in the arginine and non-arginine toothpaste differed and the arginine toothpaste also contained other components e.g pH-regulating Na-hydroxide which might have contributed to increased plaque pH. Thus a limitation of the study was the possible interaction of the different components, in small proportions, that



**Figure 2:** Interproximal, supragingival plaque pH *in situ* in sites 22/23, 25/26, 43/42 and 46/45, before and up to 15 min after a sugar rinse in caries individuals. Measurements performed before (BA) and after (AA) a 6-weeks use of the arginine toothpaste.

could participate in the modification of the pH. Such differences may, however, not have affected the outcome of this cross-over study with randomly distributed toothpastes. Also, it was reported that type of fluoride in the toothpaste used did not affect the caries increment [19].

Previous studies have shown lower plaque pH *in situ* for individuals with caries both before and after a sugar rinse in comparison with caries-free individuals [20-22]. The present findings of a less pronounced pH fall in the dental plaque in caries individuals after arginine use support previous studies reporting arginine to have an alkalogenic effect that could be used as an anti-cariogenic supplement for caries patients [23-25]. A reported potential higher alkali forming activity in the dental plaque of caries-free individuals than in individuals with caries [5,26] may be the reason why no significant increased pH was found for the no-caries group in the present study.

An importance of site-specific pH and urease activity for the individual caries risk has been suggested [26]. In this study, pH was generally higher and the areas under the curve generally smaller in the front and molar regions of the lower jaw compared with the upper front region. This finding is in line with previous reports of higher urease activity of the mandibular incisors and a lower caries prevalence in the lower jaw [27-29]. However, using arginine toothpaste resulted in significantly decreased areas under the curve in both the front and molar region of the upper jaw in the caries group. This could be beneficial for caries prevention as the urease activity is lower and the caries prevalence higher in the maxilla [27,29].

Furthermore, in the caries group the pH and buffer capacity of stimulated whole saliva were increased to the levels seen for the no-caries group after the 6-week use of arginine toothpaste. These findings are in accordance with a previous reported pilot study showing increased arginolytic activity in the stimulated saliva microbiome when using 8% arginine toothpaste [30]. They differ, however, from a study reporting no increased arginolytic activity in un-stimulated saliva of individuals with and without caries after using fluoride-free 1.5% arginine toothpaste [4]. Further studies are necessary to find out possible effects of arginine on the saliva microbiome.

## Conclusions

The outcome of this study on adult individuals support the hypothesis that the usage of a toothpaste containing 1.5% arginine together with 1450 ppm fluoride could improve alkali formation and pH in the dental plaque as well as the salivary pH and buffer capacity, compared with using a toothpaste with fluoride only. However, this finding was true only for caries individuals. No statistically significant, additional effects were seen for the no-caries individuals. Studies over longer periods may be necessary to reveal possible effects on plaque pH in adult individuals with no caries experience.

## Acknowledgement

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**Table 1:** The pH in interproximal plaque up to 15 min after a 10% sucrose rinse in no-carries and carries individuals before and after a 6-weeks period using arginine toothpaste. p-values for comparisons before (BA) and after (AA) the test period within the respective groups.

	No carries (n=14)			Carries (n=19)		
	BA	AA	p-value	BA	AA	p-value
<b>Site 22/23</b>						
Baseline pH	6.09 ± 0.18	6.15 ± 0.24	0.25	6.10 ± 0.14	6.11 ± 0.18	0.91
Max pH drop	0.89 ± 0.29	0.91 ± 0.33	0.82	0.96 ± 0.37	0.76 ± 0.49	0.01
Min pH	5.20 ± 0.36	5.24 ± 0.42	0.73	5.17 ± 0.43	5.32 ± 0.50	0.03
Final pH	5.77 ± 0.34	5.71 ± 0.34	0.62	5.66 ± 0.39	5.77 ± 0.37	0.15
AUC <sub>5.7</sub> <sup>1</sup>	4.09 ± 3.76	4.39 ± 3.39	0.74	4.64 ± 4.93	2.74 ± 2.81	0.05
AUC <sub>6.2</sub> <sup>1</sup>	10.43 ± 4.92	10.33 ± 5.44	0.94	10.88 ± 6.02	8.35 ± 4.71	0.04
<b>Site 25/26</b>						
Baseline pH	6.29 ± 0.17	6.26 ± 0.33	0.49	6.22 ± 0.22	6.31 ± 0.21	0.04
Max pH drop	1.00 ± 0.90	0.91 ± 0.41	0.95	0.87 ± 0.33	0.80 ± 0.30	0.39
Min pH	5.33 ± 0.20	5.35 ± 0.41	0.75	5.37 ± 0.31	5.48 ± 0.34	0.11
Final pH	5.92 ± 0.31	5.82 ± 0.32	0.19	5.76 ± 0.33	5.96 ± 0.33	0.02
AUC <sub>5.7</sub> <sup>1</sup>	2.99 ± 3.53	3.20 ± 3.24	0.76	3.36 ± 3.29	1.72 ± 2.28	0.06
AUC <sub>6.2</sub> <sup>1</sup>	7.57 ± 4.13	8.89 ± 4.92	0.24	8.70 ± 4.32	6.88 ± 4.20	0.05
<b>Site 43/42</b>						
Baseline pH	6.29 ± 0.15	6.38 ± 0.21	0.10	6.32 ± 0.24	6.40 ± 0.17	0.18
Max pH drop	0.72 ± 0.47	0.74 ± 0.35	0.75	0.90 ± 0.49	0.91 ± 0.46	0.94
Min pH	5.56 ± 0.44	5.64 ± 0.38	0.28	5.42 ± 0.46	5.49 ± 0.49	0.38
Final pH	6.13 ± 0.35	6.08 ± 0.25	0.59	6.02 ± 0.41	6.09 ± 0.36	0.25
AUC <sub>5.7</sub> <sup>1</sup>	1.53 ± 2.63	0.99 ± 1.71	0.28	2.63 ± 4.02	0.77 ± 1.72	0.03
AUC <sub>6.2</sub> <sup>1</sup>	5.06 ± 4.86	4.64 ± 4.01	0.65	7.54 ± 5.78	4.54 ± 3.45	0.02
<b>Site 46/45</b>						
Baseline pH	6.37 ± 0.29	6.36 ± 0.25	0.90	6.19 ± 0.20	6.38 ± 0.12	0.00
Max pH drop	0.91 ± 0.23	0.78 ± 0.29	0.13	0.73 ± 0.28	0.55 ± 0.30	0.03
Min pH	5.46 ± 0.36	5.59 ± 0.32	0.17	5.65 ± 0.31	5.66 ± 0.31	0.90
Final pH	6.08 ± 0.38	6.02 ± 0.29	0.59	6.02 ± 0.38	6.11 ± 0.32	0.15
AUC <sub>5.7</sub> <sup>1</sup>	2.02 ± 2.81	1.20 ± 1.81	0.06	1.25 ± 2.19	0.98 ± 1.98	0.69
AUC <sub>6.2</sub> <sup>1</sup>	6.97 ± 4.68	5.48 ± 3.89	0.08	5.62 ± 4.24	5.09 ± 3.92	0.62

<sup>1</sup>Area under the curve at pH 5.7 and pH 6.2 up to 15 min after the sucrose rinse.

**Table 2:** Mean stimulated saliva secretion rate, buffer capacity and pH (± SD) in no-carries and carries individuals before and after a 6-weeks period of using the arginine toothpaste. p-value for comparisons before (BA) and after (AA) the test period within the respective groups.

	BA	AA	p-value
<b>No-carries group</b>			
Secretion rate	1.96 ± 0.76	1.86 ± 0.69	0.38
Buffer capacity	9.50 ± 2.59	9.07 ± 2.97	0.31
Saliva-pH	7.63 ± 0.37	7.62 ± 0.73	0.84
<b>Carries group</b>			
Secretion rate	1.83 ± 0.88	1.86 ± 0.99	0.71
Buffer capacity	8.32 ± 3.03	9.53 ± 2.48	0.04
Saliva-pH	7.48 ± 0.53	7.62 ± 0.38	0.04

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## Availability of Data and Materials

Dataset used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Author's Contributions

HH contributed to design, planning, sample collection, data calculations and writing of the manuscript. PL contributed to the conception, planning and design of the study, and critical revision of the manuscript. AC contributed to planning and design of the study and critical revision of the manuscript. GL and WD contributed to planning, data calculations, and revision of the manuscript. SS contributed to sample collection, data calculations

and revision of the manuscript. All authors have read and approved the manuscript.

### Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committee at the Middlesex University (NSESC 1570). All procedures were in accordance with the ethical standards of this research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants were informed verbally and in writing about the purposes and procedures with the study and signed a written informed consent, when agreed to participate. No financial incentive was paid for the participants.

### Competing Interests

The authors declare that they have no competing interests.

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