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A Randomised clinical trial to determine the abrasive effect of the tongue on human enamel loss with and without a prior erosive challenge

ABSTRACT

Objectives: To investigate the abrasive effect of the tongue on human enamel loss with and without a prior dietary acid challenge in an in situ model.

Methods:
A single centre, single blind, randomly allocated, split mouth, four treatment regimen, *in situ* study in healthy adult volunteers was undertaken. Twenty four subjects wore two lower intra-oral appliances each fitted with 4 human enamel samples 6 hours/day for 15 days. The samples were treated with either 50ml orange juice or water for 5 minutes ex vivo 4x /day; prior to being licked or not licked with the subject's tongue for 60 seconds. There were 2 samples per group per subject. Surface loss was measured by contact profilometry.

Results:
23 subjects completed the study with no adverse events. The mean loss of enamel at 15 days was: 0.08 μm for water without licking, 0.10 μm with water and licking; 1.55 μm with orange juice alone, 3.65 μm with orange juice and licking. In the absence of erosive challenge, licking had no detectable effect on enamel loss $p=0.28$. Without licking, orange juice had a highly significant effect on loss compared to water, $p<0.001$. Erosive challenge followed by licking more than doubled the loss of enamel $p<0.001$.

Conclusions:
When enamel was exposed to orange juice prior to licking, tissue loss as a result of tongue abrasion of the eroded surface was increased, and double that of the erosive challenge alone. Licking enamel with the tongue had no perceptible effect on enamel loss in the absence of the erosive challenge.

Clinical Significance:
Enamel wear resulting from tongue abrasion on tooth surfaces softened by acid challenge, can be an unavoidable consequence of oral function. This may account for the pattern of erosive toothwear on palatal and occlusal tooth surfaces, reinforcing the importance of restricting the frequency of dietary acid challenge in susceptible individuals.

INTRODUCTION

Toothwear is the destruction of teeth over the course of a lifetime following exposure to a number of physical and chemical insults. Friction of exogenous material (abrasion), the effect of antagonistic teeth (attrition), forces incurred during tooth flexure (abfracation) and chemical dissolution (erosion) all contribute to various degrees [1]. Erosion, abrasion, and/or attrition rarely act alone, and often act synergistically, in the multifactorial aetiology of the condition [2]. Clinically, whilst the dominant origin of toothwear is often surmised, it is difficult to determine the part played by specific causative factors. Erosive toothwear has increased dramatically over the last couple of decades, particularly in the young adult populations and is of increasing importance for the long term health of the dentition [3]

Research has shown that when the enamel surface is challenged by acidic insult, loss of structural integrity occurs, rendering a softened tooth layer vulnerable to abrasive forces. This may cause further enamel substance loss [4]. Conversely, abrasive forces usually have no significant effect on sound tooth in an acid free environment [5], although individuals who brush more than twice a day with excessive force may suffer from abrasive toothwear and subsequent dentine hypersensitivity especially on the tooth that is brushed first and tends to be brushed for the longest [6]. One of the most destructive interactions in human toothwear, is the abrasion of erosively altered enamel [7]. Numerous studies assessing the effect of tooth brushing on eroded dental tissue indicate erosion is the dominant wear factor in toothwear, however the abrasivity of toothpaste will influence the degree of wear [8, 9]. It is rare that other possible abrasive or frictional forces are considered to impact on erosive toothwear. It is known that the acid softened zone of enamel consist of bundles of crystals separated by large spaces 10] and is thus vulnerable to the slightest abrasive or frictional influence. It has therefore been postulated that friction from the oral soft tissue and the tongue in particular [11], may contribute to the site specificity of toothwear. It may also explain the predilection for tooth wear instead of tooth loss on the palatal aspect of the upper incisors where the tip of

the tongue exerts pressure as well as the occlusal surfaces of lower first molars where the lateral borders of the tongue spread at rest [12, 13].

Studies examining the abrasive effects of the tongue on toothwear are scarce. Gregg et al [13] conducted a study *in vitro* demonstrating that enamel loss was significantly greater when acid challenge was followed by licking or ultrasonication than when acid challenge was followed by water immersion. This suggests that the tongue is exerting an abrasive effect on softened enamel. Vieira et al [14] investigated *in vitro*, the disruption of acid softened enamel by simulated tongue friction. This methodology employed toothbrushes covered with chamois leather to replicate the tongue texture and abrasive force. Again this resulted in synergistic tooth tissue loss derived from erosion and abrasion compared which was significantly greater than that achieved by erosion alone.

The aim of this *in situ* study was to investigate the abrasive effect of the tongue on human enamel surface loss in combination with and without a prior erosive challenge on the enamel surface.

The research questions asked were:

(i) Does licking tooth enamel lead to loss of tooth tissue in the absence of acidic soft drinks?

(ii) Is the loss of tooth tissue caused by acidic soft drinks enhanced by licking?

The study hypothesis was that acidic soft drinks cause enamel tissue loss by erosion and additional enamel loss is incurred due to the abrasive effects of the tongue.

MATERIALS AND METHODS

Preparation of the enamel samples

Recently extracted, caries free, human third molar teeth donated from patients aged over 18 years of either gender were used for the enamel samples. Prior to donation, each patient signed an ethically approved informed consent form, allowing their teeth to be used for research purposes. To comply with UK law,

human molars were sourced through an appropriately licensed and ethically approved Tissue Bank and were tracked and disposed of in compliance with Human Tissue Legislation.

Upon donation to the Tissue Bank, the teeth were soaked for at least 24 hours in sodium dichloroisocyanurate (20,000 ppm available chlorine) solution, cleaned, roots sectioned from the crown and pulp removed, prior to soaking for a subsequent 24 hours in sodium dichloroisocyanurate (20,000 ppm available chlorine) solution. The sections were then washed in distilled water and stored in the tooth tissue bank

Sections of enamel 4x4x2 mm were cut from the buccal surface of the crown of the tooth and embedded in epoxy resin. The samples were placed in a stainless steel jig and polished with p600 silicon carbide paper using a lapping and polishing machine, followed by hand polishing with a slurry of p1200 grit silica powder on a glass slab and 0.3 µm alpha alumina powder on a felt cloth. The samples were finally placed in an ultrasonic bath containing deionised water to remove any powder debris.

Enamel Sample Measurement

Two baseline readings of each enamel sample were taken across an area to be exposed to the study treatment using a contact profilometer (Surftest SV-200®, Mitutoyo, UK). This area was demarcated on the epoxy resin surrounding the enamel sample and the specimen identified with a unique number on the reverse. The area to be treated was left exposed by placing PVC tape over the enamel surface either side of the demarcated area leaving an enamel window for treatment. The profilometer was calibrated daily on a reference block and has been validated to an accuracy of 0.042µm for the measurement of step height enamel loss [15].

On Day 15, contact profilometry readings from the exposed area of enamel were recorded and tissue loss calculated. Prior to taking profilometric measurements, the samples were removed from the appliances and disinfected by soaking in a mixture of 0.5% chlorhexidine and 70% aqueous ethanol for a period of at least 20 minutes.

Study design

This was a single centre, single blind (blinded to the person responsible for performing the enamel sample analysis), randomly allocated, split mouth, four treatment regimen, one period, *in situ* study in healthy adult volunteers performed in a UK dental school. Favourable approval from an NHS Research Ethics Committee was obtained and the study was conducted to Good Clinical Practice guidelines as laid down by the Declaration of Helsinki. The primary objective of the study was to determine the loss of enamel tissue due to the abrasive influence of the tongue with and without prior exposure to orange juice over a 15 day period measured by contact profilometery.

Participant eligibility and randomisation

Participants aged 18 or over were invited to attend a screening visit, where those happy to take part in the study gave informed consent. Eligibility for inclusion in the study was determined following an oral soft tissue examination and evaluation of inclusion and exclusion criteria. Inclusion criteria included being in good general health and able to accommodate two lower intra-oral buccal appliances. Exclusion criteria included, susceptibility to acid regurgitation, orthodontic appliances, periodontal disease, caries, acidic medication, xerostomia and allergies to the study products.

A total of 24 subjects were enrolled in the study and custom made lower right and left buccal intra-oral appliances were constructed for all subjects, each fitted with four enamel samples (eight samples in total). Two study treatment regimens were applied per appliance and two specimens were used per regimen. Subjects were allocated a study number based on the order they were

enrolled onto the study and were randomly allocated to 4 sequence groups, 6 participants per group, using a block randomisation scheme by study staff. The randomisation scheme dictated: right or left appliance samples being treated with either an acidic or water challenge regimen and; samples licked or not licked being either anteriorly or posteriorly placed in the appliance.

Study Schedule

On each treatment day, subjects inserted both left and right intra-oral appliances before 08.00, at least 60 minutes prior to attending the study site. Subjects attended the study site 4 times a day, at 08.30 ± 30 minutes, 10.30 ± 30 minutes, 12.00 ± 30 minutes and 14.00 ± 30 minutes, where all treatments were applied *ex vivo*. There were a total of 15 treatment days, treatment days were week days only, and volunteers were allowed to take days off if necessary within the week as long as 15 days in total were completed. The study duration was 5 study weeks.

The appliances were removed from the mouth for up to 1 hour over the lunch period and worn for at least 30 minutes following the last treatment of the day, before being returned to the study site before 15.30 for cleaning. Cleaning consisted of the appliances and samples being dipped in Corsodyl® mouthrinse (0.2% w/v chlorhexidine gluconate, GSK Weybridge, Surrey, UK) for approximately 3 minutes, and rinsed in tap water.

No food or drink apart from water could be consumed whilst wearing the appliances. The subjects were not allowed to smoke or chew gum during the study. The subjects took their appliances home with them ready to place in their mouth on the following study day. When not worn, the appliances were stored in a 'moist pot' (a pot containing a damp cotton wool pad, moistened with water). Subjects were asked to perform their regular oral hygiene at home using standard allocated toothpaste (Colgate Total®, Colgate-Palmolive, Guilford, UK) and manual toothbrush (Oral B Indicator 35®, Procter & Gamble UK, Weybridge, Surrey, UK), without the appliances in place.

After completion of the study subjects were asked to attend a follow up visit within 7 days of the end of the final treatment day, returning their toothbrush

and toothpaste to the study site. At each visit, concomitant medications were checked and any Adverse Events recorded.

Treatment Regimens

There were 4 treatment regimens, two for each appliance.

Acidic Challenge Regimens:

Orange Juice only (JO) and Orange Juice and licking (JT)

1. The subject removed their appliance and an assigned study staff member soaked the appliance and samples in 50ml of orange juice agitated on an orbital shaker for 5 minutes followed by rinsing in water (Volvic®) for 30 seconds.
2. The two adjacent samples assigned to receive the acid challenge only, were securely masked off by wrapping these samples securely in parafilm®, an inert film that protected the samples (JO).
3. The appliance was returned to the subject who used their tongue to lick the remaining 2 exposed enamel samples with firm strokes in the appliance for 60 seconds at an approximate rate of 1 lick per second *ex vivo* (JT).
4. The masking parafilm® was removed and the appliance returned to the subject's mouth.

Water Challenge Regimens

Water only (WO) and Water and licking (WT)

1. The subject removed their appliance and an assigned study staff member soaked the appliance and samples in 50ml of water (Volvic) agitated on an orbital shaker for 5 minutes followed by rinsing in water (Volvic®) for 30 seconds.
2. The two adjacent samples assigned to receive the water challenge only, were securely masked off by wrapping these samples securely in parafilm®, an inert film that protected the samples (WO).

3. The appliance was returned to the subject who used their tongue to lick the remaining 2 exposed enamel samples with firm strokes in the appliance for 60 seconds at an approximate rate of 1 lick per second (WT).
4. The masking parafilm® was removed and the appliance returned to the subject's mouth.

Each of the appliances held 2 specimens for either JO and JT; or WO and WT regimens. The orange juice (Sainsbury's®, London, UK) had a pH of 3.77 and titratable acidity of 6.81.

Participants were trained to lick the enamel specimens with their tongue. The protruded tongue licked the exposed enamel face of the enamel specimen in an upward direction with the tip of the tongue with firm stokes for 60 seconds at an approximate rate of 1 lick per second.

Statistical Analysis

The primary outcome measure for the study was enamel surface loss as determined by contact profilometry. All analyses were based on loss of enamel readings averaged across the specimens fitted to the same appliance during the same period.

Statistical analyses were based on paired tests using the Hills and Armitage [16] method adapted for a split mouth design to account for the confounding factor of left/right differences. The degree to which the effect of licking was altered by orange juice (interaction) was also determined using the same paired test by comparing JT-JO with WT-WO.

Differences are reported with p-values and confidence intervals.

Statistical Power

The power assessment corresponded to a paired t analysis. With the planned sample size of 24, a mean difference of 0.57 times the standard deviation

represented how the contrast varied between subjects in the same group detectable with power 80% using a test at the conventional 5% two-sided alpha level.

RESULTS

The study was conducted in 2013. Of the 24 volunteers enrolled into the study, 16 were female and 8 were male, but 1 female participant withdrew from the study prior to the start of treatment due to work commitments, consequently all analyses were restricted to the per protocol population. There were no adverse events.

The response of the 23 participants to each of the four regimens was characterised by changes from baseline to day 15 profilometric readings, Table 1. The mean change from day 0 to day 15 being negative in each group, i.e. in the direction of loss. Each specimen was read in duplicate and the average tissue loss for the 2 specimens for each participant in each regimen determined. The loss of enamel per specimen in µm is also represented in Figure 1, the results being arranged in increasing order of loss of material following treatment with juice combined with licking (JT). The mean loss of enamel at 15 days was as follows: 0.08 µm water without licking (WO), 0.10 µm with water and licking (WT), 1.55 µm with orange juice alone (JO), 3.65 µm with orange juice and licking (JT).

Further analyses involved evaluating specific contrasts between pairs of regimens, Table 2. In every one of the 23 subjects, there was little difference between WO and WT, but a greater tissue loss with JO and a much greater loss still with regimen JT (Figure 1). Comparing the mean tissue loss caused by the regimens, in the absence of erosive challenge, licking had no detectable effect on the enamel loss ($p = 0.28$). Without licking, orange juice caused a high level of tissue loss that was significantly greater than observed in the specimens that received water ($p<0.001$). Similarly tissue loss following orange juice with licking (JT) was significantly greater than tissue loss following water with licking (WT) ($p<0.001$). Furthermore, licking more than doubled the loss of material following the erosive challenge ($p<0.001$).

DISCUSSION

The aim of this in situ study was to investigate the potential abrasive licking effect of the tongue on human enamel surface loss in combination with and without a prior acidic soft drink erosive challenge. The results show a very clear pattern that completely accords with the study hypothesis. In the absence of erosive challenge, tongue abrasion of the enamel sample had no perceptible effect on enamel tissue loss. However, when enamel specimens were exposed to orange juice prior to licking, the effect of tongue abrasion was more than double the erosive effect of the acidic challenge. The results confirm unequivocally the study hypothesis. There were 3 highly significant differences derived from the analyses between the groups and the interaction effect, these differences being all the same in all 23 subjects. The study design and protocol confirmed a pattern of tooth surface loss that was 100% consistent across all subjects. Furthermore, the effect of tongue abrasion on samples treated only with water produced a negligible difference. The study hypothesis was therefore accepted: Acidic soft drinks are recognised to cause enamel tissue loss *in situ* due to erosion and additional enamel loss is incurred due to the abrasive licking effects of the tongue. Interestingly although the combined abrasion and erosion caused significantly greater tissue loss than the erosion alone, the pattern being highly consistent across all individuals there was variation in the degree additional tissue loss seen (Figure 1). These variations likely reflect differences between study participants such as saliva/pellicle as well as differences in enamel sample which have been shown to account for variations in experimental tissue loss [17].

This *in situ* methodology study is the closest to date that researchers have developed to mimic the tongue's abrasive action on human toothwear. Measuring any form of natural toothwear is problematic due to lack of permanent landmarks or reference points in the mouth, accuracy of intra-oral measuring techniques and the sporadic nature of tooth tissue wear. Attempting to evaluate the pressures from the tongue on the teeth has been investigated by a number of researchers in other fields, who stress the role of the tongue's

numerous movements, variations in contact time and pressures exerted on the teeth [18, 19]. The lateral borders of the tongue lie at rest on the occlusal surfaces of the lower first molars and in speech for “tooth sounds” the tongue is in contact with the palatal aspect of the maxillary anterior teeth. Swallowing is initiated by the tip of the tongue being forced on to the palatal aspect of the upper incisors and remaining in this area during the entire swallow, pressures of 80g/cm^2 being exerted, which is 20 times as great on the teeth as pressures recorded at rest, 4g,cm^2 [18, 20].

Tongue licking was supervised by the study personnel at all times. Participants in the current study were trained to lick the enamel specimens with a consistently firm lick for a timed 60 seconds at an approximate rate of 1 lick per second *ex vivo*. Although the actual pressure of a strong lick may have varied across the participants, for the individual, the study aimed for an average force of 80g/cm^2 to be exerted. This representing the force exerted by the tongue on the palatal aspects of the incisor teeth during swallowing. A fixed time of 60 seconds for the duration of tongue licking was chosen in line with previous research by Vieira et al [14] and Gregg et al [13] who applied a similar regimen. In the study by Vieira et al [14], an artificial tongue was created consisting of toothbrushes covered with chamois leather. This artificial tongue was used to investigate the disruption of acid softened enamel *in vitro* by simulated tongue friction. The device was loaded with 150g and the samples abraded for 1 minute. Interestingly, the study demonstrated a trend for more enamel loss due to erosion and tongue friction than with erosion and tooth brushing under the same experimental conditions. Tongue friction on the teeth occurs more frequently than tooth brushing the teeth for individuals, and furthermore occurs during and immediately after the acid challenge. One could therefore surmise that this synergistic toothwear could be very detrimental to susceptible individuals, and this is supported by the data from this current investigation where a third of the subjects demonstrated over 4µm loss of tissue over 15 days. It is difficult to extrapolate this effect to real time toothwear as the tongue movements and contact time with the teeth are variable, as is the degree and nature of acidic challenge throughout the day and for each individual. This study

aimed to observe human enamel loss with a commonly imbibed acidic drink, with a consistent force and contact time of the tongue on the enamel surface.

This in situ methodology also allowed the researchers to standardise the acidic challenge, a commonly consumed acidic soft drink whilst not exposing the acid challenge to the natural dentition. Samples were agitated in orange juice for 5 minutes, which is a reasonable time over which to imbibe a drink. Depending on the pH of the erosive agent, short erosion times of up to 3 minutes in vitro have been found to result in a softened enamel layer of about 0.5 µm which is prone to brushing wear [5, 21]. A slightly longer time period was chosen as the oral environment tends to protect the sample against erosion, with *in vitro* results extrapolating findings *in vivo*.

In the current investigation the human enamel surfaces were polished flattened specimens. The specimens were held in the oral environment to gain a pellicle for at least 1 hour prior to investigation and during the investigation when treatment was not being performed, as this is known to influence erosive toothwear [22-24], providing an inhibitory effect against demineralisation and protection following erosion. However, the pellicle cannot protect the tooth against severe erosive challenges [24], no matter how long its formation time [25].

This study showed very convincingly that the abrasive licking effect of the tongue on enamel is negligible compared with the abrasive licking effect of the tongue after acid pre-treatment and softening of the enamel surface. The implication is that chemical action of an acidic soft drink on the enamel surface is the dominant factor for toothwear, with the tongue exerting a secondary localised effect. It follows that clinical management of this type of synergistic interaction must be focused on decreasing the frequency of acidic soft drink intake to minimise the abrasive effects of the tongue.

CONCLUSIONS

In our study licking with the tongue *per se,* has no detectable abrasive effect on enamel in the absence of acidic challenge. Nevertheless, when enamel is also

exposed to the erosive influence of acidic soft drinks, licking substantially exacerbates loss of enamel

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Table 1.

Contact profilometry readings.
Changes from day 0 to day 15. Summary statistics by regimen, based on averages of readings for 2 specimens read in duplicate for 23 participants.

Regime	Mean	Std. Deviation	95% confidence limits	
			Lower	Upper
Water only	-0.08	0.05	-0.11	-0.06
Water tongue	-0.10	0.06	-0.13	-0.08
Juice only	-1.55	0.61	-1.82	-1.29
Juice tongue	-3.65	0.93	-4.05	-3.25

Table 2

Contact profilometry readings. Changes from day 0 to day 15. Five comparisons between pairs of regimens.

Contrast	Mean difference		95% confidence limits		P-value
	Unadjusted	Adjusted	Lower	Upper	
WT vs. WO	-0.02	-0.02	-0.05	0.02	0.28
JT vs. JO	-2.10	-2.11	-2.51	-1.71	<0.001
JO vs. WO	-1.47	-1.47	-1.74	-1.19	<0.001
JT vs. WT	-3.55	-3.548	-3.96	-3.13	<0.001
Interaction	-2.08	-2.08	-2.48	-1.68	<0.001

Figure 1. Tissue loss per participant per treatment

Tissue loss (um) is shown for each treatment for each participant, with participants ordered such that tissue loss following orange juice combined with abrasion (JT) is presented in ascending order.