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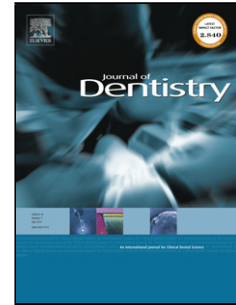
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## Accepted Manuscript

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## Timing of Dietary Acid Intake and Erosive Tooth Wear: A Case-Control Study

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### AUTHOR CONTRIBUTIONS

All authors contributed to the study design, analysis, interpretation, drafting and critically revising the manuscript. S O'Toole, D Bartlett and R Moazzez contributed to conception and data acquisition.

### Abstract

**Objectives:** There is a lack of clinical data on the impact of timing of dietary acid intake and toothbrush abrasion when attempting to control erosive tooth wear progression. The aim of this study was to estimate the association of theoretical causative factors with erosive tooth wear to inform

evidence-based guidelines. **Methods:** Using case-control study design, 300 participants with dietary erosive tooth wear and 300 age-matched controls were recruited from the restorative clinics of King's College London Dental Institute. A previously validated questionnaire was adapted to be interviewer-led and to assess frequency, timing and duration of dietary acid intake in addition to alternate drinking habits prior to swallowing. Timing of toothbrushing in relation to meals and dietary acid intake was investigated. Associations with erosive tooth wear were assessed in crude and adjusted logistic regression models. **Results:** Fruit intake between meals ( $p < 0.001$ ), but not with meals ( $p = 0.206$ ), was associated with erosive tooth wear and contrasted with acidic drinks which maintained a strong association regardless of timing of intake (OR up to 11.84 [95%CI:5.42-25.89],  $p < 0.001$ ). Prolonged fruit eating and alternate drinking habits prior to swallowing (OR 12.82 [95%CI:5.85-28.08] and 10.34 [95%CI:4.85-22.06] respectively) were as strongly associated with erosive tooth wear as three or greater daily acid intakes (OR 10.92 [95%CI:4.40-27.10]). Toothbrushing within 10 minutes of acid intake was not associated with erosive tooth wear following adjustments for dietary factors (OR 1.41 [95%CI:0.82-2.42],  $p = 0.215$ ). **Conclusion:** Significantly increased odds ratios were observed when acids were consumed between meals in this cohort of patients. Universal advice to delay brushing after meals may not be substantiated. **Clinical Significance:** Prevention should be focused on avoiding dietary acids between meals, eliminating habits which increase contact time with the acid and reducing daily intake of acidic drinks. Toothbrushing after meals was not associated with erosive wear. Toothbrushing immediately after an acid challenge requires further investigation.

**Keywords:** Erosive Tooth Wear, Risk Factors, Toothbrush Abrasion, Epidemiology

## REGISTRATION

This trial is registered on <http://clinicaltrials.gov> under ID number [NCT02449434](https://clinicaltrials.gov/ct2/show/study/NCT02449434).

## INTRODUCTION

Erosive tooth wear is the chemical-mechanical cumulative loss of dental hard tissue not caused by bacteria [1]. As the prevalence of erosive wear is increasing [2] an increased focus on prevention may prolong the long term health of the dentition. However, the interactive role of dietary erosion and abrasion and the relative significance of each effect are currently undergoing academic debate [3].

Preventive guidelines remain unclear and attention is urgently required to produce basic evidence-based preventive advice.

Frequency of dietary acid intake is hypothesised to be the most important risk factor in extrinsic tooth wear progression [4–6], and yet a clinical threshold of “safe” intake has yet to be established. Inherent protective mechanisms conferred by saliva, salivary pellicle and intra-oral ion reservoirs require depletion before dental mineral loss occurs [7]. In addition, acid clearance and intra-oral pH stabilisation occurs rapidly over 2-13 minutes [8–10]. The frequency of daily acid intake which overrides the natural defences, is unclear from the literature. Previous case-control studies have investigated either acidic beverages or fruit in isolation [5,11,12] but not the overall pattern of acid intake adjusting for confounding factors. The timing of acid intake may be important and to date has not been investigated epidemiologically. Additional foods present may buffer the acid [13]. Increased salivary flow rates at mealtimes [14] may also lower the erosive potential of the acid sufficiently to prevent demineralisation and irreversible tissue loss. However, the clinical significance of timing of acidic foods has yet to be clarified.

Furthermore, it has been suggested that salivary remineralisation after erosive challenges may confer significant protection from abrasion [15,16] and therefore the timing of tooth brushing in relation to erosive challenges may be important in tooth wear progression [17]. However, there is laboratory evidence to suggest that full remineralisation of eroded dental tissues may not be possible [18] and a large European epidemiological study failed to observe clear patterns between timing of food intake and tooth brushing [3]. Attention has been drawn to the low level of clinical evidence base behind these recommendations and the clinical significance of remineralisation time prior to abrasive forces may be relatively small [18,19]. In addition, if the buffering capacity of meals is significant, insufficient demineralisation may have occurred to enable abrasive tooth wear. The relative importance of the interactions between frequencies of dietary acid intake with and between meals, prolonged contact time with the acid and toothbrush abrasion remains to be established.

The aim of this study was to investigate the interrelationship between dietary acid intake, tooth brushing after acid intake and erosive tooth wear. It was hypothesised that the frequency, timing (between meals) and duration of acid intake are positively associated with erosive tooth wear whereas delaying brushing after meals is negatively associated with erosive tooth wear.

## **MATERIALS AND METHODS**

This was a single-centre, frequency-matched, case-control study. The study protocol was approved by West of Scotland Research Ethics Service (Reference 14/WS/0015) and written informed consent was obtained from all participants. The present study adheres to the Strengthening the Reporting of Observational Studies (STROBE) statement [20] [and is registered at clinicaltrials.gov \(Identifier number: NCT02449434\)](https://www.clinicaltrials.gov/ct2/show/study/NCT02449434)

### ***Participants***

Participants (n=600) aged 18 years or older, were recruited between May 2014 and March 2016 following referral by their general dental practitioners (GDP) for erosive tooth wear (cases, n=300) or general treatment (controls, n=300) to the restorative clinics at King's College London Dental Institute. All participants had a minimum of 20 teeth (10 in each jaw). Participants were excluded if they had missing anterior teeth, anterior crowns/bridges or cavitated caries on more than one tooth. A history of eating disorders or gastro-oesophageal reflux, bruxism or prescribed xerostomic/heartburn medication, pregnancy, involvement in other research within the past 30 days or inability to speak or understand the English language also excluded the participant from this study. Bruxism was diagnosed from the clinical appearance, signs and symptoms of attritive tooth wear; those with flattened surfaces without erosive lesions were excluded.

According to a pilot study and a previous study within our group [6], a minimum sample size of 490 participants (245 in each group) were needed. This calculation assumed the proportion of adults with high dietary acid intake (3+ times/day) was 55% among cases and 40% among controls (expected odds ratio of 2.25), case-control ratio of 1-to-1, 90% statistical power and 95% significance level.

### ***Data collection***

Data collection procedures were identical for cases and controls. The Basic Erosive Wear Examination (BEWE) index graded tooth wear on the buccal, occlusal and palatal/lingual surfaces of each tooth excluding third molars and was used to differentiate the groups. This ordinal scale graded tooth wear from 0-3 (0 = no wear, 1 = early surface loss, 2 = surface loss < 50% or specific defect, 3 = surface loss > 50%). Teeth with restorations involving >50% of the tooth, traumatised or carious teeth were excluded from the assessment. Teeth were examined under good lighting in a dental chair but without magnification. A sextant BEWE score was calculated by recording the highest score from any

surface in each sextant. The cumulative BEWE score was calculated by summing each sextant BEWE score within the range from 0-18 [21]. A trained and calibrated dentist carried out all clinical examinations. Inter-examiner and intra-examiner Kappa scores, calculated on duplicate examinations of 30 patients against a calibrated BEWE expert (DB), were 0.85 and 0.75 respectively.

Erosive tooth wear cases were defined as those with a BEWE score of 12 or higher and at least one score of 3 in a sextant whereas controls were defined as those with a BEWE score of 10 or lower and no score of 3 on any surface of any tooth (clinically classified as no or mild erosive tooth wear). Controls were age-matched on a 1:1 ratio with cases. Frequency matching was used, to yield controls with the same distribution over six age groups (18-25, 26-35, 36-45, 46-55, 56-65 and 66+ years). Cases that could not be matched were excluded from the study.

After recruitment, a trained interviewer questioned participants on potential risk factors for erosive tooth wear using an adapted version of a previously validated questionnaire [3]. Participants were asked about the frequency, timing (with meals or between meals) and duration of consumption of fruits, fruit drinks, carbonated beverages and other acidic drinks, the type of holder (cup, glass, bottle, can), whether they had a habit of retaining drinks in their mouth (sipping, swishing or holding) prior to swallowing, whether they reported to brush within 10 minutes of consuming something acidic and any associated symptomatology (i.e. sensitive teeth). Total daily frequency of acid intake was estimated as the sum of fruit (apples, citrus, grapes, berries and any other fruit) and acidic drinks (carbonated drinks, fruit drinks, any other acidic drinks eg fruit teas, wine). For timing, total daily frequency of acid intake was separated into four indicators, daily frequency of fruit intake with and between meals and daily frequency of acidic drink intake with and between meals. Duration of a single acid intake was divided into three categories (<5 min, 5-10 min and  $\geq 10$  min). Questions were standardised and participants were given the opportunity to clarify questions, ensuring comprehension before answering. Each questionnaire lasted between 5 and 10 minutes.

### **Data analysis**

All analyses were performed in the Statistical Package for Social Sciences version 22 for Windows (IBM Corporation, Armonk, New York, USA). Cases and controls were compared in terms of their demographic and clinical characteristics, using the Chi-square test for categorical variables and the t-

test for continuous measures. Cases and controls were also compared in terms of risk factors (total daily acid intake, fruit intake with meals, fruit intake between meals, acidic drink intake with meals, acidic drink intake between meals, duration of fruit and acidic drink consumption, brushing within 10 minutes of acid intake and alternate drinking habits) using the Chi-square test. Variables to be included in the logistic regression model were manually selected based upon prior theory and the research hypothesis. The variables included were: the frequency of fruit and acidic drink consumption with meals and between meals, duration of consumption, brushing after acid intake and alternate drinking habits prior to swallowing.

Using the presence or absence of severe erosive wear (cases and controls) as the dependent variable, the unadjusted, sex-and-age-adjusted and fully adjusted associations of the included variables with erosive tooth wear were estimated using unconditional binary logistic regression and reported using odds ratios (OR). As frequency matching does not require the use of conditional logistic regression during analysis [20], age (in the pre-defined groups), was included as a potential confounder.

## RESULTS

Table 1 shows the demographic and clinical characteristics of cases and controls. There were no significant differences in age between the two groups, but there were significantly more females among controls ( $p=0.003$ ). The mean BEWE score, used to differentiate the groups, was 15.0 for cases and 6.3 in controls. An increased proportion of cases reported to have dental sensitivity compared to controls (55% versus 42%,  $p<0.001$ ).

Table 2 reports the crude associations of all risk factors with erosive tooth wear. Cases were more likely to report higher total (combined daily fruit and acidic drink) acid intake (OR 13.5, 95%CI: 6.94-26.29), fruit intake between meals (3.64, 95%CI: 2.39-5.52) and acidic drink intake both with (6.92, 95%CI: 4.02-11.93) and between meals (10.19, 95%CI: 6.46-16.09) as well as taking more time to consume fruits (4.51, 95%CI: 2.47-8.24) and acidic drinks (1.72, 95%CI: 1.15-2.27). There were no statistical differences determined between cases and controls regarding fruit intake with meals.

Cases were more likely to report brushing their teeth within 10 minutes of acid intake (2.02, 95% CI:1.38-2.95). This relationship remained statistically significant when adjusting for sex and age (2.20 95% CI:1.46-3.17,  $p<0.001$ ) but not when dietary factors were fully controlled for (1.41 95% CI 0.82-

2.42,  $p=0.215$ ) in the fully adjusted model reported in Table 3. In contrast, the frequency of acid intake and the duration over which they were consumed remained significantly associated with erosive tooth wear after adjustments (Table 3). The exception to this was fruit intake with meals which remained insignificant in the fully adjusted model. The frequency of fruit intake between meals (1.95, 95%CI: 1.02-3.75 for once/day and 5.35, 95%CI: 2.51-11.43 for 2+ times/day) remained significantly associated with erosive tooth wear. Acidic drinks with meals (6.42, 95%CI: 2.97-13.91 for 2+ times/day) and acidic drinks between meals (2.49, 95%CI: 1.25-4.98 for once/day and 11.84 95%CI: 5.42-25.89 for 2+ times/day) also remained associated with erosive tooth wear after controlling for other risk factors and the other indicators of timing of acid intake.. Furthermore, cases were 2.47 (95%CI: 1.14-5.32) and 12.82 (95%CI: 5.85-28.08) times more likely to take 5-10 and >10 min to eat fruits than controls. Similarly, cases were 2.35 (95%CI: 1.14-5.32) and 2.93 (95%CI: 1.63-5.29) times more likely to take 5-10 and >10 min to drink acidic drinks than the controls. Cases were also 10.34 (95%CI: 4.85-22.06) more likely to sip, swish or hold drinks in their mouth prior to swallowing.

## DISCUSSION

These findings show that the overall daily frequency of dietary acidic intake was associated with erosive tooth wear. Interesting patterns emerged when dietary acid intake was separated by timing in relation to meals. The consumption of acidic drinks both with and between meals was independently associated with erosive tooth wear. Fruit intake between meals was also associated with erosive wear whereas no relationship was observed when fruit was consumed with meals. Additionally, the findings provide limited support for the role of tooth brushing after acid intake in the aetiology of tooth wear.

Limitations of this study need to be addressed. This study was based on hospital volunteers which may limit the ability to generalise findings beyond the study population. Dietary assessment in this study was based on current patterns of consumption. Erosive damage to the dentition may have occurred at any stage post eruption of the permanent dentition where a different diet was consumed. This is a limitation of retrospective questionnaire-based studies where existing (prevalent) and new incident) cases are difficult to identify. Longitudinal studies with multiple dietary assessments over time are recommended.

Having addressed these limitations, strong statistical relationships were observed in the data. A positive linear relationship was observed between increasing frequency of dietary acid intake and erosive wear. In this study, the odds of erosive tooth wear increased substantially when two or greater dietary acids were consumed daily. Other authors have reported similar figures in case-control studies performed in children [5,22] and adults [11,23]. Less than daily consumption of dietary acids was not associated with an increased risk of severe erosive tooth wear in this study. Caution should be exercised when interpreting this as risk will be dependent on the timing of the acid intake, the erosive potential of the acids and salivary factors. However, it may serve as a clinical indicator when assessing risk potential.

Although the potential buffering capacity of meals has been discussed in previous studies [13], this is the first study to demonstrate the impact of snacking on dietary acids. Fruit intake with meals was not statistically associated with erosive wear, similar levels of fruit intake between meals were. In addition, the odds ratios of consuming acidic drinks twice daily increased when acidic drinks were consumed between meals compared to with meals. This discovery may explain conflicting epidemiological studies, some of which have observed increased risk with dietary acids [3,23] and others which have not [24].

Acidic drink intake was a stronger predictor of erosive wear than fruit intake and support the findings of other clinical studies [6,25,26]. If the participant admitted to sipping, swishing or holding the drinks in the mouth prior to swallowing an increased OR of 10.34 was observed. Few participants (12% of the study population) spent  $\geq 10$  minutes consuming fruit at a single sitting. However this characteristic was a highly significant predictor of erosive tooth wear (OR 12.82). The comparable risk associated with a prolonged fruit eating habit is a novel and interesting finding. This emphasises the importance of contact time between the teeth and dietary acids and supports observations on alternate drinking habits in other studies [5,6,26].

No statistical differences were observed between cases and controls with respect to brushing at mealtimes. A statistically greater number of erosive wear patients brushed their teeth within 10 minutes of consuming something acidic, but this was not statistically significant when dietary risk factors were fully adjusted for. This is supported by evidence from a large epidemiological study observing no statistical relationship between timing of toothbrushing in relation to breakfast and

erosive tooth wear [3]. Although few epidemiological studies have investigated the timing of toothbrushing and erosive tooth wear, many have investigated the frequency of brushing. Although there have been studies that have reported increased erosive wear with increased frequency of brushing [23,27], other studies have shown increased risk when brushing was performed less than twice daily [26,28–31]. This may be due to the protective action of fluoride [32]. Authors have also hypothesised that brushing before a meal may confer a level of protection due to the increased presence of re-mineralising ions [17], which was not observed in this study. However, in vitro studies have observed this protective effect may be dependent on the type of fluoride used [33] and the severity of the erosive challenge [34] and this may require further investigation clinically. The results of this study would indicate that prevention advice for erosive wear should be focused on dietary advice.

This research demonstrates the importance of questioning those showing early signs of erosive wear on how they are consuming dietary acids in addition to their overall frequency of consumption. These findings help characterise dietary patterns more strongly associated with tooth wear and would suggest prevention should be focused on avoiding dietary acids between meals, eliminating habits which increase contact time with the acid and reducing daily intake of acidic drinks. These results indicate no relationship between brushing after meals and erosive tooth wear. The authors are hesitant however, to fully exclude brushing within 10 minutes of acid intake outside of meals as a risk factor based upon the results of this study. Prospective, longitudinal studies incorporating multiple dietary assessments are recommended to confirm the results of this study.

## **CONCLUSION**

In this large cohort of hospital-based patients, the predominant risk factors in the development of severe erosive tooth wear in this study were acid intake between meals, an alternate drinking method such as sipping, swishing or holding acidic drinks in the mouth prior to swallowing and eating fruit over an extended time period. Brushing after meals was not associated with erosive tooth wear suggesting universal preventive advice to delay brushing after meals is not substantiated.

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Table 1: Comparison of demographic and clinical characteristics of participants

Characteristics	Cases		Controls		p value <sup>a</sup>
<i>Gender, n (%)</i>					0.003
Men	162	54%	125	42%	
Women	138	46%	175	58%	
<i>Age groups, n (%)</i>					1.000
18-25 years	31	10%	33	11%	
26-35 years	67	22%	67	22%	
36-45 years	68	23%	66	22%	
46-55 years	66	22%	66	22%	
56-65 years	44	15%	44	15%	
66+ years	24	8%	24	8%	
<i>Age in years</i>					0.788
Mean $\pm$ SD	44.07 $\pm$ 14.17		43.76 $\pm$ 14.71		
Range	18-74		18-75		
<i>BEWE score</i>					<0.001
Mean $\pm$ SD	15.01 $\pm$ 2.30		6.27 $\pm$ 2.79		
Range	12-18		0-10		
<i>Self-reported sensitivity, n (%)</i>					<0.001
No	134	45%	194	58%	
Yes	166	55%	106	42%	

<sup>a</sup> Chi square and t tests were used to compare proportions and scores, respectively.

Table 2: Crude associations of investigated risk factors

Characteristics	Cases		Controls		OR <sup>a</sup>	[95% CI]	p value
	n	(%)	n	(%)			
<i>Total acid intake (Combined daily fruit and acidic drink intake)</i>							
1 per day or less	11	4%	76	25%	1.00	[Reference]	
2 per day	31	10%	92	31%	2.33	[1.09-4.94]	0.028
3+ per day	258	86%	132	44%	13.50	[6.94-26.29]	<0.001
<i>Fruit intake with meals</i>							
Less than once daily	191	63%	174	58%	1.00	[Reference]	
1 per day	74	25%	86	29%	0.78	[0.54-1.14]	0.200
2+ per day	35	12%	40	13%	0.79	[0.48-1.31]	0.372
<i>Fruit intake between meals</i>							
Less than once daily	62	21%	107	36%	1.00	[Reference]	
1 per day	82	27%	119	39%	1.19	[0.78-1.81]	0.419
2+ per day	156	51%	74	25%	3.64	[2.39-5.52]	<0.001
<i>Acidic drinks with meals</i>							
Less than once daily	130	43%	206	69%	1.00	[Reference]	
1 per day	87	29%	75	25%	1.84	[1.25-2.69]	0.002
2+ per day	83	28%	19	6%	6.92	[4.02-11.93]	<0.001
<i>Acidic drinks between meals</i>							
Less than once daily	71	23%	186	62%	1.00	[Reference]	
1 per day	89	29%	78	26%	2.99	[1.99-4.49]	<0.001
2+ per day	140	47%	36	12%	10.19	[6.45-16.09]	<0.001
<i>Brush within 10 minutes of acid intake</i>							
No	205	68%	244	81%	1.00	[Reference]	
Yes	95	32%	56	19%	2.02	[1.38-2.95]	<0.001
<i>Duration of fruit consumption</i>							
<5 min	181	61%	215	72%	1.00	[Reference]	
5-10 min	31	10%	29	10%	1.27	[0.74-2.19]	0.389
>10 min	57	19%	15	5%	4.51	[2.47-8.24]	<0.001
Does not eat fruits	31	10%	41	13%			
<i>Duration of acidic drink consumption</i>							
<5 min	65	22%	84	28%	1.00	[Reference]	
5-10 min	57	19%	37	12%	1.99	[1.18-3.37]	0.010
>10 min	161	53%	121	40%	1.72	[1.15-2.27]	0.008
Does not have drinks	17	6%	58	19%			
<i>Alternate drinking habits prior to swallowing</i>							
No	178	59%	227	76%	1.00	[Reference]	
Yes	105	35%	15	5%	8.56	[4.43-16.57]	<0.001
Does not have drinks	17	6%	58	19%			

<sup>a</sup> Binary logistic regression was fitted and odds ratios (OR) reported.

**Table 3: Adjusted regression models showing frequency of fruit and acidic drink intake in relation to meals and explanatory variables.**

Characteristics	Sex-and-age adjusted models			Fully adjusted model		
	OR <sup>a</sup>	[95% CI]	p value	OR <sup>a</sup>	[95% CI]	p value
<i>Fruit with meals</i>						
Less than once daily	1.00	[Reference]		1.00	[Reference]	
1 per day	0.80	[0.55-1.17]	0.256	1.36	[0.75-2.45]	0.316
2+ per day	0.77	[0.46-1.28]	0.309	1.99	[0.92-4.32]	0.083
<i>Fruit between meals</i>						
Less than once daily	1.00	[Reference]		1.00	[Reference]	
1 per day	1.25	[0.81-1.92]	0.310	1.95	[1.02-3.75]	0.017
2+ per day	4.14	[2.68-6.37]	<0.001	5.35	[2.51-11.43]	<0.001
<i>Acidic drinks with meals</i>						
Less than once daily	1.00	[Reference]		1.00	[Reference]	
1 per day	1.84	[1.25-2.70]	<0.001	1.81	[0.97-3.37]	0.061
2+ per day	7.12	[4.09-12.39]	<0.001	6.42	[2.97-13.91]	<0.001
<i>Acidic drinks between meals</i>						
Less than once daily	1.00	[Reference]		1.00	[Reference]	
1 per day	3.20	[2.11-4.87]	<0.001	2.49	[1.25-4.98]	0.010
2+ per day	10.75	[6.72-17.18]	<0.001	11.84	[5.42-25.89]	<0.001
<i>Brush after acid intake</i>						
No	1.00	[Reference]		1.00	[Reference]	
Yes	2.20	[1.46-3.17]	<0.001	1.41	[0.82-2.42]	0.215
<i>Duration of fruit consumption</i>						
<5 min	1.00	[Reference]		1.00	[Reference]	
5-10 min	1.46	[0.84-2.56]	0.310	2.47	[1.14-5.32]	0.022
>10 min	5.19	[2.80-9.62]	<0.001	12.82	[5.85-28.08]	<0.001
<i>Duration of acidic drink consumption</i>						
<5 min	1.00	[Reference]		1.00	[Reference]	
5-10 min	2.14	[1.25-3.66]	0.005	2.35	[1.14-4.81]	0.020
>10 min	1.83	[1.22-2.76]	0.004	2.93	[1.63-5.29]	<0.001
<i>Alternate drinking habits</i>						
No	1.00	[Reference]		1.00	[Reference]	
Yes	9.54	[5.32-17.10]	<0.001	10.34	[4.85-22.06]	<0.001

<sup>a</sup> Binary logistic regression was fitted and odds ratios (OR) reported. The fully adjusted model included all variables listed in the table plus sex and age groups as explanatory variables.