Tooth Wear in Irish Teenagers:

A Laboratory and Epidemiological Study

Volume I of II

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Declaration

The research presented in this thesis was carried out by the undersigned. No part of the research presented here has been submitted in support of an application for another degree or qualification of this or any other University or Institute of learning.

Máiréad Antoinette Harding

Dedication

To my very dear family, friends and colleagues, for you have accompanied me on this journey. To my very dearest Sorcha and Iseult, we have shared so much and from you I am still learning.

The Journey is the reward

Chinese Proverb

Abstract

Introduction

This thesis addresses the challenge of tooth wear in the permanent dentition. The aim of the work was to investigate the characteristics, development and determinants of tooth wear among Irish schoolchildren with a view to identifying modifiable risk factors to inform future preventive strategies. The importance of this work lies in the fact that in developed countries there is an increasing dentate and ageing population. The maintenance of a dentition, which is fit for purpose and aesthetic from ‘cradle to grave’ is an important goal in contributing to quality of life generally and specifically oral health related quality of life in all age groups. Tooth wear, although a condition rather than a disease poses a threat to the integrity of the dentition and can be challenging to treat.

This research project used two different study designs in two separate populations. Sixteen-year-old teenagers participated in a cross sectional study. Participants first contacted at age 5-years and subsequently examined at age 12- and 14-years made up the longitudinal study. Both studies allowed the investigation of the determinants of wear at single points in time, and in addition the longitudinal study provided deeper insight into the progression of wear over time. The cross sectional study also included detailed analysis of saliva characteristics and their association with wear.

Methods

Two studies cross sectional (examination at age 16) and longitudinal (examinations at age 5, 12, 14). A trained and calibrated clinical examiner (MH) carried out all examinations. On each fieldwork occasion, children or their parents completed a demographic profile and questionnaire on their usual oral hygiene practices, questions on dietary choices, health, medication and exercise, in that way the studies were similar. In addition, unstimulated and stimulated saliva samples were collected according to a standardised protocol from the 16-year-olds. Two indices were used, that described by Bardsley et al., (2004) and a more discriminate index described by Bartlett et al., (2011b). The index described by Bardsley et al., (2004) was used in all three episodes of fieldwork, and in addition, the more refined index described by

Bartlett et al., (2011b) was used when conducting the examinations with the participants in the longitudinal study.

Outcome measures for tooth wear included the proportion with tooth wear, the proportion with tooth wear on specific surfaces, the number of teeth affected and whether tooth wear was confined to dental enamel or where dentine was visible. The choice of explanatory variables for both the cross sectional and longitudinal study included demographic factors, tooth brushing habits, dietary choices, general health and lifestyle factors. Salivary flow rate was determined gravimetrically for all who provided a sample. Analysis of the fluoride, phosphate and calcium concentration in both stimulated and unstimulated saliva was carried out using ion chromatography. The Bradford protein assay was used to determine protein concentration and protein carbonyl concentration (protein oxidation marker) was determined spectrophotometrically. Protein spots were observed using gel electrophoresis and identification of the protein spots used mass spectrometry. Both chemical and biochemical analyses were conducted for a selected subset of participants who had either no wear or moderate wear to explore differences in salivary profiles. Tooth wear scores and questionnaire responses collected at the earlier examinations in the longitudinal study were also included as explanatory variables. In all statistical analyses significance was accepted at $p<0.05$.

Results

The prevalence of tooth wear with dentine visible was 44% in the 16-year-old teenagers. For the 16-year-olds the flow rates for unstimulated and stimulated saliva were 0.51 ml min^{-1} and 2.05 ml min^{-1} respectively. Although, there was no difference in the salivary flow rates based on tooth wear scores, the selected group with moderate tooth wear had a significantly higher mean protein carbonyl concentration ($p<0.0001$) and a significantly higher mean total calcium concentration ($p<0.002$) in unstimulated saliva. In addition the moderate tooth wear group had a significantly lower mean protein concentration for stimulated saliva ($p<0.0001$). The 2-DE images prepared for a small group of subjects who were selected based on their tooth wear score and their protein carbonyl concentration differed between the individuals with tooth wear and those without. Mass spectrometry, identified one of the particular proteins of interest as the constant region of IgA. Analysis of the demographic factors, tooth brushing habits, dietary choices, general health and lifestyle factors in the cross sectional study indicated that participants who brush their teeth after breakfast are significantly more likely to have tooth wear scores that are lower than participants who do not brush after breakfast ($p<0.03$). Participants who indicated that they bit their nails were significantly more likely to have higher tooth wear scores on buccal/labial surfaces than those who did not ($p<0.03$). The number of buccal/labial surfaces affected by tooth wear was significantly lower in individuals who eat apples between once a week and once a day than participants who eat apples less than once a week or never ($p<0.002$). Participants who indicated they were asthmatic had more buccal surfaces affected than participants who were not asthmatic ($p<0.04$) and participants who reported having a dry mouth had more buccal surfaces affected than participants who did not ($p<0.03$). In the longitudinal study participants who were identified with tooth wear into dentine at age five had more tooth wear at age 14 for a number of the selected tooth wear outcomes (all $p<0.05$). In addition, tooth wear at age 14, for the outcome 'dentine visible on the incisal surface of permanent incisors' was positively associated with tooth wear at age 12, ($p<0.02$).

Discussion

The results illustrate the multifactorial aetiology of tooth wear and suggest the need for an holistic approach integrating oral health with general health promotion on diet and lifestyle choices. The need to maintain a balanced diet and individualised oral hygiene advice is apparent. The biochemical and physical correlates of perceived dry mouth generate interesting and useful hypotheses for future research on the role of protein oxidation and saliva quality in tooth wear.

Conclusion

This research has indicated the impact of dietary factors, salivary factors and the previous experience of tooth wear on the teenage or early permanent dentition. The longitudinal study indicates that from the very earliest age the primary dentition provides information on the future health of the permanent dentition. Thus early intervention focussed on the prevention of tooth wear and based on the correlates identified in this thesis should provide the basis for future work.

Abbreviations and glossary of terms

1-DE	One-dimensional electrophoresis
2-DE	Two-dimensional electrophoresis
Alcopops	Ready mixed alcoholic spirits
AM	Dr Alexander Milosevic (Inter-examiner gold standard, Bardsley et al., 2004 index)
BEWE	Basic Erosive Wear Examination (Bartlett et al., 2008)
BMI	Body Mass Index
Ca^{2+}	Calcium ion
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CC	Creative commons – images to share
CREC	Clinical Research Ethics Committee
CUTH	Cork University Teaching Hospitals
ETI	Exact Tooth Wear Index (Bartlett et al., 2011b)
F^-	Fluoride ion
Fluoridated (F)	Participants who had a life time exposure to a fluoridated home water supply
GORD	Gastro-oesophageal reflux disease
HA	Hydroxyapatite
H_3PO_4, $H_2PO_4^-$ HPO_4^{2-} and PO_4^{3}	The four ionic forms of phosphate
IC	Ion chromatography

IPG	Immobilised pH gradient
MALDI	Matrix-assisted laser desorption/ionization (mass spectrometry)
Medical Card	Used as an indicator of disadvantage status
MH	Máiréad Harding (the Examiner / Researcher)
Mod TWI	Modified Tooth Wear Index described by (Bardsley et al., 2004)
Mr	Relative molecular mass (relative to carbon 12)
MS	Mass spectrometry
NDNS	National Diet and Nutrition Survey
NHANES	National Health and Nutrition Examination Survey
Non-fluoridated	Participants who had a lifetime exposure to a non-fluoridated home water supply
OHSRC	Oral Health Services Research Centre
OR	Odds ratio
Part	Participants who had exposure to a fluoridated water supply for part of their lifetime
pH	Molar concentration of hydrogen ions in solution; a measure of the acidity or alkalinity of the solution
ppm	parts per million
RoI	Republic of Ireland
ROS	Reactive oxygen species
RR	Relative risk
Soft drink	See the UK BSDA information graphic included in this section

Stimulated saliva	Stimulated whole mouth saliva, which is the mixture of secretions collected
(SD)	Standard deviation
SD	Dr Soha Dattani (Inter-examiner gold standard, Bartlett et al, 2011b index)
SSB	Sugars-sweetened beverages
TOF	Time of flight
tp0	Time point zero
tp1	Time point one
tp2	Time point two
tp3	Time point three
TWI	Tooth Wear Index (Smith and Knight, 1984)
TY	Transition year
Unstimulated saliva	Unstimulated whole mouth saliva, the mixture of secretions that enter the mouth in the absence of exogenous stimuli. It is composed of secretions from parotid submandibular, sublingual, minor mucous glands, the gingival crevicular fluid, desquamated epithelial cells and food residues.
VEDE	Visual Erosion Dental Examination

A note on

Terminology used in the research project

When the term ‘no tooth wear’ is used, the descriptor means no obvious wear in enamel is visible. The term ‘moderate tooth wear’ indicates tooth wear where dentine is visible. Measurement is determined with a clinical examination, with the teeth dried, in the presence of good lighting, without additional magnification. The terms tooth wear ‘with dentine visible’ and ‘tooth wear into dentine’ are used. Both terms mean that at the examination, enamel loss had progressed sufficiently so that dentine appeared to be obviously exposed. However, in the absence of an histological examination, that state could not be ascertained with 100% certainty.

Unit of measurement salivary ions

The concentrations of salivary ions, calcium, phosphate and fluoride are presented in the text as milli moles per litre (mmol l^{-1}). In the tables both the term mmol l^{-1} and parts per million (ppm) are presented. The inclusion of the unit parts per million in the tables permits comparison with older data.

Decimals and percentages

In the results section, the values calculated were rounded to two decimal places in the text and in the tables, where applicable. All analyses were conducted to at least four decimal places. Percentages were rounded up to whole numbers.

Both IBM SPSS® Version 22 and Microsoft Excel® software packages were used to calculate means and standard deviations, and rounding was not conducted until the end of the calculation. The consequence of this is that in tables summing of rows or columns may not exactly equal the presented total number or percentage.

Statistical values and significance

In the research undertaken, the number of outcome and explanatory variables was considerably large. In an effort to maintain clarity in the text, the researcher (MH) may mention that values were significant, which means the result was significant at a value of at least $p<0.05$. All of the tables presented contain where appropriate, the mean value with standard deviation (SD), or the Odds ratio, or ratio of means with

95% confidence intervals. In addition, the tables prepared from the results of the regression analyses provide the interpretation of the result and the direction of the association.

Age in the thesis

The children in this research are referred to as 5-year-olds, 12-year-olds, 14-year-olds and 16-year-olds. All of the participants referred to as 5-year-olds, were 5-years of age when first invited to participate. Thus, the vast majority were 12-years-old and 14-years-old when seen again at each time point in the longitudinal study. In the cross sectional study children who were in transition year (TY) in school were selected, thus some were 15-years-old and some 17-years-old but the majority were 16-years-old. In the thesis the children and their ages may also be referred to as, age 5-, 12-, 14- and 16-years or 5, 12, 14 and 16.

Saliva parameters measured

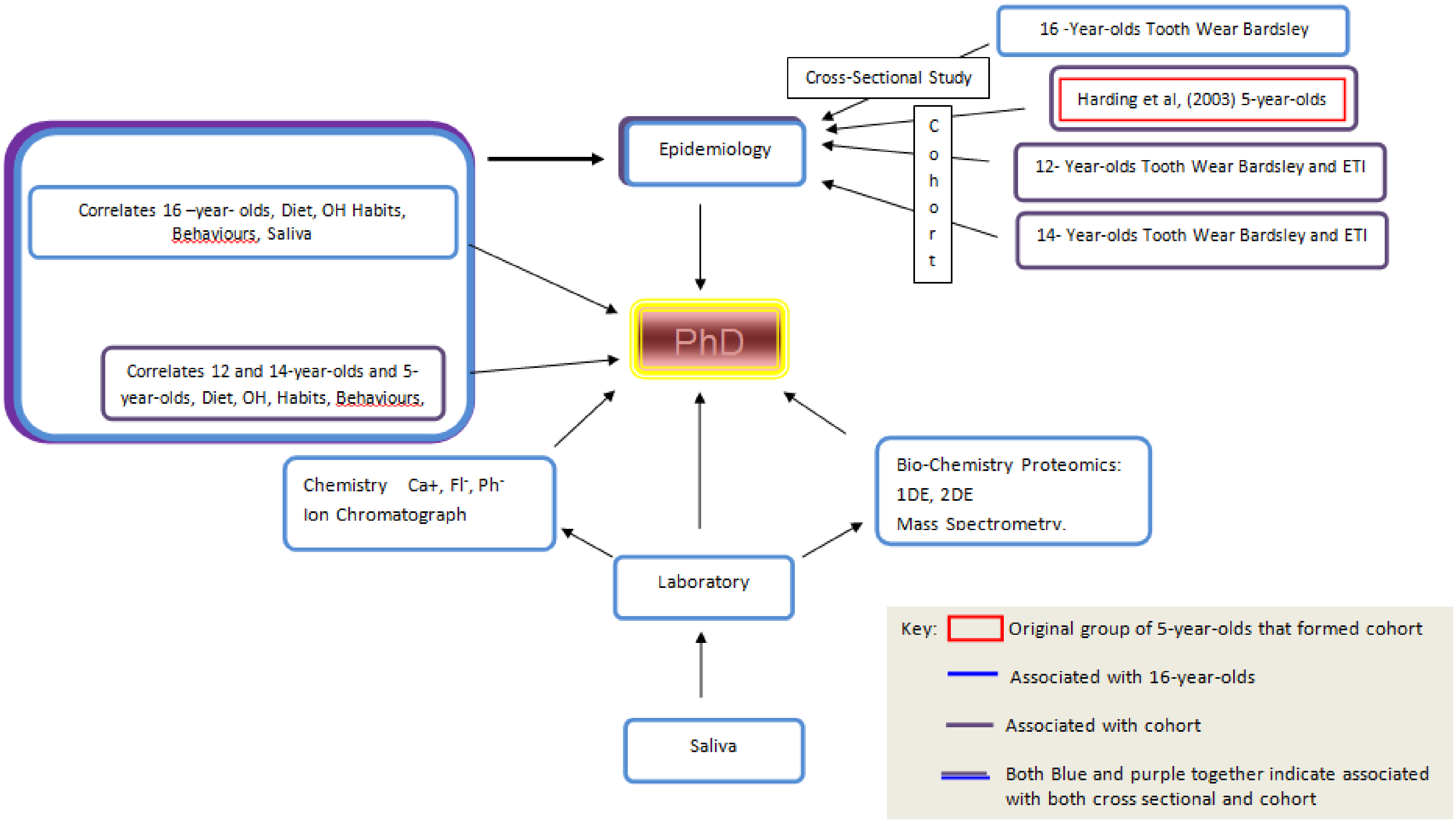
In the cross sectional study, the parameters measured for saliva were; flow rate, protein concentration, carbonyl concentration, fluoride, phosphate and calcium concentration in both resting and stimulated saliva. For a selected small group 1-DE and 2-DE analysis were conducted. Saliva collection was conducted at the participants school. The need to preserve as much saliva as possible for the analysis of protein concentration meant that neither buffering capacity or pH were determined.

Water fluoridation in Ireland

Fluoridation of public piped water supplies was introduced to Ireland through the Health (Fluoridation of water supplies) Act 1960. Approximately 73% of the Republic of Ireland is served by a fluoridated public piped water supply (Whelton et al., 2006).

The above image reproduced from the British Soft Drinks Association (BDSA) website visually depicts the range of drinks considered as soft drinks. The same descriptors were used in this research project. http://www.britishsoftdrinks.com/soft-drinks [Accessed April 7th 2015]

Schematic representation of the research project



Chapter 1 **Introduction**

1.1 Introduction

In this chapter, the key themes of the research project are introduced. In chapter 2, the literature that informed both the epidemiological and laboratory methods, is presented and discussed. This is followed by the methods adopted and the results achieved. The final chapters discuss the importance of the study findings, the value that could be achieved from the early identification of tooth wear and the potential value of the salivary profile in tooth wear. The thesis closes with recommendations for future research.

Tooth wear is not a new condition, but masked in previous years or deemed of lesser concern because of a high caries prevalence. It was actually described in one of the first dental textbooks in the late 1700's (Hunter, 1803), GV Black (1908) estimated that the prevalence of dental erosion was less than 0.1%, at the turn of the last century (Black, 1908). Anthropologists have identified tooth wear in dental remains unearthed from archaeological sites (Eshed et al., 2006; Kaidonis, 2008; Liu et al., 2010). Anthropologists regard the human jaws and the dentition as an extremely rich source of information. The shape and size of the teeth and jaws have provided invaluable information for the study of the evolution of human kind. More particularly from the point of view of this research project, the prevalence and pattern of tooth wear in human remains of those who lived in different millennia have been regarded as an important information source (Forshaw, 2014). They have provided information on the type of diet our distant ancestors consumed during life. Extensive wear on the occlusal surfaces of premolar and molar teeth has been useful to help distinguish between the hard fibrous foods consumed during the era of the human hunter gatherer and the softer plant foods consumed as agricultural practice advanced (Forshaw, 2014). Whilst, archaeological excavations allow the study of teeth and jaws from different eras, in more recent times epidemiological studies are being undertaken with increasing frequency to investigate the problems of tooth wear in living populations. Interestingly the study of the extent and pattern of tooth wear and the association with dietary patterns in different cultures has also been a feature of many of these studies. Indeed, although dental caries and periodontal disease have dominated the interests of the dental scientific community over the last century, and certainly dental caries for some child and teenage populations is still a major dental public health problem (Petersen, 2005; 2009). There are more and more studies now being undertaken to measure the prevalence of tooth wear and its link with modern day lifestyles and dietary choices.

Tooth wear is a natural physiological phenomenon, and is not associated with bacterially mediated destruction of the dentition. The increased retention of teeth into middle and old age clearly means that the clinical evidence for the presence of the condition is now more apparent (Harford, 2009; Kelly et al., 2000; Whelton et al., 2007). This together with increased longevity adds to the observed increase in the prevalence of the condition in the population (Central Statistics Office, 2011). These two factors contribute to the emergence of tooth wear as a major dental public health problem and heightened interest from researchers and clinicians. In younger populations, there are a number of factors that may have contributed to the increased interest and prevalence. One of the factors given for the increase is the increase in sales of carbonated/ fizzy (acidic) drinks and the increased frequency of acidic intakes. Evidence for these issues will be reviewed in chapter 2.

The evidence to support interventions for the prevention of tooth wear are limited (UK Department of Health, 2014). Perhaps this is a missed opportunity as early identification of risk factors and risk indicators for tooth wear could assist in maintaining a sound, healthy and functioning dentition throughout life. Surveillance is a key element of public health. Early identification of risk factors and risk indicators for tooth wear, which are common to other conditions and diseases supports both an upstream and common risk factor approach to health promotion and prevention (Watt, 2007; Watt and Sheiham, 2012). This holistic approach is important when both life expectancy and the retention of natural teeth are increasing (Harford, 2009; Central Statistics Office, 2011; Kelly et al., 2000; Whelton et al., 2007). Different methods are being developed to manage the ageing dentition with an emphasis on respecting the needs and abilities of the particular individual (Ericson, 2004; Pontefract, 2002; World Health Organisation, 2013b). For successful outcomes in population oral health, the importance of a life course approach needs to be emphasised.

1.1.1 Tooth wear in the context of this research project

In this research project, the term used is tooth wear; this term has been used in many anthropological studies. Other researchers use the term tooth surface loss (TSL), erosive tooth wear or lately acid wear. Tooth wear in this research emphasis the combined aetiologies of abrasion, attrition and erosion. The term tooth wear is selected since, (i) both studies in the research are epidemiological studies, (ii) a specific diagnosis at the time of the clinical examination was not made and (iii) the aetiologies do not occur alone but in varying combinations that are synergistic.

The aetiologies of abrasion, attrition, erosion and abfraction are defined by the manner in which tooth mineral is lost from the tooth surface.

Attrition: The progressive loss of hard tooth tissue caused by chewing or grinding of opposing teeth.

Abrasion: The progressive loss of hard tooth tissue caused by mechanical factors other than chewing or tooth-to-tooth contacts.

Erosion: The progressive loss of hard tooth tissue by chemical dissolution that does not involve bacteria, the acid involved may originate from within (intrinsic), or externally such as diet or place of work (extrinsic) (Pindborg, 1970).

Abfraction: The loss of hard tooth tissue when excessive cyclic, non-axial tooth loading leads to cusp flexure and stress concentration in the vulnerable cervical region of teeth. Such stress is thought to directly or indirectly contribute to the loss of cervical tooth substance (Michael et al., 2009).

When enamel demineralises it becomes more susceptible to both abrasion and attrition, so although erosion may be considered the dominant aetiology, the other processes co-exist (Lussi et al., 2011a). Tribology the science and engineering of interacting surfaces in relative motion including friction, lubrication and wear is frequently used to emphasise that abrasion, attrition and erosion are unlikely to act independently and more commonly interact together (d'Incau et al., 2012; Lewis and Dwyer-Joyce, 2005; Mair, 1992). Thus, tooth wear and the loss of dental hard tissue depends on complex mechanisms, synchronous or sequential, synergistic or additive that can make it difficult to identify the true origin (d'Incau et al., 2012). Abfraction as an aetiology was excluded in this research, because no participant was older than 17-years (Michael et al., 2009).

1.1.2 Epidemiology of tooth wear

In epidemiology, cross sectional studies are an ideal means of establishing prevalence, and statistical analysis can assist in identifying associations between particular explanatory or independent variables and outcome or dependent variables. Cross sectional studies are not a means to establish that the explanatory variables caused the outcome. Longitudinal or cohort studies can be either prospective or retrospective. Longitudinal studies add to our knowledge through demonstrating associations and the sequencing of events.

This research consists of two studies; (a) A cross sectional study with a stratified, random sample of 16-year-old transition year students in the counties of Cork and Kerry in Ireland, and (b) A prospective longitudinal study in which the cohort was examined at age 5-years (Harding et al., 2003), age 12-years (Harding et al., 2010) and age 14-years. Conducting a study with teenagers permits the investigation of cumulative wear on the early permanent dentition, which by age 16-years has had a nine or ten-year exposure to the oral environment (Berkovitz et al., 2009). The combination of a cross sectional study and a longitudinal study enabled MH to address some of the challenges faced by researchers in the field of tooth wear and epidemiology (Bartlett et al., 2008; Bartlett et al., 2011b; Ganss and Lussi, 2008; Huysmans et al., 2011; Milosevic, 2011; Young et al., 2008).

Systematic reviews and a meta-analysis of the prevalence of tooth wear and tooth erosion for both the primary and permanent dentition have been conducted (Kreulen et al., 2010; Salas et al., 2014; Van't Spijker et al., 2009). Although much of the 'newer' epidemiology of tooth wear in Europe in particular child and teenage populations was originating from the UK in the 1990's, research was also emerging from Switzerland and the Nordic countries, along with the development of the Lussi index to measure dental erosion (Lussi et al., 1991). There was some work from the US (Bartlett et al., 1999; Deery et al., 2000). The many indices that have been developed for the measurement of tooth wear will be discussed in chapter 2.

1.1.3 Dental hard tissues

Teeth are composed of three mineralised tissues (enamel, dentine and cementum) that surround an inner core of loose connective tissue, the dental pulp. Enamel is of ectodermal origin while dentine, cementum and dental pulp are of ectomesenchymal origin. Dentine forms the bulk of the tooth and is covered coronally by enamel and in the root by cementum. Tooth enamel is thickest over the cusps and incisal edges and thinnest at the cervical margin. Over the cusps of unworn permanent teeth, the enamel is approximately 2.5 mm in thickness, becoming very thin at the cervical margin. The thickness of enamel varies between teeth and between individuals. Both enamel and dentine contain a carbonated calcium-deficient hydroxyapatite (HA) usually notated as $Ca_{10} (PO_4)_6(OH)_2$, (Lussi et al., 2011a). The hydroxyl ion, the end member of the complex apatite group, can be replaced by fluoride, chloride or carbonate. The hardness and the resistance of enamel to wear are attributed to the intricate crystallite orientation of the enamel prisms. Dental enamel is resistant to wear with an annual wear rate calculated at 30 to 40 µm in one study (Lambrechts et al., 1989), and between 15µm and 10.7µm over a 12-month period for abrasion and attrition, calculated using study models (Pintado et al., 1997; Rodriguez et al., 2012). On the other hand, the solubility of tooth mineral, apatite is dependent on the pH (hydrogen ion concentration) and the levels of the minerals present. The critical pH for apatite dissolution is 5.5 (Stephan, 1944), in the pH range 2 – 6 there is a tenfold increase in apatite solubility for each unit change in pH. Tooth enamel exposed to acid on a continual basis will lead to exposure of the underlying organic matrix of dentine. The effect on the dentine matrix is not yet fully understood, because of its higher organic content. Both the hardness and the solubility of tooth enamel add to the complexity in measuring tooth wear *in vivo*.

1.1.4 Saliva - maintaining oral health and minimising tooth wear

The importance of saliva to oral health and the dental hard tissues possibly is best appreciated through the ravages and discomfort that develop in its absence (Young et al., 2001). Saliva is produced by the three major paired salivary glands, the parotid, submandibular and sublingual and the many minor salivary glands (Figure 1-1), it is the first line of defence in the oral cavity. The submandibular gland contributes predominantly to resting saliva, while the parotid contributes to stimulated saliva.

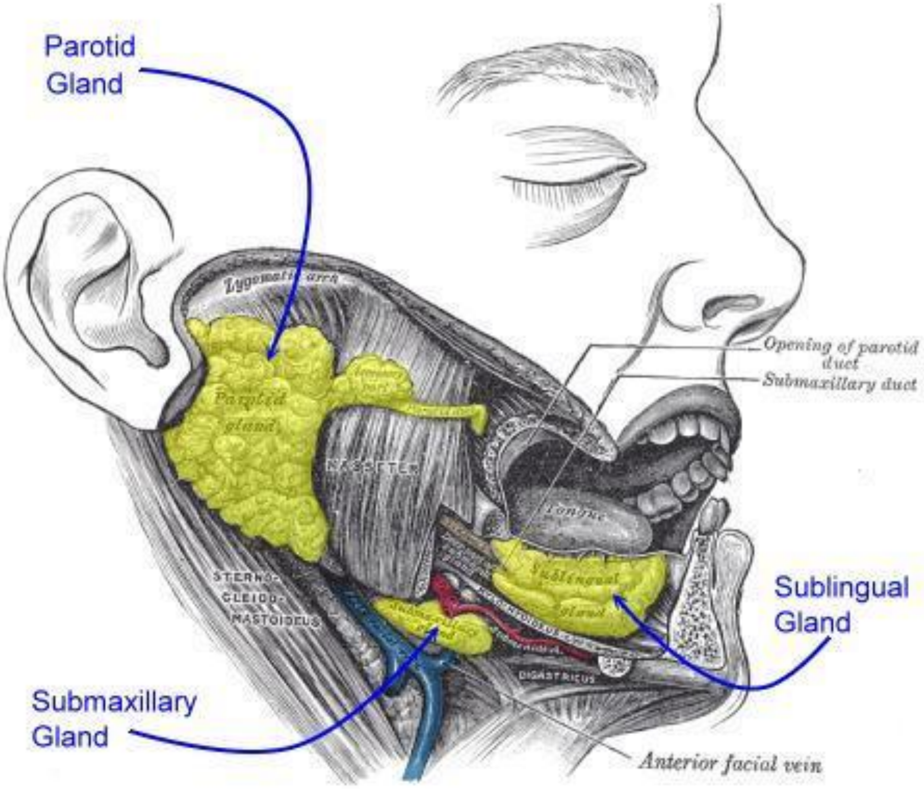


Figure 1-1: Cross section displaying the three major saliva glands

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When the functions of saliva are considered, it is evident why the absence may have such deleterious effects on both the hard and soft tissues of the oral cavity. By what means saliva that is almost entirely water (99%) with only ~ 1% electrolytes, proteins and glycoproteins can have such a significant impact makes it the subject of much research. The focus of research on the protein profile of saliva has grown with the development of new methods and proteomic technologies (Helmerhorst et al., 2006; Helmerhorst and Oppenheim, 2007; Hu et al., 2007; Oppenheim et al., 2007; Yan et al., 2009). Saliva bathes the hard and soft tissues in a thin layer of no more than 10 µm in thickness. Despite coating surfaces in a thin film that is only microns in thickness and being 99% water, saliva quality and quantity effect tooth wear. Both the quantity and quality of saliva have been considered with respect to both erosive tooth wear and tooth wear associated with attrition (Al-Dlaigan et al., 2002b; Bartlett et al., 1998). The prevalence of tooth wear in young teenagers and the multifactorial aetiology of tooth wear prompted the author to investigate whether alterations in salivary proteins at a relatively early time point in the life course would offer some reasons.

1.1.4.1 Calcium, phosphate and fluoride

As previously, mentioned calcium and phosphate are the key components of the tooth mineral, hydroxyapatite. The calcium and phosphate ions present in saliva are essential in maintaining tooth mineral integrity, and the presence of the protein statherin prevents their precipitation in solution. Phosphate in saliva contributes in a small way to the buffering ability of saliva. The presence of fluoride in saliva at a constant low level in the prevention of dental caries is undisputed, in particular the process of promoting remineralisation and reducing demineralisation (Featherstone, 1999). Water fluoridation, is considered the ideal public health measure in caries prevention; since its effectiveness does not require conscious daily cooperation (Centers for Disease Control and Prevention, 1999).

1.1.5 Oxidative modifications of salivary proteins

Oxidative stress arises from an excess of reactive oxygen species (ROS) over antioxidant defences and a break down in the biochemical equilibrium of redox homeostasis. This leads to cellular damage, which includes damage to proteins, lipids and DNA. The production of ROS can be attributable to a number of factors including lifestyle and nutrition (Regano et al., 2008). The presence of oxidatively stressed proteins in saliva is a focus of salivary proteomics research. The most general and well used biomarker of oxidative modification and protein damage is protein carbonyl content (Dalle-Donne et al., 2003b). This is because oxidative damage leads to the oxidation of amino acid residues on proteins, and the formation of protein carbonyls Figure 1-2 and Figure 1-3 (Levine et al., 1994). These protein carbonyls can be either ketones or aldehydes, which are organic compounds which incorporate a carbonyl functional group, C=O.

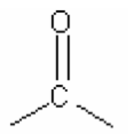

Figure 1-2: Carbonyl group, a carbon double bonded to an oxygen

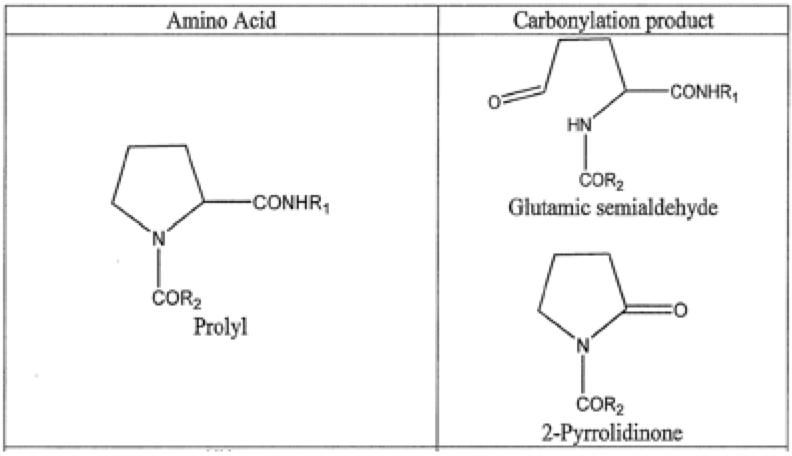

Figure 1-3: Aldehyde or ketone formation through carbonylation of proline (Dalle-Donne 2003a)

1.1.6 Lifestyle, health, habits and practices

Elements of lifestyle, general health, oral hygiene and dietary habits and practices are all associated with tooth wear. Since the preceding factors have been associated with tooth wear, then a multidisciplinary and multiagency collaboration should be a consideration in the prevention and management of tooth wear. Lifestyles and dietary intake by choice or by circumstance impact on general health as well as tooth wear. Given the impact of diet and because tooth wear is cumulative (Van't Spijker et al., 2009) then an investigation of the interplay of the many aetiological factors is essential. Such investigation also permits effective preventive interventions and therapies to be explored. The ultimate goal for oral health should be to maintain a healthy, pain free, functioning dentition minimising the requirement for complex restorative interventions at an advanced age. This philosophy fits with an overall health philosophy of adding '*life to years and not just years to life*'.

1.2 Location of the fieldwork in this research

The research project has two separate studies, the cross sectional study was conducted in the counties of Cork and Kerry. The areas selected were based on the fluoridation status of the school attended and whether the school had a transition year. The longitudinal study conducted in Cork City and County, Southern Ireland (Figure 1-4). The 16-year-old teenagers in the cross sectional study did not participate in the longitudinal study at any time point. They are samples from different populations.

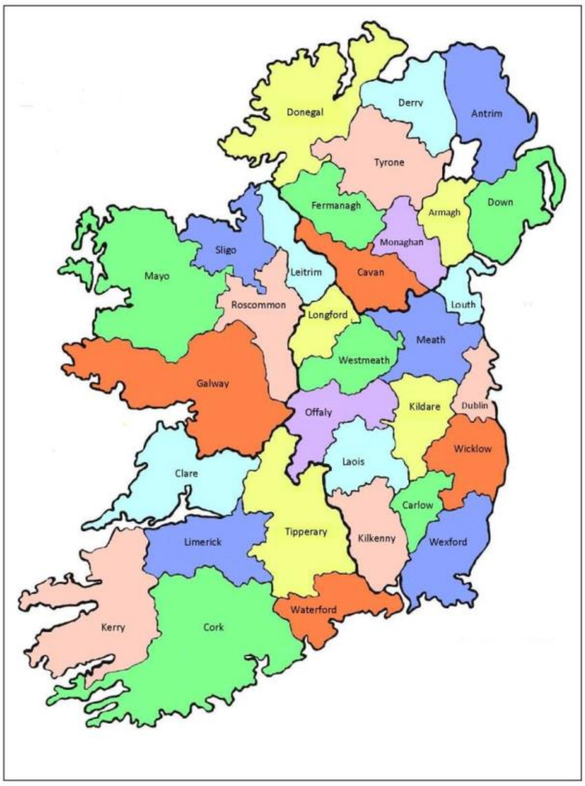

Figure 1-4: Shaded blue and brown areas show the counties of Cork and Kerry

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1.3 Relevance

The relevance of tooth wear to oral health and dental public health encouraged MH to undertake the research presented in this thesis. The observation of tooth wear on a regular basis in child and teenage patients, who presented for care, challenged MH. What was best to manage the condition identified at an early stage and why did some individuals with similar habits and practices not have the consequence of those habits and practices expressed in the same way on the dentition. MH suspected that some of these answers were contained within the quality and quantity of saliva. Early identification could perhaps delay the relatively young age at which some clients are committed to a restorative pathway, experience pain or resign themselves to unsatisfactory dental aesthetics. The identification of salivary properties varying with the presence of tooth wear could assist in explaining some of the individual variation observed. Given that salivary proteins are important contributors to maintaining salivary ion homeostasis, lubrication and buffering capacity, it was important to investigate their contribution. Although consensus may not exist, that tooth wear is a dental public problem, failure to intervene at an early stage is perhaps but delaying the time at which significant investment of scarce resources will be required.

1.4 Aim of the research project

The aim of this research project is to investigate the characteristics, development and determinants of tooth wear among Irish school children.

The aim will be achieved using data collected from two different studies; a cross sectional study and a longitudinal study. The objectives are set out in full at the end of the literature review (p 49).

Chapter 2 **Review of the literature**

2.1 Structure and background to the literature review

The growing interest in tooth wear in the permanent dentition of children, teenagers and young adults is evidenced by the now increasing volume of epidemiological studies appearing from across the globe (Teo et al., 1997; Deery et al., 2000; Ogunyinka et al., 2001; Arnadottir et al., 2003; Luo et al., 2005; Peres et al., 2005; Lussi and Jaeggi., 2006; Saerah et al., 2006; Harding et al., 2010; Al-Zarea., 2012; Bartlett et al., 2013; Fung and Messer, 2013). For this research project, literature searching encompassed PubMed, Google Scholar, textbooks, international conference proceedings, personal communications, the databases of professional organisations, journals and journal 'alerts'. The bibliographic lists at the end of searched papers were also hand checked to ensure completeness. In comparison to dental caries, there are no agreed index ages at which tooth wear is recorded. The literature review therefore includes the various child and teenage age groups reported in the literature. There are two recently completed systematic reviews of tooth wear. The reviews established that, tooth wear increased with age in the primary dentition (Kreulen et al., 2010), similarly tooth wear increased in the permanent dentition for adults (age 20 years and over) (Van't Spijker et al., 2012). In addition to the absence of key ages when tooth wear is reported there is the absence of a single agreed index for the measurement of tooth wear (Kreulen et al., 2010; Van't Spijker et al., 2012). Although of late this is being remedied and work is proceeding in that area, albeit focussed on erosive tooth wear (Bartlett et al., 2008). Non-carious cervical lesions do not form part of this literature review. They are usually reported in older populations (Wood et al., 2008). However, a recent large multicentre study across general dental practices in Europe identified non-carious cervical lesions in the youngest age-group examined (18- 25 years) (Bartlett et al., 2013). This finding could be a consequence of changing patterns of tooth wear. It may also be a consequence of the means by which tooth wear was measured, again it may centre on the fact that the examining dentists were looking for the condition, which is something previously discussed by

Bartlett et al., (1999) when discussing the differing perspectives between Europe and the US.

The review includes the measurement and prevalence of tooth wear, the studies undertaken to measure salivary factors including salivary ions, proteins and oxidative stress and the risk factors and indicators for tooth wear. The review was limited to epidemiological studies for the permanent dentition conducted with children or teenagers unless there was an absence of such information, or development of tooth wear indices necessitated their inclusion. In the absence of *in vivo* studies, some *in vitro* or *in situ* studies were reviewed.

2.1.1 Choice of search terms

The search strategy involved using the keywords either alone or in combination. Tooth wear was included as both two words and as a single word. The terms erosion, and erosive and eros* were also used, because frequently tooth wear in teenagers is assigned to erosion.

2.1.1.1 Measurement and prevalence of tooth wear

("measurement"[All Fields]) AND ("tooth wear"[MeSH Terms] OR ("tooth"[All Fields] AND "wear"[All Fields]) OR "tooth wear"[All Fields]) AND ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms])

2.1.1.2 Tooth wear and saliva and ions and proteins

Tooth wear [All Fields] AND ("saliva"[MeSH Terms] OR "saliva"[All Fields]) AND ("tooth erosion"[MeSH Terms] OR ("tooth"[All Fields] AND "erosion"[All Fields]) OR "tooth erosion"[All Fields] OR ("dental"[All Fields] AND "erosion"[All Fields]) OR "dental erosion"[All Fields])

("tooth wear"[MeSH Terms] OR ("tooth"[All Fields] AND "wear"[All Fields]) OR "tooth wear"[All Fields]) AND ("tooth).

2.1.1.3 Dental erosion and saliva

("saliva"[MeSH Terms] OR "saliva"[All Fields]) AND ("ions"[MeSH Terms] OR "ions"[All Fields]) AND ("tooth erosion"[MeSH Terms] OR ("tooth"[All Fields]

AND "erosion"[All Fields]) OR "tooth erosion"[All Fields] OR ("dental"[All Fields] AND "erosion"[All Fields]) OR "dental erosion"[All Fields])) AND (Review[ptyp])

2.1.1.4 Tooth wear and salivary proteins

("saliva"[MeSH Terms] OR "saliva"[All Fields]) AND ("proteins"[MeSH Terms] OR "proteins"[All Fields]) AND ("tooth wear"[MeSH Terms] OR ("tooth"[All Fields] AND "wear"[All Fields]) OR "tooth wear"[All Fields])

2.1.1.5 Tooth wear and diet

Tooth wear including dental erosion and diet in PubMed: tooth wear [All Fields] AND ("diet"[MeSH Terms] OR "diet"[All Fields]) AND ("tooth erosion"[MeSH Terms] OR ("tooth"[All Fields] AND "erosion"[All Fields]) OR "tooth erosion"[All Fields] OR ("dental"[All Fields] AND "erosion"[All Fields]) OR "dental erosion"[All Fields]) AND erosion[All Fields]

2.1.1.6 Tooth wear and alcohol

("tooth wear"[MeSH Terms] OR ("tooth"[All Fields] AND "wear"[All Fields]) OR "tooth wear"[All Fields]) AND ("tooth erosion"[MeSH Terms] OR ("tooth"[All Fields] AND "erosion"[All Fields]) OR "tooth erosion"[All Fields] OR ("dental"[All Fields] AND "erosion"[All Fields]) OR "dental erosion"[All Fields]) AND ("ethanol"[MeSH Terms] OR "ethanol"[All Fields] OR "alcohol"[All Fields] OR "alcohols"[MeSH Terms] OR "alcohols"[All Fields])

2.1.1.7 Tooth wear and carbonated drinks

("tooth wear"[MeSH Terms] OR ("tooth"[All Fields] AND "wear"[All Fields]) OR "tooth wear"[All Fields]) AND ("tooth erosion"[MeSH Terms] OR ("tooth"[All Fields] AND "erosion"[All Fields]) OR "tooth erosion"[All Fields] OR ("dental"[All Fields] AND "erosion"[All Fields]) OR "dental erosion"[All Fields]) AND ("carbonated beverages"[MeSH Terms] OR ("carbonated"[All Fields] AND "beverages"[All Fields]) OR "carbonated beverages"[All Fields] OR ("carbonated"[All Fields] AND "drinks"[All Fields]) OR "carbonated drinks"[All Fields]).

2.2 Measurement of tooth wear

Exploration of the distribution and determinants of a condition is contingent on the use of a valid and reliable index to measure the condition of interest. The simplest method of measuring any condition is by a count of the number of cases of its occurrence. The simple count works best with unusual conditions of low prevalence and becomes less useful as the prevalence of the condition increases. Case definition also comes into play as the point at which a condition is deemed present or absent will vary according to detection methods and the definitions used. The use of an epidemiological index should help to discriminate between degrees of severity. The measurement methods and criteria used to assign a particular score, form an integral part of the index. Indices to measure tooth wear can be based on the aetiology of the wear, the site of the wear or the appearance. There is, as this chapter will indicate, quite a debate about indices for the measurement of tooth wear and case definition (Bardsley, 2008; Ganss et al., 2006; Ganss and Lussi, 2008). Measurement may be, by a trained clinical examiner either with or without magnification or using instrumentation such as profilometry (Schlueter et al., 2011). The type of tooth wear discussed in this research is macro wear, identified by a trained clinical examiner, with the assistance of good lighting and a dry tooth surface. The buccal/labial, palatal/lingual and occlusal/incisal surface of the permanent teeth in both the maxillary and mandibular arches were examined.

2.2.1 Tooth wear Indices

Tooth wear indices were initially developed to report wear in the permanent dentition in adults, modifications have been made for use in the primary dentition and the permanent dentition in teenage populations. This has led to much debate on the design of an ideal index (Ganss and Lussi, 2008; Schlueter et al., 2011). The most widely used index in epidemiological studies is the Tooth Wear Index (TWI) first described in 1984 (Smith and Knight, 1984), (Table 2-1).

Table 2-1: Smith and Knight Tooth wear Index (TWI)

Score	Surface	Criteria
0	B/L/O/I	No loss of enamel surface characteristics
	C	No loss of contour
1	B/L/O/I	Loss of enamel surface characteristics
	C	Minimal loss of contour
2	B/L/O	Loss of enamel exposing dentine for < $^1/_3$rd of the surface
	I	Loss of enamel just exposing dentine
	C	Defect less than 1mm in depth
3	B/L/O	Loss of enamel exposing dentine for ≥$^1/_3$rd of the surface
	I	Loss of enamel and substantial loss of dentine
	C	Defect less than 1-2 mm in depth
4	B/L/O	Complete enamel loss - pulp exposure – secondary dentine exposure
	I	Pulp exposure or secondary dentine exposure
	C	Defect more than 2 mm in depth - pulp exposure - secondary dentine exposure

B=Buccal, L=Lingual, O=Occlusal, I=Incisal C=Cervical

The TWI was initially developed for use in an adult population and has subsequently been used, in its complete or modified forms, in studies involving both primary and permanent teeth in adolescents and the elderly (Milosevic et al., 1994; O'Brien, 1994; Donachie and Walls, 1996; Smith and Robb, 1996; Bartlett et al., 1998; Kelly et al., 2000; Whelton et al., 2006; Whelton et al., 2007; Harding et al., 2010; Health and Social Care Information Centre, 2011). The original five level ordinal scale codes wear on buccal/labial, cervical, palatal/lingual and occlusal/incisal surfaces of all teeth that are present. Zero (0) indicates no wear and four indicates at or near pulpal exposure. The score assigned is irrespective of how the tooth wear occurred. The TWI (Smith and Knight, 1984) records the more severe forms of wear with grade three (3) indicating wear over $^2/_3$rds of a surface exposing dentine and grade four (4), secondary dentine or pulpal exposure. Smith and Knight, (1984) used the index in a series of investigations which reported on the prevalence of tooth wear firstly in a patient population referred to a dental school and secondly in a study undertaken in general dental practice (Smith and Knight, 1984; Smith and Robb, 1996). Smith and Knight (1984) used the term pathological tooth wear to describe those participants with severe levels of wear likely to require restorative intervention to maintain function. Donachie and Walls (1996) were critical of the choice of the word pathological when they used the index in adults over 45-years. They noted that there was a significant increase in tooth wear with increasing age on all cervical tooth surfaces and on all occlusal/incisal surfaces, in both the maxillary and mandibular arch. They also noted a significant increase in wear on the lingual aspect of the maxillary anterior teeth that would be termed pathological. Steele and Walls (2000) went on then to describe a tooth wear index with partial recording for older adults.

The TWI (Smith and Knight, 1984) although it is the most widely used index, it was not the first index. It is a modification of the index described by Eccles (1979), (Table 2-2). Eccles index (Eccles, 1979) was designed from the examination of 72 clinical records; the index records erosion on the buccal/facial, occlusal and palatal tooth surfaces using the terms Class I, II, IIIa, IIIb, IIIc and IIId, dependent on site and appearance. It was however considered overly complicated, particularly for the more severe lesions. Smith and Knight (1984) did not include the class divisions in the TWI and recorded all wear irrespective of aetiology and did not focus singularly on erosion. Despite the improvements that the TWI made to Eccles index (1979) there are still challenges to be addressed, particularly in young and teenage populations.

Table 2-2: Eccles Index

Class	Surface	Criteria
Class 1		Early stages of erosion; absence of developmental ridges; smooth surfaces of maxillary incisors and canines
Class 2	Facial	Dentine involved for less than 1/3rd of the surface; two types: **Type 1 (commonest):** ovoid-crescentic in outline, concave in cross section, differentiate from wedge shaped abrasion lesions **Type 2:** Irregular lesion entirely within crown, punched out
Class 3a	Facial	More extensive destruction of dentine affecting anterior tooth part of the surface, but some are localised and hollowed out
Class 3b	palatal/lingual	Dentine eroded for more than 1/3rd of the surface area; incisal edges translucent; dentine is flat or hollowed out often extending into secondary dentine

Class	Surface	Criteria
Class 3c	Incisal/occlusal	Surfaces involved into dentine, appearing flattened or with cupping; undermined enamel restorations are raised above the surroundings
Class 3d	All	Severely affected teeth, where both labial and lingual surfaces may be affected; teeth are shortened

With the TWI (Smith and Knight, 1984) tooth wear present in enamel is recorded with a single grade. This is reasonable when one considers that the index was originally designed to record the restorative need in a population. A single score in enamel may be less appropriate in the primary or early permanent dentitions, or in a preventive treatment paradigm. If tooth wear is identified at different stages in enamel, it allows the opportunity for early identification and a focus on preventive therapies. The temporal relationship of an index developed in 1984 (Smith and Knight, 1984) and a population examined in 1995 at age 45 years and older must be considered with respect to the present. The populations most probably have different food choices available and somewhat different lifestyles (Brug and Klepp, 2007; Lawrence and Worsley, 2007; Smith and Robb, 1996). The recent literature review on the prevalence of tooth wear (Kreulen et al., 2010) in children and teenagers converted the various indices used to record tooth wear in child and teenagers (up to age 18-years) to a common denominator to permit comparison. The common denominator was ‘dentine visible’, the results indicated that tooth wear increased with age in the primary dentition but an increase with age to 18 years in the permanent dentition was not substantiated (Kreulen et al., 2010). The reviews that have been carried out acknowledge that there are difficulties when recording tooth wear *in vivo.* The following have all been identified as challenges; firstly, the different terminologies used to describe erosion, abrasion or attrition. Then, the difficulties acknowledged in defining a reference point, deciding whether dentine is visible and thirdly, trying to agree on a suitable common denominator for comparisons when different indices are used (Azzopardi et al., 2000; Bartlett and Dugmore, 2008; Ganss and Lussi, 2008; Ganss et al., 2011). Berg-Beckhoff et al., (2008) in a narrative review discussed the properties of the ideal index (Burt and Eklund, 2005) and the challenges that arise with tooth wear including that tooth wear is a normal component of ageing. If the ideal index to record tooth wear existed or if tooth wear was easily quantified it is likely that the number of available indices would be less. The Basic Erosive Wear Examination (BEWE) index (Table 2-3) was introduced as a tool for general practice to allow comparison with other more, discriminate indices (Bartlett et al., 2008; Young et al., 2008), however it is not possible to make direct comparisons between the BEWE (Bartlett et al., 2008), and the TWI (Smith and Knight, 1984). The Exact Tooth wear Index (ETI) (Bartlett et al., 2011b) (Table 2-6) was constructed in an attempt to overcome some of the challenges expressed with respect to the measurement of tooth wear in epidemiological studies particularly in young populations. The number of levels at which tooth wear could be recorded in dental enamel was increased, to allow for the recording of the lateral spread of tooth wear in enamel. The design of the index is such that the scores can be combined when required to allow comparison with the TWI (Smith and Knight, 1984). A recent workshop albeit on dental erosion concluded that there was still research required in the area of epidemiological indices (Lussi et al., 2011b; Shellis et al., 2011). Tooth wear a condition rather than a disease shares similar challenges to those experienced with the measurement of periodontal disease and early caries lesions (Pitts, 2004; Leroy et al., 2010).

2.2.1.1 Basic Erosive Wear Examination (BEWE) Index

The aim of the BEWE was to develop an index to promote a consensus within the scientific community and provide an internationally accepted, standardised and validated index that allowed the integration of results from previous studies. The index was developed for use with adults in general dental practice to raise awareness of dental erosion, erosive tooth wear and to assist in its management. The scoring system is such that the most severely affected surface in each sextant is recorded on a four-level ordinal scale (0–3) and summated. In a new departure for tooth wear indices, the cumulative score is matched to risk levels which are to guide the management of the condition, however similar to Smith and Knight’s (1984) impression of pathological wear risk levels may need to be reviewed (Young et al., 2008), (Table 2-3).

Table 2-3: The scores for the BEWE Index

Class	Most severely affected surface in each sextant	Criteria
0		No erosive tooth wear
1		Initial loss of surface texture
2*		Distinct defect, hard tissue loss <50% of the surface area
3*		Hard tissue loss ≥50% of the surface area

* in scores 2 and 3 dentine is often involved

When the sextants are summated, the maximum score is 18, the table setting out the risk level is then consulted (Table 2-4) and (Table 2-5).

Table 2-4: Organisation of the teeth in sextants for BEWE

Sextant 1 Teeth 17 - 14	**Sextant 2** Teeth 13 - 23	**Sextant 3** Teeth 24 - 27	Score = Sum
Sextant 4 Teeth 37 - 34	**Sextant 5** Teeth 33 - 43	**Sextant 6** Teeth 44 - 47	

Table 2-5: Risk levels associated with BEWE

Risk Level	Cumulative score for all sextants	Management
None	≤ 2 *	Routine maintenance and observation Repeat at 3-year intervals
Low	Between 3 and 8 *	Oral hygiene and dietary assessment, and advice, routine maintenance and observation Repeat at 2-year intervals
Medium	Between 9 and 13 *	Oral hygiene and dietary assessment, and advice, identify the main aetiological factor(s) for tissue loss and develop strategies to eliminate respective impacts. Consider fluoridation measures or other strategies to increase the resistance of tooth surfaces. Ideally, avoid the placement of restorations and monitor erosive wear with study casts, photographs, or silicone impressions Repeat at 6–12-month intervals

Risk Level	Cumulative score for all sextants	Management
High	14 and above *	Oral hygiene and dietary assessment, and advice, identify the main aetiological factor(s) for tissue loss and develop strategies to eliminate respective impacts. Consider fluoridation measures or other strategies to increase the resistance of tooth surfaces. Ideally, avoid restorations and monitor tooth wear with study casts, photographs, or silicone impressions. Especially in cases of severe progression consider special care that may involve restorations Repeat at 6–12-month intervals

* May need to be reviewed

The BEWE has been used in both adult and adolescent populations (Arnadottir et al., 2010; Bartlett et al., 2013; Vered et al., 2014) along with efforts to validate it (Holbrook et al., 2014). The BEWE was also modified for use in the primary dentition (Mantonanaki et al., 2013), although one would have to question the need to record the lower anterior sextant in five-year-old children. The low levels of both inter-examiner and intra-examiner agreement identified in general practice suggest that BEWE results be interpreted with some caution (Dixon et al., 2012). Given the ease of use of the index, it is surprising that the authors associate a time interval with respect to repeating the examination in the first two risk levels. One would think it should be used at each opportunity that presents. It would also seem that the multifactorial nature of tooth wear is not actually considered. With respect to epidemiological studies some researchers assert that tooth wear of a specific aetiology recorded, and all wear should be collectively reported as tooth wear or tooth surface loss (TSL) (Milosevic, 2011).

2.2.1.2 Exact Tooth wear Index ETI

The ETI (Bartlett et al., 2011b) was also developed to overcome some of the limitations of the TWI (Smith and Knight, 1984); similar to it, it makes no mention of possible or probable aetiologies. The ETI (Bartlett et al., 2011b) is based on the TWI (Smith and Knight, 1984) and grades wear on the buccal/labial, cervical, palatal/lingual and the occlusal or incisal surfaces of each tooth. Each tooth is given a score first for wear of enamel and then for dentine. The severity of wear is graded at five levels in enamel and six in dentine with a range from 0 through to $<{}^{1}/_{10}{}^{th}$, $<{}^{1}/_{3}{}^{rd}$, ${}^{1}/_{3}{}^{rd}$ to ${}^{2}/_{3}{}^{rds}$, $>{}^{2}/_{3}{}^{rds}$ for both enamel and dentine, and for dentine only the exposure of secondary dentine or pulpal exposure (Table 2-6). The index has been used to a lesser degree than the BEWE (Bartlett et al., 2011b; Fares et al., 2009), and challenges have presented with the lower levels when scoring tooth wear in enamel, at the lower levels (Table 2-6).

Table 2-6: Exact Tooth Wear Index (Bartlett et al., 2011b)

(A) ETI for Enamel	
0	No tooth wear: no loss of enamel characteristics or change in contour
1	Loss of enamel affecting less than $<{}^{1}/_{10}{}^{th}$ of the scored surface
2	Enamel loss affecting between $<{}^{1}/_{10}{}^{th}$ and ${}^{1}/_{3}{}^{rd}$ of the scored surface
3	Enamel loss affecting at least ${}^{1}/_{3}{}^{rd}$ but less than ${}^{2}/_{3}{}^{rds}$ of the scored surface
4	Enamel loss affecting ${}^{2}/_{3}{}^{rds}$ or more of the scored surface
(B) ETI for Dentine:	
0	No dentinal tooth wear: no loss of dentine
1	Loss of dentine affecting less than $<{}^{1}/_{10}{}^{th}$ of the scored surface
2	Dentine loss affecting between $<{}^{1}/_{10}{}^{th}$ and ${}^{1}/_{3}{}^{rd}$ of the scored surface
3	Dentine loss affecting at least ${}^{1}/_{3}{}^{rd}$ but less than ${}^{2}/_{3}{}^{rds}$ of the scored surface
4	Dentine loss affecting ${}^{2}/_{3}{}^{rds}$ or more of the scored surface, no pulpal exposure
5	Exposure of secondary dentine formation or pulpal exposure.

2.2.1.3 Simplified Erosion Partial Recording System (SEPRS)

The SEPRS is a relatively new index for use in teenagers, it focuses on the buccal and lingual surfaces of the maxillary anterior teeth and the occlusal surfaces of the first permanent molars (Hasselkvist et al., 2010). The SEPRS added scoring of the occlusal surface of the first permanent molar to the index designed by (Johansson et al., 1996) (Table 2-7). Documenting all tooth wear present on these surfaces as erosion will, however, over report erosion and overlook wear that is associated with the aetiologies of attrition and abrasion. It may also contribute to a victim blaming approach to prevention, rather than one firmly rooted in the tenets of the Ottawa charter for health promotion (World Health Organisation, 1986). There are a number of similarities between the ETI (Bartlett et al., 2011b) and the SEPRS (Hasselkvist et al., 2010), particularly in trying to capture the changes that may occur in enamel on the maxillary anterior teeth. However, in an epidemiological study focussing only on erosion ignores possible synergies and risk factors acting in concert.

Table 2-7: Simplified erosion partial recording systems (SEPRS)

	Ordinal scale used for grading severity of dental erosion on buccal and palatal surfaces of maxillary anterior teeth
0	No visible changes, developmental structures remain. Macro-morphology intact.
1	Smoothened enamel, developmental structures have totally or partially vanished. Enamel surface is shiny, matt, irregular, ‘melted’, rounded or flat. Macro-morphology generally intact.
2	Enamel surface as described in grade 1. Macro-morphology clearly changed faceting or concavity formation within the enamel, no dentinal exposure.
3	Enamel surface as described in grades 1 and 2. Macro-morphology greatly changed (close to dentinal exposure of large surfaces) or dentin surface exposed by $^1/_3{}^{rd}$
4	Enamel surface as described in grades 1, 2 and 3. Dentin surface exposed by > $^1/_3{}^{rd}$ or pulp visible through the dentin.
	Ordinal scale used for grading cuppings on occlusal surfaces of first permanent molars
0	No cupping/intact cusp tip
1	Rounded cusp tip. Changed morphology compared to the assumed original anatomy at time of eruption.
2	Cupping ≤1 mm
3	Cupping >1 mm
4	Fused cuppings: at least two cuppings are fused together on the same tooth

2.2.2 Monitoring tooth wear

The monitoring of tooth wear for an individual or in an intervention study or taking measurements in a longitudinal study in a defined time period provides challenges. Firstly, because indices may not be sufficiently discriminatory e.g. TWI (Smith and Knight, 1984) or the risk level is based on summated sextant scores e.g. BEWE. Other difficulties are when the index focusses only on erosion or another aetiology and because there is a degree of normal physiological tooth wear that occurs, which produces a rising baseline. The challenges faced when recording tooth wear in enamel and dentine and the ability of a clinical examiner to accurately record it have been expressed by a number of authors (Berg-Beckhoff et al., 2008; Ganss and Lussi, 2008; Holbrook and Ganss, 2008; Ganss et al., 2011; Milosevic, 2011; Schlueter et al., 2011). However, despite reservations of visual diagnosis or recording wear at the dentine level, Mulic et al., (2010) compared the visual erosion dental examination (VEDE) with BEWE and found that the highest agreement was score 0 (no wear) and score 3 exposure of dentine. Ganss et al., (2006) demonstrated that the examination of teeth by examiners, which was then followed by the histological examination, indicated high specificity (approximately 88%) with lower sensitivity (approximately 65%) for ‘dentine exposed’. Importantly though, is that despite dentists scoring a tooth as ‘dentine exposed’ when in fact there was still an enamel layer of 0.5mm thickness present, 89% of the examiners were in agreement. One therefore must be quite explicit in the reason why measurement is being carried out.

The different indices in either their entirety or modifications and the studies in which they were used in children and adolescent populations are set out in Table 2-8. It is apparent from the table that despite the shortcomings mentioned for the TWI (Smith and Knight, 1984), it is the most frequently used index, either in its totality, partial or modified forms.

Table 2-8: Indices used in child and adolescent studies

Index	Year	Teeth Scored Permanent (P adult) or Primary (pr primary)	Surfaces Scored	Modifications of or Use of Index
(Eccles, 1979)	1979	P	Buccal, Palatal and Occlusal/Incisal	(Järvinen et al., 1988) (Smith and Knight, 1984)
(Smith and Knight, 1984)	1984	P and pr	Buccal, (Cervical) Palatal and Occlusal/Incisal	(Millward et al., 1994) (Milosevic et al., 1994) (Bartlett et al., 1998) National Surveys UK, , US (O'Brien, 1994) (Williams et al., 1999) (Deery et al., 2000) (Walker et al., 2000) (Al-Dlaigan et al., 2001b) (Al-Majed et al., 2002) (Nunn et al., 2003) (National Survey, England, Wales, NI (ONS) ., 2005) (Bardsley et al., 2004) (Dugmore and Rock, 2004) (Luo et al., 2005) (Centers for Disease Control and Prevention, 2005)

Index	Year	Teeth Scored Permanent (P adult) or Primary (pr primary)	Surfaces Scored	Modifications of or Use of Index
				(Whelton et al., 2006) (El Karim et al., 2007) (de Carvalho Sales-Peres et al., 2008) (Harding et al., 2010) (Bardolia et al., 2010) (Gurgel et al., 2011) (Health and Social Care Information Centre, 2011) (Abu-Ghazaleh et al., 2013) (Health and Social Care Information Centre, 2015)
(Linkosalo and Markkanen, 1985**)**	1985	P		(Linkosalo and Markkanen, 1985)
(Aine et al., 1993**)**	1993	pr	Tooth is scored Scores for erosion present in gastro-oesophageal reflux	
(Johansson et al., 1996**)**	1996	P and pr	Maxillary Buccal, Palatal	(Hasselkvist et al., 2010)
(Lussi, 1996**)**	1996	P	Full mouth,	(van Rijkom et al., 2002) (Truin et al., 2005) (Jaeggi and Lussi, 2004) (El Aidi et al., 2008)

Index	Year	Teeth Scored Permanent (P adult) or Primary (pr primary)	Surfaces Scored	Modifications of or Use of Index
(Larsen et al., 2000**)**	2000	P	Buccal, Palatal, Occlusal/Incisal/ and cervical. In addition photographs and models	(Luo et al., 2005)
(O'Sullivan, 2000**)**	2000	P and pr	Buccal, Palatal, incisal incisors And first molars maxillary and mandibular based on (Eccles, 1979) combination of surfaces affected	(Caglar et al., 2005) (Peres et al., 2005) (Wiegand et al., 2006) (Correr et al., 2009) (Wang et al., 2010) (Aguiar et al., 2014)
(Al-Malik et al., 2001b**)**	2001	Pr	Buccal, Palatal maxillary incisors	(Al-Malik et al., 2001a) (Harding et al., 2003)
(Bartlett et al., 2008**)**	2008	P and pr	Buccal, Palatal	(Bartlett et al., 2013) (18-25 youngest age-group) (Arnadottir et al., 2010) (Mantonanaki et al., 2013) (Vered et al., 2014)

2.3 Previous reviews of tooth wear

As previously mentioned, Kreulen et al. (2010) conducted a systematic review of tooth wear in children and adolescents, searching the literature from 1980 to 2008. From 29 reviewed papers, they reported a wide range in the prevalence of tooth wear involving dentine. The prevalence ranged from 0% to 82% for deciduous teeth in children up to 7 years. In the selected studies, tooth wear was recorded with nine different indices. They concluded that tooth wear increased with age in the primary dentition. They were unable to substantiate that tooth wear in the permanent dentition increased with age up to 18 years. A number of narrative reviews on tooth wear including erosive tooth wear have been published. Nunn et al., (2003) using data collated from the 1993 UK children's dental health survey (O'Brien, 1994) and the dental report of the two National Diet and Nutrition Surveys (NDNS) (Hinds and Gregory, 1995; Walker et al., 2000), compared prevalence data that indicated, similar to Kreulen et al., (2010) that dental erosion increased between different age cohorts. All three studies reviewed by Nunn et al., (2003) had used the same index. Taji and Seow (2010) conducted a narrative review of erosion in children and articulated the challenges that present with measurement. They also concluded that erosion is unlikely to be the only aetiology present and reported on the associated dietary and personal factors. Salas et al., (2014) recently concluded in a systematic review and meta-regression analysis that the estimated prevalence of erosive wear in the permanent teeth of children and adolescents is 30.4%; this type of analysis is a new departure for tooth wear methodology. They also indicated that in the analysis there was high heterogeneity between studies. They made the point that the TWI (Smith and Knight, 1984) presents the highest prevalence rates of dental erosion. However, the TWI (Smith and Knight, 1984) was never designed to measure erosion. Perhaps the review of Salas et al., (2014) points to the fact that, making distinctions based on aetiology in an epidemiological study is erroneous.

Figure 2-1 and Figure 2-2 both demonstrate tooth wear present at age 12 on maxillary permanent incisors of one subject in the longitudinal study carried out in this research. The reduced crown height, chipping along the incisal edge and loss of enamel surface characteristics on the labial surface are evident (Figure 2-1). Examining the image would suggest that there is possibly merit in retaining the 3d score of Eccles (1979) 'severely affected teeth, where both labial and lingual surfaces may be affected'; 'teeth are shortened'. However, unless quantified it would be of little value in determining tooth wear between time points. In Figure 2-2 the loss of enamel with evidence of a retained rim of enamel at the gingival margin can be seen in this 12-year-old participant in the longitudinal study.

Figure 2-1: Tooth wear present on the labial and incisal surfaces of maxillary incisors, age 12

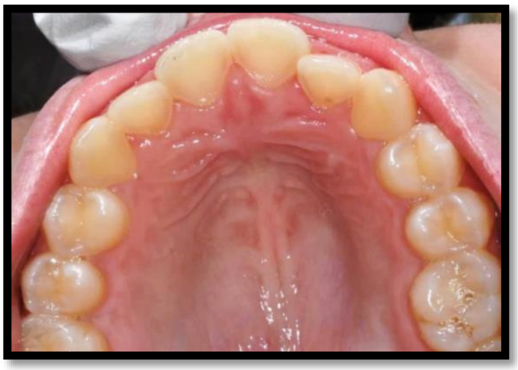


Figure 2-2: Tooth wear present on the palatal surfaces of maxillary incisors, age 12

2.4 *Prevalence of tooth wear in Ireland*

In Ireland, tooth wear was first recorded in adults in the mid-20th Century (Hooton et al., 1955) and since then two national surveys of adult oral health recorded the prevalence of tooth wear (O'Mullane and Whelton, 1990; Whelton et al., 2007). The national survey of children's oral health in Ireland recorded tooth wear in the 12-year-old and 15-year-old age groups (Whelton et al., 2006). In the studies conducted by Whelton et al., (2006) and (2007), the same modified TWI was used to record tooth wear in both studies. The index was also used in the adult dental health survey in the UK (Kelly et al., 2000) and England, Wales and Northern Ireland (Health and Social Care Information Centre, 2011). The prevalence of tooth wear available from the Irish national surveys (Whelton et al., 2006; Whelton et al., 2007) is presented in Table 2-9 and in Figure 2-3 for the 15-year-old age group.

Table 2-9: Levels of maximum tooth wear by age group and gender in Ireland

Age	Gender	N	% Maximum tooth wear			% Any Wear
			Mild	Moderate	Severe	
12	Male	1879	18.5	1.0	0.2	19.6
	Female	1982	14.8	0.5	0.0	15.3
15	Male	1772	29.9	1.6	0.2	31.7
	Female	1745	25.8	1.2	0.5	27.5
16-24	Male	510	39.1	4.5	0.2	43.7
	Female	681	31.2	0.9	0.3	32.4
35-44	Male	363	65.2	15.2	1.7	82.1
	Female	596	63.0	6.5	0.8	70.3
65+	Male	208	48.1	38.1	8.5	94.7
	Female	198	59.8	25.8	5.7	91.3

Mild: tooth wear just exposing the dentine
Moderate: tooth wear exposing the dentine for more than one third of the individual surface
Severe: complete loss of tooth enamel, with the pulp or secondary dentine exposed

The majority of tooth wear at age 15 years was in the mild category, with dentine just exposed.

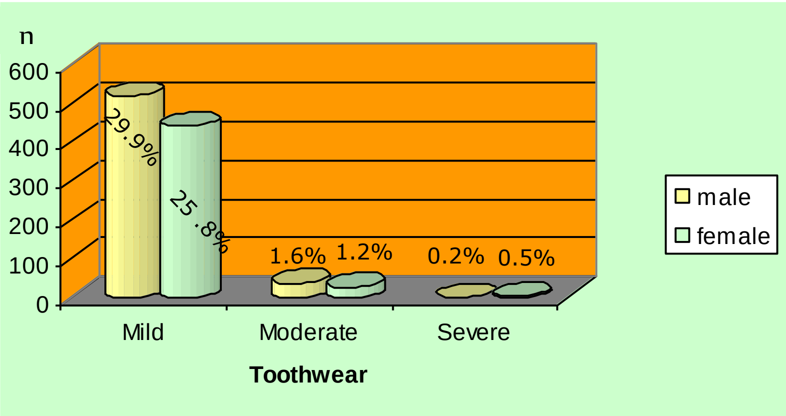


Figure 2-3: Tooth wear at the different levels at age 15 in Ireland

A number of local studies of tooth wear have been conducted in Ireland in both the primary and the permanent dentition (Farvardin, 2000; Harding et al., 2003; Harding et al., 2010). Harding et al., (2003) determined the prevalence and correlates of dental erosion in the primary dentition of five-year-old children in Cork. The index used was that described by (Al-Malik et al., 2001b). The prevalence reported in the study conducted by Harding et al., (2003) was similar to that reported in the UK children's dental health survey (O'Brien, 1994). The indices used were similar but not identical. Consenting participants from the five-year-old study were examined again at age 12 (Harding et al., 2010) and the prevalence was determined for the permanent dentition using a modification of the TWI (Smith and Knight, 1984). The index used was that described by Bardsley et al., (2004). The prevalence of tooth wear measured with the Modified TWI (Mod TWI) (Bardsley et al., 2004) was 38%. The prevalence of tooth wear with exposed dentine on the first permanent molars was 10% (Harding et al., 2010).

The UK children's dental survey of 1993 (O'Brien, 1994) was the first national survey to record tooth wear in the primary dentition. At that time the researchers focused on tooth erosion, postulating that the observed increase in tooth wear was associated with the increased availability of acidic beverages (O'Brien, 1994). Subsequent child dental health surveys in 2003 (Office of National Statistics., 2005) for the UK and in 2013 (Health and Social Care Information Centre, 2015) for England, Wales and Northern Ireland recorded tooth surface loss. The Adult survey conducted in 1998 in the UK (Kelly et al., 2000) the adult dental health survey 2009 in England, Wales and Northern Ireland (Health and Social Care Information Centre, 2011) recorded tooth wear, using a modified TWI (Smith and Knight, 1984) index (Steele and Walls, 2000). The index was similar to that later adopted in the national surveys in Ireland (Whelton et al., 2006; Whelton et al., 2007).

2.5 Progression of tooth wear

A logical next stage to an increasing interest in the prevalence of tooth wear in younger populations is the determination of the increment and associations (Dugmore and Rock, 2003; El Aidi et al., 2010; El Aidi et al., 2011). The ability to quantify the development of new lesions in teenage populations could assist a life-course preventive approach to the management of tooth wear. Dugmore and Rock (2003) demonstrated that at a subject level, new or more advanced lesions were seen in 27% of 12 year-olds over a two-year period, and reversals were observed in 4.1% of participants. The complexity and the interplay of factors associated with erosive tooth wear was demonstrated by El-Aidi et al., (2010), who found that in 1.5 years the incidence in 10–12 year-olds was 24.2%, and 18% of participants with erosion at baseline were recorded without erosion at the second time point. Both Dugmore and Rock (2003) and El Aidi et al., (2010) acknowledged the challenge that reversals present to the analysis of the data, and that further research is required. Certainly, more thought needs to be directed towards the discriminatory nature of the index, quantifying and recording the amount of tooth wear in 1.5 years with a clinical examination is challenging. A robust analytical means of managing reversals in tooth wear analysis are still in their early stages. Harding et al., (2010) reported that tooth wear on the occlusal surface of first permanent molars was associated with tooth wear/erosion to dentine on the primary incisors at age 5 (odds ratio of 5.06, 95% CI (1.32–19.39) (Figure 2-4).

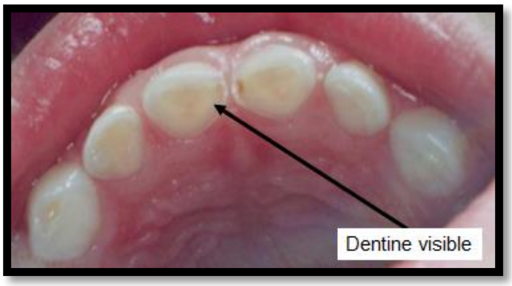


Figure 2-4: tooth wear/erosion to dentine on the primary incisors at age 5

Ganss et al., (2001) examined orthodontic study models and reported an incidence of 18% over 5 years. V examined two different age groups, 10–13 and 15–16 year-olds, and concluded that the prevalence of 'smooth bordered' tooth wear was higher in the older age group. The analysis of the progression of tooth wear faces similar challenges to those expressed by researchers involved with longitudinal studies of dental caries, and stipulating how potential changes are handled (Slade and Caplan, 1999; Shellis et al., 2011). Such work requires greater interdisciplinary collaboration and discussion of statistical methods.

2.6 Guidelines

Guidelines to assist with determining appropriate strategies for the management of tooth wear and erosion are available (O'Sullivan and Milosevic, 2008; Department of Health, 2014).The evidence based toolkit (Department of Health., 2014) acknowledges that the occurrence of some tooth wear is normal. The toolkit provides guidance and acknowledges that not all tooth wear is preventable and reintroduces the term pathological tooth wear.

2.6.1 Summary of the information from guidelines on tooth wear

- The available evidence to date relates to associations and is largely limited to cross sectional, laboratory and *in situ* studies. Thus, further research in this area is recommended.
- Prevention of tooth wear according to the need of individual patients
    - Document and re-document the sites of tooth wear/erosion over time
    - Diet analysis and counselling including particular habits and behaviours with foods and drinks
    - Medical history, note the presence of gastro-oesophageal reflux and vomiting
    - Counsel on the use of oral hygiene products

2.7 Index ages and age groups

In population surveys of oral disease in children and teenagers, the preferred index ages are 5, 12 and 15 years (WHO, 2013a). For tooth wear, different age groups are regularly selected. This along with the choice of different measurement indices makes comparisons between studies problematic (Kreulen et al., 2010; Salas et al., 2014).

2.8 Saliva and tooth wear

The oral cavity is the only site in the body where mineralised tissues are exposed to the outside environment; protection of the dental hard tissues is provided by the salivary glands and the acquired enamel pellicle. The secretion of saliva by the major and minor salivary glands is tightly regulated through neurotransmitter stimulation in what is considered a two-step process (Edgar et al., 2013). The first step involves secretion of an isotonic primary fluid by the acinar cells. The second stage, occurring in the duct system, involves the re-absorption of most sodium (Na^+) and chloride (Cl^-) ions found in the primary fluid, along with the secretion of potassium (K^+) and bicarbonate (HCO_3^-) ions. However, in humans, these two steps appear to be reasonably complicated (Baum et al., 2011). The ion fluxes leading to saliva formation are mediated through multiple ion transporters and channels. The final saliva that enters the mouth is markedly hypotonic. The three paired major salivary glands (parotid, submandibular, sublingual) produce generally similar but nonetheless unique secretions. The parotid produces a secretion that is serous and watery in consistency. The secretions of the submandibular gland are mixed both serous and mucinous and the sublingual gland again is mixed but predominantly mucinous. The minor mucous glands, present throughout the oral cavity produce a viscous secretion, due to their glycoprotein content (Edgar et al., 2013). The consequence of multiple processes in saliva production mean that whole mouth saliva, the fluid in the mouth, is not the simple sum of these individual gland secretions. Proteins found in gland saliva can adhere to both the mucosa and the dentition, be subjected to proteolysis or deglycosylation, and aggregate into complexes (Helmerhorst and Oppenheim, 2007). The absence of saliva and the ravages thus produced by xerostomia secondary to systemic conditions in the older person, in particular the occurrence of tooth wear, are discussed by Young et al.,(2001). In addition to the importance of the quantity of saliva, the quality of saliva also impacts on the presence of tooth wear (Bartlett et al., 1998; Al-Dlaigan et al., 2002a; Saerah et al., 2012) in younger populations, as determined in cross sectional and case control studies.

2.8.1 Saliva type

Depending on the purpose for which saliva is to be analysed, there are several types of saliva that can be collected: individual secretions from the major and minor salivary glands, as well as whole saliva (Screebny and Vissink, 2010). It can be either unstimulated (resting), which is the saliva secreted continuously in the absence of an exogenous stimulus, or under stimulated conditions, with different means of stimulating secretion being adopted (Baum et al., 2011). Whole saliva, which represents a mixture of all the saliva available in the oral cavity, is often used to investigate the functions of saliva, including the measurement of the salivary ions calcium, phosphate and fluoride (Oliveby et al., 1989; Dawes and Dong, 1995; Cury et al., 2005; Fukushima et al., 2011).

2.8.2 *Functions of saliva*

The primary function of saliva is to aid in the pre-processing of food in concert with tooth-mediated mastication. The various functions of saliva – its ability and its versatility – result from its water, protein and ion content (Mandel, 1987; Carpenter, 2013; Edgar et al., 2013). Saliva is now recognised as being capable of meeting the demand for inexpensive, non-invasive and easy-to-use diagnostic aids for oral and systemic diseases (Wong, 2012).

2.8.2.1 Ion reservoir

Saliva is supersaturated with calcium and phosphate electrolytes, the proline-rich proteins and statherin help to keep these ions in solution and serve as vehicles for the transfer of calcium to the enamel surface for remineralisation. Other important electrolytes present are sodium, potassium, magnesium, chloride and bicarbonate. Fluoride is present in saliva at constant low levels, which tend to be higher in areas with a fluoridated water supply and with the use of fluoride products (Buzalaf, 2011). Jarvinen et al., (1991) noted that participants with erosive tooth wear had less calcium and inorganic phosphate in saliva. The research went on to conclude that individuals with low salivary calcium concentrations have a higher critical pH (pH-value at which hydroxyapatite starts to dissolve) (Stephan, 1944; Dawes, 2003) and may be more susceptible to demineralization than participants with higher calcium concentrations (Jarvinen et al., 1991). Saliva velocity differs in different areas of the mouth, with unstimulated saliva velocity much less on labial maxillary incisors than on lingual mandibular incisors (Dawes, 2008).

2.8.2.2 Protection

There is a balance of de- and re-mineralisation that, in concert with the adsorbed pellicle proteins and acquired enamel pellicle protects the teeth from decalcification. It also slows down the abrasive and attritive damage caused by food during mastication (Siqueira et al., 2012). The visco-elastic properties of saliva that provide protection through lubrication are largely attributable to the presence of glycoproteins (Edgar et al., 2013). The antimicrobial and agglutination functions of saliva are attributable to proteins such as sIgA and lyzozymes and glycoproteins, including mucins (Screebny and Vissink, 2010). Statherin which inhibits calcium phosphate precipitation from supersaturated solution, experiences rapid degradation in the oral cavity (Li et al., 2004). The salivary secretions of the submandibular and sublingual salivary glands tend to be quite viscous and their excretions form a protective lubricant layer against attrition (Van Nieuw Amerongen and Veerman, 2002; Van Nieuw Amerongen et al., 2004; Edgar et al., 2013). The secretions of these glands also contribute to buffering capacity. Altered levels of sIgA in saliva have been used as a marker of stress (Tsujita and Morimoto, 1999), however it may only be short lived expression (Tsujita and Morimoto, 2002).

2.8.2.3 Taste

Saliva plays a critical role in taste function as a solvent for food, a carrier of taste-eliciting molecules and, through its composition, in permitting foodstuffs to interact with taste buds. Sourness is the taste that detects acidity and the habit of seeking out sourness is related to a child’s food habits and preferences (Skinner et al., 2002). It has also been suggested that the fondness for bitterness is aligned with the individuals protein profile (Dsamou, 2012) and remarkably an infant’s salivary profile (Morzel et al., 2013). Research has also indicated that changes in salivary protein profile occurs with taste stimulation (Mounayar et al., 2014).

2.8.2.4 Excretion

The excretory function of saliva is not well refined, as many of the products excreted are reabsorbed in the gastrointestinal tract (Buzalaf, 2011).

2.8.2.5 Maintaining water balance

Under conditions of dehydration, salivary flow rate is reduced. Normal salivary flow rate is established at approximately 0.3–0.4 ml min^{-1} for unstimulated/resting saliva and approximately 1.5 ml min^{-1} for stimulated flow rate. These levels are reduced during times of dehydration (Edgar et al., 2013). Mouth dryness signals back to the hypothalamus through osmoreceptors and urine production can be reduced. Saerah et al., (2012) identified that tooth wear was associated with the rate of oral rehydration, using a case control study with 16-year-old students in Malaysia.

2.8.2.6 Salivary proteins

Most salivary proteins contain an oligosaccharide chain and are termed glycoproteins, they are multifunctional and share generic roles such as mucosal defence, protection against organisms, lubrication and acquired pellicle formation (Kaufman and Lamster, 2002; Siqueira et al., 2012; Morzel et al., 2013). Most of the protein secreted into saliva originates in acinar cells (approximately 80%), with approximately 20% secreted by duct cells. Although collectively they are termed glycoproteins, some of the proteins are individually identified as amylase, mucins, histatins, proline-rich proteins, statherin, cystatin and immunoglobulins (Oppenheim et al., 2007). Salivary mucins have a role in a non-immune protection of the oral cavity, protecting against desiccation and environmental insult, providing lubrication and an antimicrobial effect against potential pathogens, both viral and bacterial. Approximately 2,290 proteins have been identified in human whole saliva - this would suggest a value in investigating whole saliva for varying conditions (Yao et al., 2003; Yan et al., 2009; Siqueira et al., 2012; Wong, 2012). Research undertaken by Lamanda et al., (2007) identified that proteins that were located at a particular protein band and within a particular mass range were associated with salivary buffering ability.

2.8.2.7 Immunoglobulins

More than 85% of salivary immunoglobulins belong to the IgA subclass and along with IgG make up approximately 5 to 15% of salivary proteins. They are a specific type of protein with an important role in mucosal protection. IgA present in saliva functions to inhibit bacterial adherence and the colonisation of mucosal surfaces and provides a broad-spectrum defence system (Van Nieuw Amerongen and Veerman, 2002; Van Nieuw Amerongen et al., 2004; Screebny and Vissink, 2010).

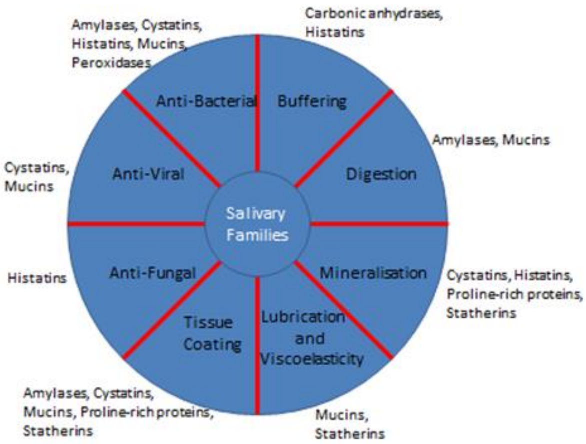


Figure 2-5: The multifunctionality of salivary proteins (Levine, 1993)

The molecular weights of the salivary proteins vary from the low molecular weight histatins to the larger molecular weight mucins.

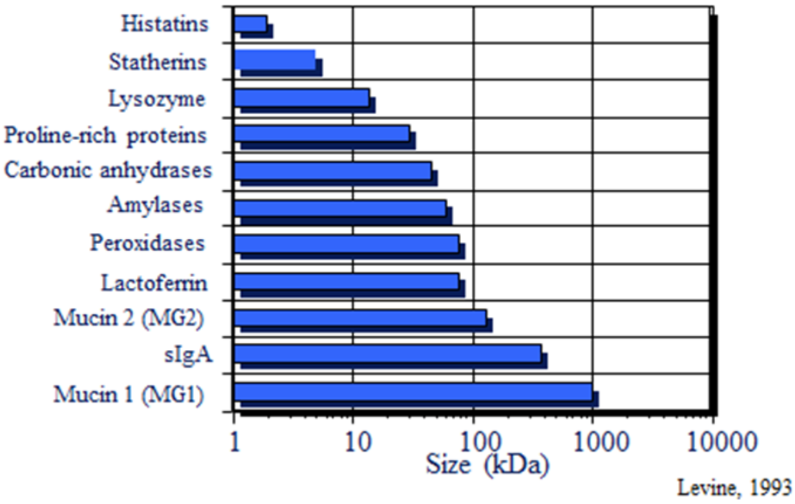


Figure 2-6: Estimated MW's (kDa) of the major salivary proteins

2.8.3 Salivary ions and remineralisation of enamel

In vitro research has demonstrated that remineralisation can occur in an eroded lesion, once an area of subsurface porosity exists and a 'crater' is already present (Amaechi and Higham, 2001). The calcium and phosphate ions present in saliva are essential in maintaining tooth mineral integrity. For dental caries, the presence of fluoride at a low level in saliva and dental plaque inhibits demineralisation, promotes remineralisation, and leads to the formation of a less soluble hydroxyapatite. The bioavailability of fluoride is dependent upon various factors such as fluoride administration, fluoride formulation, and salivary secretion rate. The fluoride concentration of whole saliva is higher than that of submandibular/sublingual duct saliva and parotid duct saliva. Both submandibular/sublingual duct saliva and parotid duct saliva are lower in concentration than plasma saliva. However, both follow plasma fluoride concentration, although at a lower concentration (Buzalaf, 2011; Zero et al., 1992 a). Zero et al., (1992b) identified baseline values of salivary fluoride of 2.2 µmol l^{-1} (0.04 ppm) in a population living in an area with fluoridated water (approximately 0.9 ppm).

2.8.4 Saliva flow rate

Saliva flow rate is one of the most important factors in determining saliva content and the concentration of the different ions, sodium, potassium, phosphate, calcium, magnesium, chloride, bicarbonate (Carpenter, 2013; Carpenter et al., 2014). The timing of saliva collection is important due to circadian rhythms exhibited in both flow and composition (Dawes, 1972). However, (Crossner, 1983) has suggested that as far as flow rate is concerned the salivary glands seemed to be fully developed at the age of 15, and flow rate determined under standardised conditions was relatively stable, not only during daytime and from day to day but also over a longer period of time, which differs from the data collected by Dawes et al., (1972) and Edgar et al., (2013) . Furthermore, Crossner (1983) went on to say that neither the time of day when the sample was taken nor the timing in relation to the last meal were found to be of significant importance to the flow rate. Crossner (1983) also identified that boys consistently had higher saliva flow rates than girls. The concentration of bicarbonate and the ability of saliva to neutralise acids can increase threefold with stimulation rising from 5 mmol l^{-1} to 17 mmol l^{-1} (Khan and Young, 2011). The oral lesions such as caries, abrasion, erosion that exist with hyposalivation demonstrate the important role that saliva plays in maintaining the oral cavity in a healthy state (Dawes, 2008). An increase in the prevalence of tooth wear was identified with a specific diagnosis of salivary hypo function in older age groups (Navazesh et al., 1992). The velocity of saliva differs in different areas of the mouth, for example the velocity of unstimulated saliva is much less on labial maxillary incisors, than on the lingual mandibular incisors, and associated with this too is a difference in the thickness of the acquired enamel pellicle (Dawes, 2008; Siqueira et al., 2012).

2.8.5 Saliva and enamel pellicle

Saliva plays a role in protecting the dentition against abrasion and erosion by contributing calcium-binding proteins to the acquired enamel pellicle. The pellicle provides a renewable lubricant, which offers some protection against abrasion and attrition: within seconds of an abraded surface being exposed to saliva, the pellicle begins to re-form. The acquired enamel pellicle in turn also protects against attrition by providing a lubricant layer. Given that the enamel pellicle is composed principally of salivary proteins and the protective role of enamel pellicle research in this arena is growing (Carpenter et al., 2014; Siqueira et al., 2012; Vukosavljevic et al., 2014). Lussi and Ganss (2014) highlighted the importance of saliva properties in protecting the dental hard tissues from tooth wear particularly erosive tooth wear. The properties identified as important were flow rate, pH, bicarbonate, buffer base, calcium, phosphorus and protein content (Lussi and Ganss, 2014). Protective effects, however, can be overwhelmed by acidic challenges. Enamel pellicle, despite the protection it affords, is only one microns in thickness, and large acidic quantities cannot be diluted by the residual salivary volume approximately 0.8ml. Enamel is susceptible to dissolution once the pH of saliva reaches the critical pH, between 5.5 and 6.5 with respect to tooth mineral. Dissolution is inversely related to phosphate and calcium concentration (Kavanagh and Svehla, 1998; Dawes, 2003). As saliva is super saturated with respect to tooth mineral, softened enamel may experience some tooth remineralisation, particularly in the presence of fluoride (Zero and Lussi, 2005). Khan and Young (2011) demonstrated *in vitro* that erosion in adults was affected by variations of the thickness of the acquired salivary pellicle. Sites of greatest thickness had the lowest incidence of cervical erosion. Carpenter et al., (2014) in an *in situ* study identified that the calcium concentration in the acquired enamel pellicle was 50% less in participants with dental erosion than the controls. In addition statherin a calcium binding protein was 35% less also in the participants with dental erosion. Wang et al., (2010) examined the salivary characteristics of a sub set of 12- and 13-year-old school children in Southern China using the O’Sullivan erosion index (O'Sullivan, 2000). They observed that the flow rate, pH, bicarbonate, buffer base, calcium, phosphorus and urea values of unstimulated and stimulated saliva were similar between the dental erosion group and control group. Saerah et al., (2012) in a case-control study in 16-year-old Malaysian schoolchildren found that hydration and viscosity of saliva were associated with tooth wear recorded with the TWI (Smith and Knight, 1984). Milosevic et al., (2004) alluded to the importance of saliva in their large study in the North West of England. Although, Milosevic et al., (2004) did not measure saliva, they concluded that absence of conclusive evidence that the risk factors were strongly linked to the outcome of erosion or wear might indicate that other, factors such as saliva influences the erosive process. Jarvinen et al., (1991) reported that calcium and phosphate levels in saliva were lower in erosion cases than in controls using univariate analysis. In the subsequent logistic regression, however these factors were not associated with erosion. Jarvinen et al., (1991) suggested as indicated previously that the lower levels were probably associated with other salivary factors such as lower unstimulated salivary flow rate. Carlsson et al., (1985) identified in an adult population that the calcium concentration of the saliva of those with occlusal tooth wear was higher than their control group with no tooth wear. The mean age of their participants being 42-years (range 31–61-years) for males and 34-years (range 16–45-years) for females. They suggested that those with erosion can have an increased mucin content whereby the deposition of calcium, which normally repairs small defects in enamel is prevented.

2.8.6 Proteomics and saliva

Saliva is so readily accessible that researchers are now adopting proteomic methods. These methods are being used, to identify structure and function and to quantify salivary proteins. They can then be used as biomarkers of diseases or conditions and herald exciting times in prevention (Al-Tarawneh et al., 2011; Vukosavljevic et al., 2014; Wong, 2012).

2.8.6.1 Oxidative stress and reactive oxygen species

Oxygen radicals are reactive molecular species produced by one-electron reduction of oxygen. Approximately 4% of oxygen consumed by mitochondria is converted to the reactive oxygen species (ROS) hydrogen peroxide and superoxide during oxidative phosphorylation. These ROS are converted through a number of distinct processes to highly reactive harmful hydroxyl radicals. The availability of ROS can then damage proteins, lipids and DNA (Sies, 2015). Therefore, the presence of ROS is often used as an indication that protein damage has occurred. The oxidative modification of enzymes and structural proteins present in saliva have been shown to play a role in several human diseases such as Alzheimer's disease, chronic lung disease, diabetes and periodontal disease (Battino et al., 1999; Dalle-Donne et al., 2003b; Carnelio et al., 2008; Su et al., 2009). As research and interest in the area of oxidative stress grows, terminology such as dietary oxidative stress, postprandial oxidative stress, physiological oxidative stress, photo oxidative stress, radiation-induced oxidative stress, oxidant stress and pro-oxidant stress now appear in the literature (Sies, 2015). Quantifiable measurement of oxidative stress and ROS in biological systems avails of the presence of the protein carbonylation fingerprint. Carbonyl groups are composed of a carbon atom double-bonded to an oxygen atom. An irreversible post-translational modification (PTM) occurs with carbonylation that leads to a loss of protein function (Madian and Regnier, 2010). The formation of protein carbonyl groups (aldehydes and ketones) on protein side chains particularly of the amino acids proline, arginine, lysine and threonine are then available as a marker of oxidative stress (Dalle-Donne et al., 2003a; Aschner et al., 2011).

2.8.6.2 Carbonylation as a biomarker

Recent proteomic analysis of human whole saliva has shown that salivary biomarkers could contribute to the detection of local and systemic diseases, but the standardisation of sampling procedures needs further development (Castagnola et al., 2011). High levels of oxidative stress are implicated in a number of chronic conditions including diabetes mellitus and neurodegenerative diseases (Aschner et al., 2011). For the detection of oral cancer, researchers have developed salivary biomarkers by using proteomic, genomic, miRNA34 and metabolomic technologies. These salivary biomarkers have been evaluated and confirmed in Indian and Serbian populations (Aschner et al., 2011; Baum et al., 2011; 2010; Wong, 2012). Establishment of clear mechanisms showing how salivary biomarkers can reflect disease states in the oral cavity and elsewhere in the body could further develop the scientific acceptance of this emerging field (Wong, 2012).

2.8.7 Measurement of salivary proteins

2.8.7.1 2-DE

Electrophoresis is frequently used in proteomics; it is a process used to separate complex mixtures of proteins and to purify proteins for use in further applications. Polyacrylamide gels (PAGE) show only weak interactions with proteins in the presence of sodium dodecyl sulphate (SDS) and thus do not absorb the proteins (Lovric, 2011). In polyacrylamide gel electrophoresis, proteins migrate in response to an electrical field through pores in the 'gel matrix'; the pore size decreases with increasing acrylamide concentration. The combination of pore size and protein charge, size, and shape determines the migration rate of the protein (Gallagher, 2001), 2-DE is a valuable technique that allows quantitative comparisons and functional probing of proteins. A drawback of 2-D SDS PAGE is that high-molecular-weight (>120 kDa) and very low-molecular-weight (<10 kDa) protein species can only be poorly resolved or do not enter the separation at all (Ruhl, 2012).

2.8.7.2 Absorption spectrophotometry

Absorption spectrophotometry measures the characteristics of substances to absorb electromagnetic radiation. The region of the electromagnetic spectrum that is most useful for the investigation of biological systems lies between wavelengths of about 240 nm and 800 nm. The concentration of protein carbonyls in saliva samples can be derived using absorption spectrophotometry using the Beer Lambert law, which states that the concentration of an absorbing substance is linearly related to its absorbance.

2.8.7.3 Mass spectrometry

To establish the chemical structure of proteins and peptides, mass spectrometry is used; data collected can then be referred to existing databases and named (Lovric, 2011).

2.8.7.4 Mascot score

Mascot is a free software search engine that interprets mass spectrometry data to identify proteins from primary sequence databases. The prevailing experimental method for protein identification is a bottom-up approach, where a protein sample is typically digested with the amino acid *trypsin* to form smaller peptides. Peptides usually fall within the limited mass range for measurement with a typical mass spectrometer, hence the requirement for trypsin digestion. The mass spectrometer measures the molecular weights of peptides in a sample. Mascot then compares these molecular weights against a database of known peptides. The more peptides Mascot identifies from a particular protein, the higher the Mascot score for that protein (http://www.matrixscience.com/).

2.8.8 Measurement of inorganic ions

2.8.8.1 Ion exchange chromatography

Ion chromatography (IC) determines the presence and concentration of substances over a wide range. An advantage of IC is the range of ions that can be analysed in a single analysis. With IC, the sample is pumped with the eluent onto the ion exchange column and the ions are then attracted to the charged stationary phase of the column. The charged eluent elutes the retained ions which then pass through the detector and are depicted as peaks on a chromatogram. The time at which the ion elutes is used in the identification of the ion and the area under the curve is used in calculating its concentration. Ion chromatography has been used with saliva samples to determine anion concentrations in human saliva (Chen et al., 2004a; 2004b), phosphate, chloride, nitrate, acetate, lactate, and thiocyanate anions present in saliva were all measured (Chen et al., 2004a; 200b). Chen et al. (2004) did not investigate the concentration of fluoride ion using ion chromatography, while Abudiak et al., (2012) reported using IC to determine fluoride concentration in plaque.

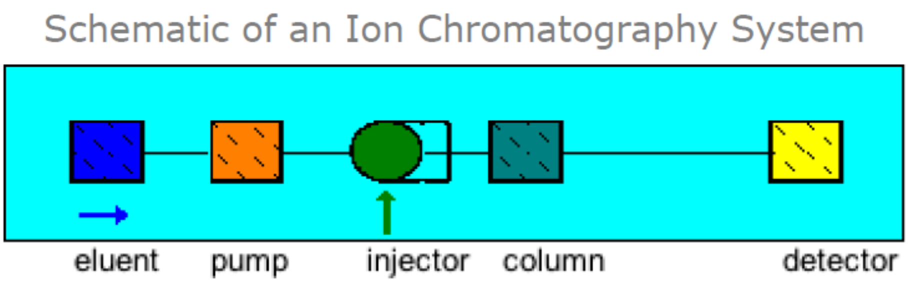


Figure 2-7: Ion chromatography schematic

2.9 Risk factors and indicators for tooth wear

In the following paragraphs, risk factors and protective factors for tooth wear will be discussed under the headings: diet, soft drink consumption, chemical factors, alcohol, fluorides, gastro-oesphageal reflux (GORD) and sociodemographic factors.

2.9.1 Diet

Acidic foods and drinks and the manner in which they are consumed are implicated as the primary aetiological factor in the presence of tooth wear particularly erosive tooth wear in children and adolescents (Lussi and Ganss, 2014; Nunn et al., 2003; Taji and Seow, 2010). Evidence derived from *in vitro* and *in situ* studies have also proved convincing (Attin et al., 2005; Barbour and Lussi, 2014; Davis and Winter, 1980; Lussi and Ganss, 2014; Millward et al., 1994). In the absence of intervention studies, it has been difficult to demonstrate the clear effect of acidic foods and drinks in epidemiological studies. Many studies however, have identified associations between acidic foods and drinks and tooth wear in children and adolescents. Bartlett et al., (2011a) used the ETI to measure the prevalence of tooth wear in a convenience sample of University attending subjects. The recommendations subsequent to the study were that an emphasis be placed on the dietary message to reduce the frequency of consumption of acidic food and drink. Analyses of the data at the enamel level and at the dentine level indicated different associations. The authors concluded that the associations were associated with the different constituent proportions of the dental hard tissues. A review of soft drinks and dental health conducted by Tahmassebi et al., (2006) suggest a protocol to encourage the sensible use of drinks and the modification of drinks to render them less harmful would be appropriate. Barbour et al., (2011) discuss the use of *in vitro* models for the screening and prediction of the erosive potential of drinks.

2.9.2 Soft drink consumption

The narrative in many countries is that an increasing prevalence of tooth wear in children and adolescents is associated with increasing soft drink consumption and associated drinking habits. This is true also of countries where erosive tooth wear is only of recent times being acknowledged (Hamasha et al., 2013). The consumption of soft drinks in Ireland steadily increased between the years 1986 to 2008 from just under 400 million litres to just below 900 million litres (Beverage Council of Ireland, 2009), with an average consumption of approximately 200 litres/person or approximately 600 mls daily/person (Table 2-10). It is possible that it is an expression of parents' and teenagers' knowledge of carbonated (fizzy) beverages being an 'unhealthy' choice (Safefood, 2009), that contributed to the downward trend in 2008 of soft drink consumption (Table 2-10).

Table 2-10: Soft drink consumption (litres (l)/person) 2002 – 2008 (Ireland)

	2002	2003	2004	2005	2006	2007	2008
million litres	792	783	786	800	839	886	898
% change	+2.5	-1.1	+0.4	+1.8	+0.4	+5.6	-3.02
l / person	202	196	193	193	199	206	206

The International data for carbonated soft drink consumption in 2002 placed Ireland in second position behind the US (Figure 2-8) (Euromonitor). It will be interesting to note whether the downward trend continues in the sale of carbonated beverages. The substitutes such as bottled water, dilutables and juices however, may be problematic for oral health. Research from the UK indicates that sports drink consumption fell by 2.5% in 2012. However, the per capita estimation of soft drink consumption in the UK for 2013 was 230 litres per person, an increase of 4% on the previous year (British Soft Drinks Association, 2015). Recent research from Ireland indicates that the consumption of non-diet soft drinks for children aged nine varied with mother's educational attainment. A non-diet soft drink was provided in the previous 24-hour period by 63% of mothers' with a lower educational attainment as opposed to 40% graduate level (Williams et al., 2009). Whether soft drinks are the principal aetiological factor at population level still requires confirmation.

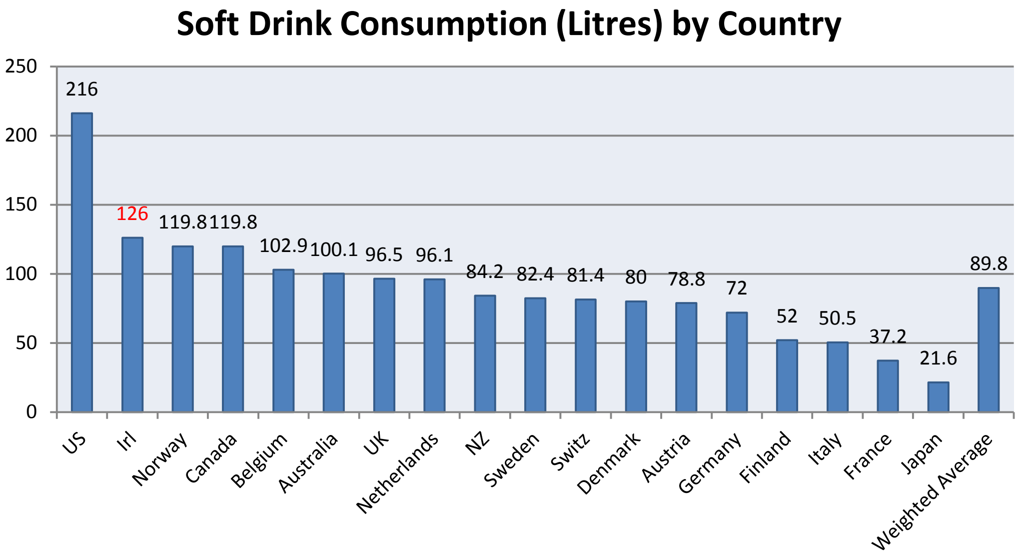


Figure 2-8: Soft drink consumption by country data for 2002

2.9.3 Chemical factors: pH, titrateable acidity

In vitro and *in situ* studies have been important in pointing out the erosive potential of particular foods and beverages. (Lussi, 2014; Lussi and Ganss, 2014). A recent clinical study, albeit in adults, demonstrated that the pH drop at the tooth surface in adults was greater in those with erosion than those without (Lussi et al., 2012). Research suggests that key variables that are associated with erosive potential are the type and concentration of the acid, pH, pKa, duration of the exposure and stirring rate (Lussi and Ganss, 2014; Zero and Lussi, 2005).

2.9.4 Alcohol consumption

Although the legal age for alcohol consumption in Ireland is 18 years-old, it is known that individuals younger than that are experimenting with alcohol. A third of Irish 15–17 year-olds reported having been drunk in the previous thirty days (Kelly et al., 2012; Nic Gabhainn S, 2007). Al-Dlaigan et al., (2001a) concluded from the examination of a cluster sample of 14-year-old students in Birmingham (UK) that the presence of wear on the buccal/labial and palatal/lingual surfaces was associated with the consumption of beer along with other acidic foods and drinks. The incidence of erosive tooth wear on first molar teeth in an adolescent population was positively associated with alcoholic mixed drinks (Aidi et al., 2011). Alcopops, introduced in the 1990s, became a palatable means for adolescents to experiment with alcoholic spirits. Alcopops combine the erosive potential of both the alcoholic spirit and carbonated drink or juice. They are considered to have a higher erosive potential than cider (Rees and Griffiths, 2002), another alcoholic beverage with which adolescents experiment. The astringency of alcohol has the ability to further contribute to tooth wear by aggregating salivary proteins producing a loss of lubricant properties of saliva and attrition and abrasion compounding erosion (Wilson M, 2009).

2.9.5 Fluoride use

The advent of artificial water fluoridation (Centers for Disease Control and Prevention, 1999) and the introduction of fluoridated tooth pastes in the 1970s have played an important role in the global decline of dental caries. The impact of fluorides on tooth wear is less obvious. However, presently, considerable *in situ* and *in vitro* research is focussed on polyvalent metal ions and the use of stannous fluoride and titanium fluoride products in the prevention on tooth wear. Studies indicate that the stannous fluoride products act by depositing a protective barrier layer onto the enamel surface and by interacting with the natural pellicle to provide enhanced resistance of the pellicle itself against erosive acid challenges. After treatment with stannous fluoride containing products, teeth become coated with an invisible, metal-rich barrier layer that is likely composed of precipitates, such as $Sn_3F_3PO_4$, $Ca(SnF_3)_2$ or $SnOHPO_4$ (Lussi and Ganss, 2014). However, Larsen (2001) with a series of *in vitro* studies of enamel erosion concluded that the effect of even high fluoride concentrations is limited (Larsen, 2001).

2.9.6 Gastro-oesphageal reflux disease (GORD)

Analysis in observational studies and case reports have demonstrated an association between tooth wear in the permanent and primary dentitions and the symptoms of gastro-oesophageal reflux (GORD) (Aine et al., 1993; Lussi and Ganss, 2014; Marsicano et al., 2013; Murakami et al., 2011). In a small case series study with 18 adults diagnosed with gastro-oesphageal disease, including bulimia and six controls, erosive tooth wear measured on study models was higher in the group with GORD (Tantbirojn et al., 2012).

2.9.7 *Socio-demographics*

Social class, place of domicile and the receipt of social benefits have all indicated an association with tooth wear, although the direction has varied between studies. Researchers have used different methods to define socio economic status or socio-demographics, Al-Dlaigan et al., (2001b) used the consumer classification ACORN (CACI, 2014) in the UK and found a higher proportion in the lowest group (striving) with erosion. Bardsley et al., (2004) also in the UK used the Townsend Index, a measure of material deprivation, and controlled for domestic water fluoridation. They concluded that tooth wear on smooth surfaces was more prevalent in deprived areas that were not fluoridated. El Karim et al., (2007) identified a high prevalence of dental erosion among 12–14 year-old Sudanese school children. In that population, dental erosion was associated with high socioeconomic status. The attendance at a private school was the proxy measure for high socioeconomic status. In the primary dentition, Harding et al., (2003) used ownership of a medical card, and identified a higher proportion of tooth wear/erosion in the group with a medical card. A medical card in Ireland is means tested and was used as a surrogate for disadvantage. The most frequent reason for medical card entitlement at the time of this study was a low income (Health Service Executive, 2014). El Aidi et al., (2010) indicated that the incidence of erosion on the upper central incisors of adolescents in the Netherlands was associated with lower socio-economic status (SES). SES was based on income, level of education and home ownership. Dugmore and Rock (2003) identified differences in the prevalence of dental erosion for socioeconomic status and ethnicity. There was less erosion in the least deprived white Caucasian group and erosion experience increased with decreasing deprivation in the non-Muslim Asian group. van Rijkom et al., (2002) reported more erosive tooth wear, described as smooth bordered wear in the least deprived. The impact of socio-demographic factors on tooth wear is complicated by the fact that not only are the tooth wear indices variable, but so too are the measures used to determine the socio-demographics of the population. If the same index and method of establishing socio-demographic factors were reproducible across the populations it may have been possible to make a clear statement given the variety of sources.

2.10 Conclusions at the end of the literature review

Reviews of epidemiological studies indicate that tooth wear increases with age for the primary dentition (Kreulen et al., 2010) and for the permanent dentition in adults (Van't Spijker et al., 2009). Risk factors for tooth wear have been identified through observational epidemiology, and therefore causation is difficult to substantiate. The increasing prevalence of tooth wear that is documented is also associated with the increased volume of acidic foods and beverages consumed internationally. However, neither direct evidence nor time trends are available. Contradictory associations are identified by researchers for tooth wear, such as in the case of sociodemographic characteristics (Dugmore and Rock, 2005; van Rijkom et al., 2002). These differences may arise however because of the different populations selected, and because a single agreed index has not been identified. There are at least 11 indices and a considerable number of modifications used to record tooth wear in children and teenagers (Table 2-8). This multitude of indices makes comparison across studies difficult, and although research is progressing with the BEWE index, this new index was designed for use in adult populations (Bartlett et al., 2008). Recording tooth wear in children and teenagers requires an index that is discriminatory, to allow for changes over the lifespan to be identified. Use of the ETI (Bartlett et al., 2011b) tries to address some of the challenges. Saliva despite being more than 99% water, has a considerable range of functions, given the relatively small amount that is known with respect to tooth wear it is timely to investigate the properties of saliva with respect to tooth wear.

Appraisal of the existing literature has identified gaps in the knowledge. This research project attempts to address some of those deficits.

2.11 Statement of Aim

The aim of this research is to investigate the characteristics, development and determinants of tooth wear among Irish school children.
The aim will be achieved with the following research objectives using data that is collected in either the cross sectional study or the longitudinal study.

2.11.1 Statement of objectives addressed in the cross sectional study

1. Estimate the prevalence and pattern of tooth wear in 16-year-old transition year students.
2. Investigate tooth wear levels and associations with demographic factors.
3. Investigate tooth wear levels and associations with oral hygiene practices and dietary factors.
4. Investigate tooth wear levels and associations with the properties of resting and stimulated saliva.

2.11.2 Statement of objectives addressed in the longitudinal study

1. Investigate whether tooth wear at age 14 and the tooth wear increment between age 12 (tp1) and 14 (tp2) are associated with demographic factors, dietary factors, oral hygiene practices, or lifestyle practices using data collected at three time points in a longitudinal study.
2. Investigate whether tooth wear at age 14 and the tooth wear increment between age 12 (tp1) and 14 (tp2) are associated with tooth wear recorded when the participants were examined at earlier time points.

2.12 Hypotheses to be tested

With the above aims, the following hypotheses will be tested using the data collected in both the cross sectional study and the longitudinal study.

2.12.1 The cross sectional study

H_0 There is no difference in the tooth wear levels between male and female 16-year-old students

H_0 There is no difference in the tooth wear levels between 16-year-old students of different socio-economic groups

H_0 There is no difference in the salivary properties of 16-year-old students according to their tooth wear levels

Salivary properties include: flow rate, protein concentration, protein carbonyl concentration, protein identification and the inorganic ions fluoride, phosphate and calcium

H_0 There is no difference in tooth wear levels between 16-year-old students who frequently consume acidic foodstuffs and 16-year-old students who consume them less often.

H_0 There is no difference in tooth wear levels between 16-year-old students who brush twice each day and 16-year-old students who brush less frequently

H_0 There is no difference in the tooth wear levels between 16-year-old students living in fluoridated areas and 16-year-old students living in non-fluoridated areas.

2.12.2 The longitudinal study

H_0 There is no association between the levels of tooth wear at age 14 and the demographic factors of participants at age 5, 12 or 14 years at the subject level.

Demographic factors include: gender, family hold a medical card, exposure to a fluoridated piped water supply.

H_0 There is no association between the levels of tooth wear at age 14 and the oral hygiene practices of participants at age 5, 12 or 14 years.

Oral hygiene practices include: the frequency of brushing and the timing of tooth brushing.

H_0 There is no association between the levels of tooth wear at age 14 and the dietary habits or lifestyle choices of participants at age 5, 12 or 14.

Dietary habits and lifestyle choices include: the choice of acidic drink, the frequency with which the drink is consumed and the typical method of drinking; eating apples or citrus fruits and the frequency with which they are eaten; the frequency of sweet and savoury food consumption; and baby bottle weaning.

H_0 There is no association between the levels of tooth wear at age 14 and the general health or medical conditions of participants at age 5, 12 or 14.

General health and medical conditions include: asthma, complaining of a 'dry mouth', taking vitamins or medicines and having frequent tummy upsets or vomiting.

H_0 There is no association between the levels of tooth wear at age 14 and the clinical outcomes of participants at age 5 or 12

Clinical outcomes include: the presence of tooth wear, tooth wear in enamel only or tooth wear extending into the dentine or the count of the number of teeth affected by tooth wear.

H_0 There is no association between the maximum increment score for tooth wear between age 12 and 14 and demographic factors, oral hygiene practices, dietary habits or lifestyle choices, general health or medical conditions and the clinical outcomes of participants at age 5 or 12.

H_0 There is no association between the mean increment for tooth wear between age 12 and 14 and demographic factors, oral hygiene practices, dietary habits or lifestyle choices, general health or medical conditions and the clinical outcomes of participants at age 5 or 12.

Chapter 3 Materials and Methods

3.1 Introduction

Chapter 3 outlines the materials and methods used in this research project. The project includes two studies – a cross-sectional study and a longitudinal study. In the cross-sectional study, the participants were transition year students, aged approximately 16 years. The longitudinal or cohort study examined a cohort of children at three time points. The cohort was established from school children first examined at age five (n=202), (Harding et al., 2003). The participants at the subsequent time points were children whose parents or guardians had consented to being contacted at a future time. Participants were re-examined at age 12 (n=123) and age 14 (n=83). The examinations at the three age time points are referred to as time point 0 (tp 0) age 5, time point 1 (tp1) age 12, and time point 2 (tp2) age 14.

The materials and methods are set out in two parts, first for the cross-sectional study and then for the longitudinal study. To avoid duplication, the ethical approvals required for both the cross-sectional study and the longitudinal study are described together.

3.2 Ethical approvals for both studies

The clinical research ethics committee (CREC) of The Cork University Teaching Hospitals (CUTH) granted permission to conduct each part of the research (Protocol numbers: OHSRC 00500, OHSRC 01005, OHSRC 00806 and OHSRC 00409) (Table 3-1) and [Appendices 1-5].

Table 3-1: Protocol numbers associated with each element of the thesis

Protocol Number	Project
OHSRC 00500[~]	Dental erosion in the primary dentition Published: (Harding et al., 2003) (Appendix 2)
OHSRC 01005*	Tooth wear in 16-year-olds; Transition Year Students (Health Research Board) (Appendix 3)
OHSRC 00806[#]	Follow on from OHSRC 00500, Participants examined at age 12 (tp1) Published:(Harding et al., 2010) (Appendix 4 and 4a)

OHSRC 00409#	Follow on from OHSRC 00806, Participants examined at age 14 (tp2) (Appendix 5)

~ Initial contact for cohort study participants, *Cross-sectional study age 16, # Longitudinal study

3.3 Cross sectional study: dental examination, collection of saliva samples and completion of a questionnaire

To assist with understanding the components of the cross sectional study, Figure 3-1 sets outs the process of recruitment through to examination and Figure 3-2 sets out the design and flow of work throughout the study

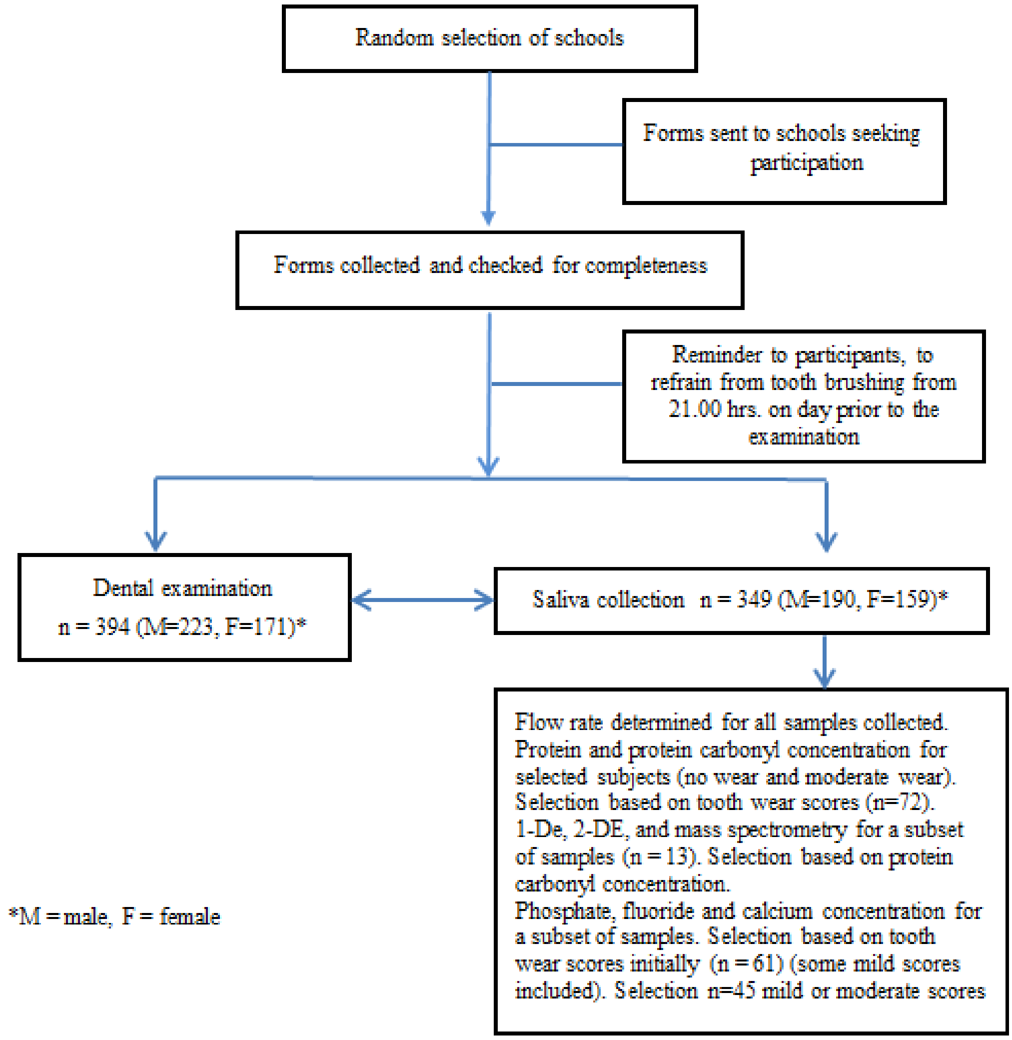


Figure 3-1: Flow chart of the process in the cross sectional study of 16-year-old schoolchildren

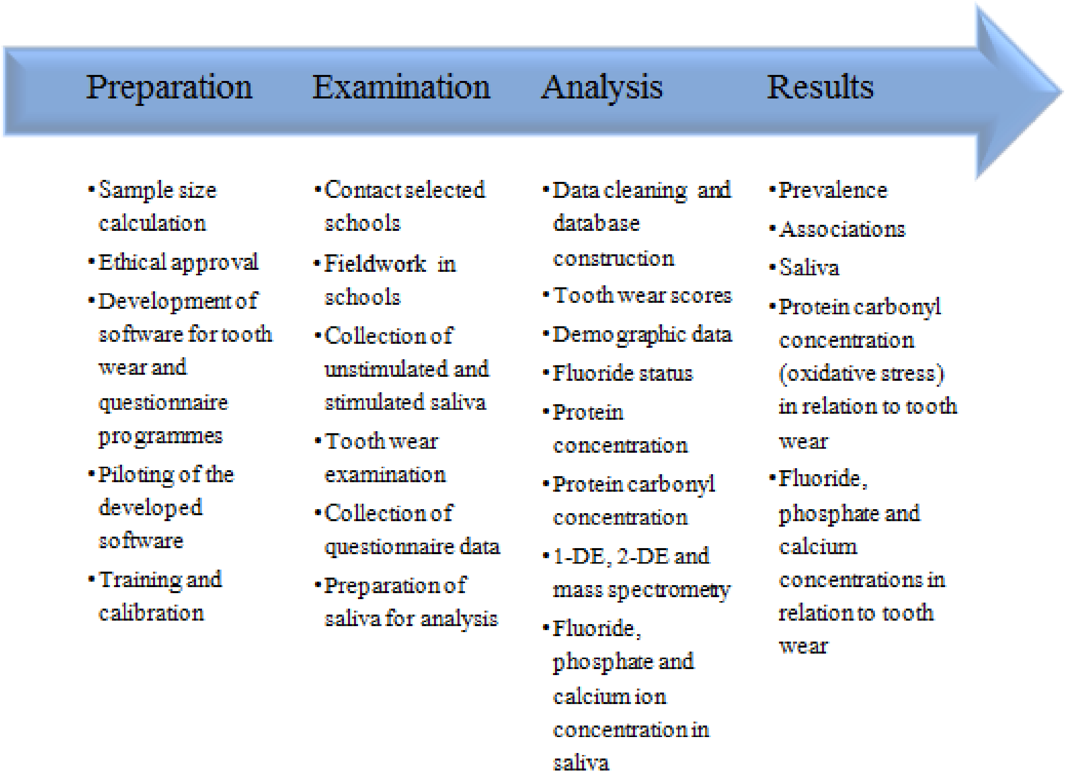


Figure 3-2: The stages involved in the cross sectional study

3.3.1 Sample

The sample was a multi-stage random sample, stratified on the basis of geographic location of the post primary school attended. Schools with a transition year (TY) were selected randomly in counties Cork and Kerry from the list of schools obtained from the Department of Education and Science. The school was the main unit of sampling. A school was selected based on whether or not it received a fluoridated public piped water supply. The school address provided the fluoridation status of the general area, however the home address of the child dictated the ultimate fluoridation status assigned to each participant. The final fluoridation status of the participant was determined from the demographic data provided and with recourse to the city and county local authorities when necessary. Only the schools with a transition year class were invited to participate. The majority of students in transition year will be 16-years-old, but there will be some 15- and 17-year-olds. They are referred to collectively as '16-year-olds'. The TY academic year is designed to assist the student transitioning from the junior cycle to the senior cycle in secondary school education. The mission statement of TY is: '*To promote the personal, social, educational and vocational development of pupils and to prepare them for their role* *as autonomous, participative and responsible members of society'* (Department of Education and Science, 2004).

3.3.1.1 Sample size calculation

In the absence of Irish data at the time at which this study was planned, calculation of sample size was based on sample sizes in cross-sectional studies conducted in the UK. UK teenagers are likely to be somewhat similar to Irish teenagers in terms of tooth wear as measured in this study. The UK studies selected involved 14-year-old school children in Birmingham and Liverpool (Al-Dlaigan et al., 2001b; Milosevic et al., 1994) respectively. In Birmingham, 418 children aged 14-years were examined, the results indicated that 51% had moderate erosion, described as loss of enamel with visible dentine for $<{}^{1}/_{3}{}^{rd}$ of the surface (Al-Dlaigan et al., 2001b). In the Liverpool study, 30% (n=1035) of children aged 14-years had tooth wear into dentine (Milosevic et al., 1994). Using these results to estimate the proportion of teenagers in Cork and Kerry with tooth wear into dentine with a margin of error of 0.05, 95% confidence limits and assuming a prevalence of 51%, a required sample size of 384 schoolchildren. To investigate whether there was a difference in tooth wear levels between students living in fluoridated and non-fluoridated areas required groups of 384 in each, but a power calculation to show a difference between fluoridated and non-fluoridated groups was not conducted.

3.3.2 Development of direct data-entry software for the dental examination and subject questionnaire

A direct data-entry system was proposed for the research that required development of a prototype and piloting. A randomly-selected group of teenagers (15–17 years) was recruited to test the prototype and assess whether this age group were comfortable using direct data entry and a handheld personal computer (PC) under survey conditions to enter the data (Harding et al., 2006) (Figure 3-3). The oral hygiene, dietary habits and behaviours questionnaire proposed for the main study was also piloted by the randomly selected group of teenagers. In addition software designed to allow direct entry of the clinical examinations to a laptop was also piloted. The laptop used for the clinical examinations also functioned as a server for the student's PC. The student questionnaire data were backed up to the laptop. All data from the laptop were backed up to an external drive.

Figure 3-3: Hand held device hosting the e-questionnaire developed for the study

3.3.3 Training and calibration in the measurement of tooth wear for the cross sectional study

The index chosen to record tooth wear was descriptive and partial (Bardsley et al., 2004). The teeth scored were the six anterior permanent maxillary and mandibular teeth (maxillary right canine to maxillary left canine, mandibular left canine to mandibular right canine) on the buccal/labial, palatal/lingual and incisal surfaces and the occlusal surface of the four first permanent molar teeth (Bardsley et al., 2004). The index is a modification of the Tooth Wear Index (TWI) (Smith and Knight, 1984), and is referred to in this research as a Modified TWI (Mod TWI). In this research project, tooth wear scores into enamel were included. Tooth wear was reported at the level of the individual; the highest score recorded in the mouth dictated the score for the participant. Participants were then allocated to groups with ‘no’, ‘mild’ or ‘moderate’ tooth wear. In order to do this, the scores were grouped such that ‘No wear’ were participants with no tooth wear evident, i.e., no score was >0, ‘mild tooth wear’ were participants with obvious tooth wear in enamel, i.e., no score >2 and ‘moderate tooth wear’ were participants where tooth wear had extended into dentine, i.e., scores ≥3 (Table 3-2).

Table 3-2: Tooth wear scoring system for 16-year-old students

Score	Description	Analysis Group
0	No wear evident	No wear
1	Loss of surface characteristics	Mild
2	Wear in enamel but no dentine is visible	Mild
3	Wear in dentine $\leq {}^{1}/_{3}{}^{rd}$ dentine visible	Moderate
4	Wear in dentine $\geq {}^{1}/_{3}{}^{rd}$ dentine visible	Moderate
R	Missing/restored/ could not be assessed	None

Dr Alex Milosevic (AM) acted as the 'gold standard' for this project and provided training and calibration. The first part of the training took place in Liverpool (UK) in January 2006 over two days where the author, Máiréad Harding (MH), examined photographs and models for tooth wear. In February 2006, a second session over a further two days took place at St. Aidan's post primary school, Ballyvolane, Cork. During training, detailed discussion occurred until MH could confidently categorise the level of tooth wear present. The full range of tooth wear scores were demonstrated: from normal tooth surface anatomy to loss of enamel surface characteristics, to loss of enamel without dentine visible, to loss of enamel with dentine visible. For the calibration programme at St. Aidan's post primary school, Cork. Both AM and MH individually examined the same 12 participants. An inter-examiner Kappa statistic (K) of 0.74 was obtained, this value indicated substantial agreement and provided confidence to continue with the study (Landis and Koch, 1977). K is a chance-corrected measure of agreement between two raters, when each examiner independently classifies each of a sample of participants into one of a set of mutually exclusive and exhaustive categories.

3.3.4 Fieldwork

Contact was made with the randomly selected schools and their co-operation sought. The study requirement of the availability of a small room with the use of a power socket, two tables and some chairs was explained to school personnel, including the school principal and the Board of Management. Each 16-year-old student then received the following:

- Letter explaining the study to the parent or guardian and the student
- Form setting out the study in detail seeking informed consent from the parent or guardian and assent from the teenager
- A medical questionnaire so that those for whom the examination would be contra-indicated could be excluded (see section 3.5.2 below)
- Demographic data form requesting the student's name, age, address, previous addresses and the means by which they receive / received their water supply.

The completed forms were returned to the school. Only participants who provided written positive consent and assent were examined. Appendix [6] contains the fieldwork documents used in the cross sectional study. Inclusion and exclusion criteria setting out who could participate were prepared.

The sequence of examination proceeded as follows:

1. Validate informed consent
2. Check that the confidential medical history questionnaire and demographic data form are completed
3. Check the inclusion and exclusion criteria
4. Collection of saliva
5. Students to commence the oral hygiene, dietary habits and behaviours questionnaire electronically
6. Soft tissue examination
7. Tooth wear examination.

3.3.4.1 Informed Consent

The parent or guardian of each child was fully informed with regard to the nature of the study. Consenting parents or guardians were asked to complete the informed consent form [Appendix 6]. The teenage participants were also given the opportunity to decide for themselves whether they wished to participate. Consenting participants then completed the assent form [Appendix 6]. The confidential medical history questionnaire [Appendix 6] was completed and consulted prior to the intra-oral examination.

3.3.4.2 Inclusion and Exclusion Criteria to be followed

Inclusion Criteria:

1. Participants must be students in transition year, for whom the parent or guardian consented and they themselves assented
2. Participants must be in good general health without any known allergy to commercial dental products or cosmetics
3. Participants must be able and willing to co-operate in all study procedures.

Exclusion Criteria:

1. Participants expressing any unwillingness, inability, or lack of motivation to participate in the study procedures as set out in the protocol
2. Any subject whose parent or guardian does not provide consent or they themselves do not assent
3. Severe generalised gingival bleeding or pain
4. Oral pathology including, but not limited to, acute ulcerative gingivitis, acute herpetic gingivostomatitis
5. Participants with a history of cardiac surgery, rheumatic fever, or other circumstances for which a bacteraemia induced by probing the gingiva could pose a threat
6. Any other condition which the investigator feels should preclude participation in the study.

3.3.4.3 Pathology

If any oral conditions that required urgent attention were observed at the time of the examination, the parent/guardian was informed. The presence of any soft tissue abnormalities present were noted on the soft tissue record form [Appendix 6].

3.3.4.4 Parameters to be recorded

3.3.4.4.1 Tooth wear

The choice of index permitted the recording of tooth wear on both smooth (buccal/labial and palatal/lingual) and occlusal/incisal surfaces (Bardsley et al., 2004; Milosevic, 2011).

3.3.4.4.2 Whole mouth saliva

Unstimulated and stimulated whole mouth salivary samples were collected from consenting participants [Appendix 7]. Flow rate, both unstimulated and stimulated, were determined for all consenting participants. Protein concentration, carbonyl concentration and the concentration of calcium, phosphate and fluoride ions were determined for selected samples based on tooth wear scores.

3.3.4.5 Clinical examination equipment requirements and diagnostic systems

3.3.4.5.1 Equipment requirements

Lighting: A Daray light with halogen bulb set to the brightest setting was used, the light was clamped to the side of the table and angled for ease of use, a replacement light was carried at all times (Daray Lighting Ltd: Commerce Way, Stanbridge Road**,** Leighton Buzzard**,** Bedfordshire LU7 8RW, UK).

Dental chair: Participants were examined in a supine position; reclined on a portable dental chair for the examination (ADU – 01 Aseptichair (Portable Dental Chair); ADC – 08 (Portable Operator Stool) Aseptic 8333 216th Street S.E. Woodinville, WA 98072 USA).

Hand instruments: Silvered front surface size 4 mirrors and a sterile CPI probe (if required) were used for each subject. Teeth were dried using cotton wool rolls.

3.3.4.5.2 Infection control requirements

Both the examiner and recorder wore personal protective equipment; a facemask was changed at least every hour, single use, latex-free and powder-free examination gloves were used for the examination of each participant. A fresh disposable paper sheet was used under each set of instruments. Protective barriers were used on light handles, and head rests. Each participant wore a pair of protective spectacles that were disinfected after each examination. After each participant the light handles and dental chair were disinfected. All contaminated 'soft' waste was disposed of in a standard hazardous waste 'yellow bag'. Used probes and mirrors were placed in a rigid container and transferred for decontamination and sterlisation.

3.3.4.5.3 Diagnostic systems

A descriptive partial index was used to record tooth wear, as proposed by Dr Alexander Milosevic (Bardsley et al., 2004), with enamel scores included. The index teeth were dried using cotton wool rolls. Examination commenced with the maxillary right first permanent molar and moved to the maxillary right permanent canine, the maxillary right lateral incisor, the maxillary right central incisor, the maxillary left central incisor, the maxillary left lateral, the maxillary left canine, the maxillary left first permanent molar. Examination then moved to the mandibular left first permanent molar along to the six mandibular anterior teeth and finished with the mandibular right first permanent molar. The Federation Dentaire Internationale (FDI) numbering system was used in charting: 1.6, 1.3, 1.2, 1.1, 2.1, 2.2, 2.3, 2.6, 3.6, 3.3, 3.2, 3.2, 3.1, 4.1, 4.2, 4.3, and 4.6 (Figure 3-4). When a tooth was absent or could not be scored the letter ‘R’ recorded.

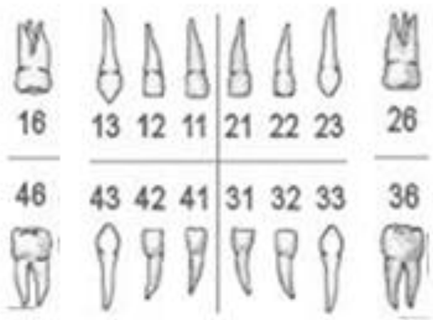


Figure 3-4: Index teeth selected in the Mod TWI (Bardsley et al., 2004)

3.3.4.6 Saliva collection equipment and method

Both unstimulated and stimulated whole mouth saliva samples, were collected from consenting participants. From here on in the research, unstimulated and stimulated saliva refers to unstimulated and stimulated whole mouth saliva. Saliva collection occurred prior to the mid-morning break in all schools. To enable salivary fluoride levels to equilibrate, participants were asked to refrain from tooth brushing from 21.00 hours the previous evening. The methods used for the collection of unstimulated saliva and stimulated saliva conformed with established protocols used at the Oral Health Services Research Centre [Appendix 7].

3.3.4.6.1 Collection equipment

Funnels, Eppendorf tubes®, test tube holders, wax pellets, disposable latex-free and powder-free examination gloves, disinfectant wipes, brown tape, an indelible marker, timer, ice packs and Styrofoam containers were all required. Each Eppendorf tube®, was marked with the participant identification number, the participant’s initials, date of birth, the date and time the sample was taken and the sample type (stimulated or unstimulated). The length of time over which the sample was collected was also recorded.

3.3.4.6.2 Unstimulated whole mouth saliva

First, unstimulated saliva was collected via passive drool; participants sat quietly in a circle with their heads tilted a little forward and their backs to each other to minimise embarrassment and afford some privacy. Each participant was provided with a pre-weighed labelled tube, a funnel and a disposable wipe. Students were first asked to swallow the saliva in their mouths, and then to drool that which collected in their mouths into the test tube via the funnel for at least 5 minutes or until 2 ml of saliva was collected. The Eppendorf tubes® were then collected from participants, double-checked for completeness of identification, and placed with ice packs in a Styrofoam box.

3.3.4.6.3 Stimulated whole mouth saliva

Participants remained seated and were given a wax pellet to chew for one minute and then asked to swallow the saliva that had collected in their mouth. After this, participants continued to chew on the wax pellet and drool the saliva as it accumulated in the mouth into a fresh pre-weighed and pre-labeled tube over a three minute time period. At the end of the three minutes the tubes were collected from the participants, double checked for completeness of identification and placed with ice packs in a Styrofoam box.

3.3.4.6.4 Estimation of saliva flow rate

Collected samples, both unstimulated and stimulated, were transported on ice in Styrofoam boxes and within 24 hours to the OHSRC. The receptacles were then re-weighed with their saliva content. The weight of collected saliva and the time taken to collect it were used to determine the flow rate. Samples were then frozen at -20^0 Celsius until required for analysis. All receptacles were weighed prior to the collection of saliva and re-weighed after saliva collection. The flow rate was determined gravimetrically (saliva density 1g ml^{-1}) and expressed in ml min^{-1} (Equation 3.1)

Equation 3.1: Determination of flow rate in ml min^{-1}

$$\left(\frac{weight\ of\ the\ test\ tube\ and\ saliva - weight\ of\ the\ empty\ test\ tube}{time}\right)$$

3.3.4.6.5 Saliva proteomics analyses and protein identification

Not all samples were selected for this component of the research. The samples that were selected were selected based on either the participant having no tooth wear or moderate tooth wear (the participants selected with moderate tooth wear had tooth wear with dentine exposed on both anterior and posterior teeth) (Table 3-3). Limiting factors in extending this component to all participants were cost and the equipment required.

Table 3-3: Samples for determination of protein concentration and protein carbonyl concentration

Saliva of participants with no tooth wear	Saliva of participants with moderate tooth wear
n=29	n=43

The workflow for the process of protein identification is set out below in Figure 3-5.

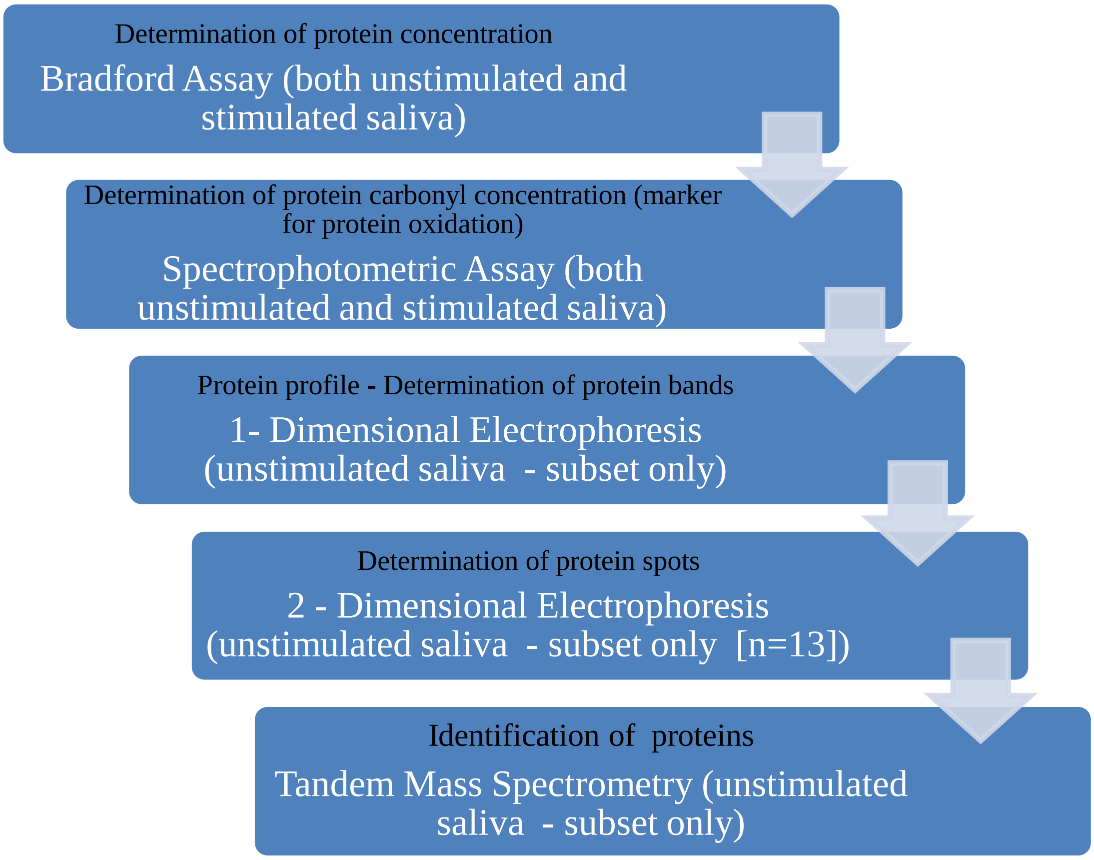


Figure 3-5: Work flow leading to protein identification

3.3.4.6.6 Protein concentration

Participants were grouped according their tooth wear score and protein concentration. The protein concentration for samples was determined using the Bradford assay with bovine serum albumen (BSA) as standard (Figure 3-6) (Bradford, 1976) at the School of Biochemistry and Cell Biology, UCC.

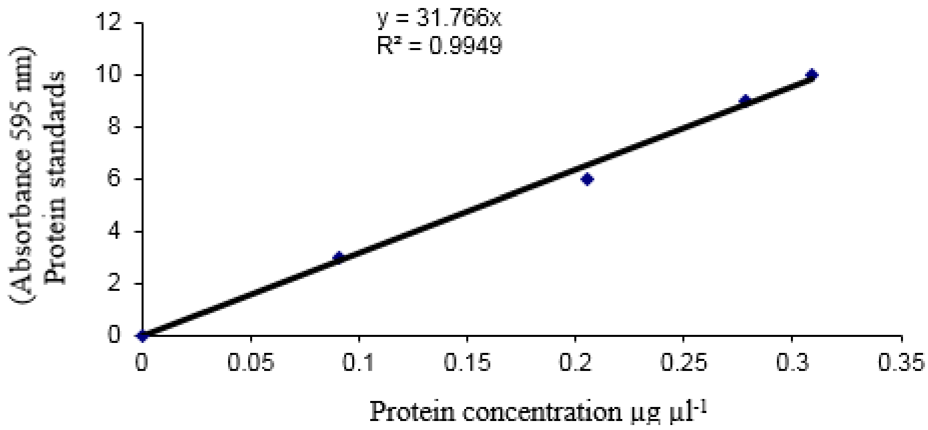


Figure 3-6: Bradford assay standard curve generated with BSA

3.3.4.6.7 Protein carbonyl concentration, Reactive Oxygen Species

Protein carbonyl concentration was determined spectrophotometrically using the reaction between 2, 4-dinitrophenylhydrazine (DNPH) and protein carbonyl groups (Figure 3-77), (Levine et al., 1994).

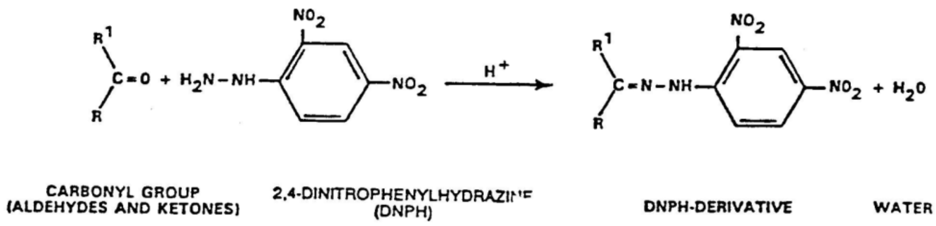


Figure 3-7: The reaction of 2,4-DNPH with a carbonyl group

The concentration of protein carbonyl present was calculated using the Beer-Lambert Law, which states that absorbance (Abs) is proportional to the concentration (**c**) of the absorbing molecules, the length of light-path (path length) through the medium and the molar extinction coefficient.

Absorbance = ε (extinction coefficient of DNPH) * c (concentration) * (path length)

Absorbance at wavelength 371 nm
ε = 22/mM•cm for DNPH
c = to be determined
path length = 1cm

thus: $c = Absorbance \times path\ length \div$ ε.

For each tooth wear group the carbonyl concentration was ranked from highest to lowest.

3.3.4.6.8 1-DE and 2-DE SDS PAGE

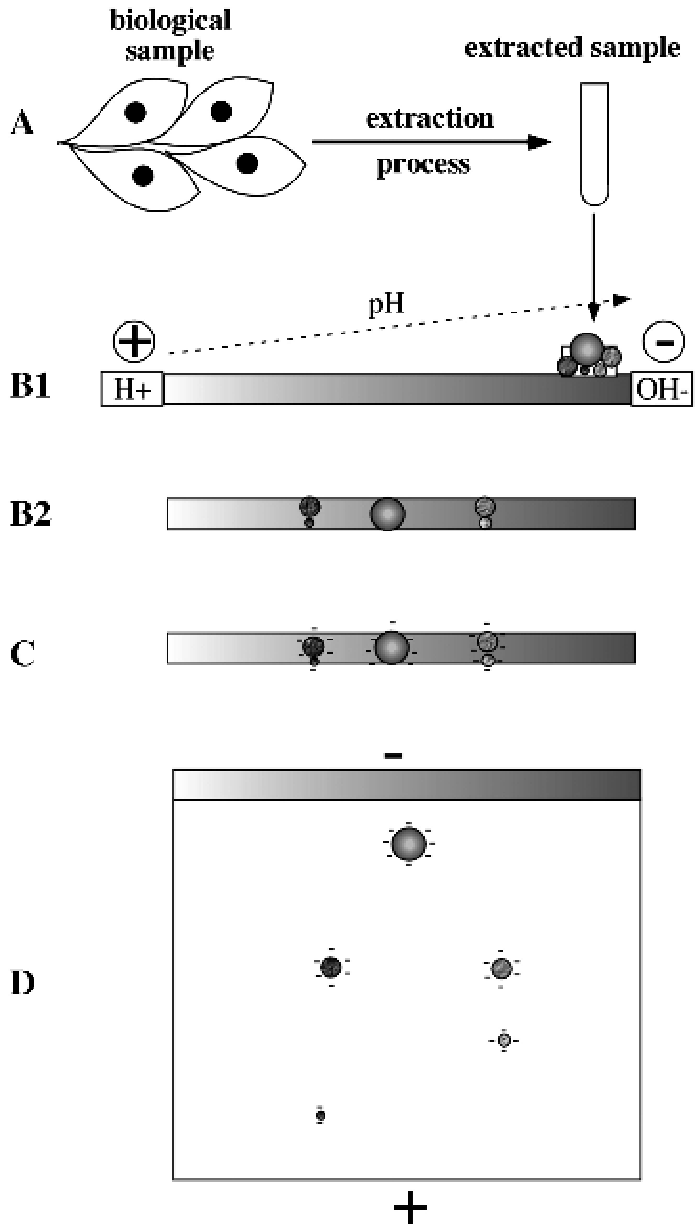
Electrophoresis is the migration of charged molecules in solution in response to an electric field. Both 1-DE and 2-DE SDS PAGE (Laemmli, 1970) were carried out. Saliva sample selection for this element was based on the participant's tooth wear score and the protein carbonyl concentration. Participants selected either had no tooth wear or moderate tooth wear, 13 samples were selected. Six participants with no tooth wear were selected. These were the three participants with the lowest protein carbonyl concentration and no tooth wear, and the three participants with the highest carbonyl concentration (n=6 out of n=29). Seven participants with moderate tooth wear were selected: three with the lowest and four with the highest protein carbonyl concentration (n= 7 out of 19) (Table 3-4).

Table 3-4: Choice of saliva samples for 1-DE and 2-DE

Saliva of participants with no tooth wear	Saliva of participants with moderate tooth wear
n=3 low protein carbonyl concentration	n=3 low protein carbonyl concentration
n=3 high protein carbonyl concentration	n=4 high protein carbonyl concentration

For electrophoresis, proteins were first derivatised with DNPH (Levine et al., 1994), followed by precipitation with 10% trichloroacetic acid. Protein pellets were recovered by centrifugation at 10,000 g for three minutes, washed with ethanol-ethyl acetate (1:1) to remove excess DNPH, and allowed to stand for 10 minutes. Pellets were redissolved in 6M guanidine solution, and centrifuged again to remove any insoluble material. Supernatants were retained for 1-DE (Laemmli, 1970) and 2-DE gel electrophoresis. For consistency, triplicate samples were analysed per sample. Gels were silver stained to reveal protein bands after separation (Rabilloud, 1990). For 2-DE, proteins were first resolved in IPG strips, and then separated in orthogonal 12% SDS PAGE gels yielding a two-dimensional array of individual protein spots.

Figure 3-8 shows diagrammatically the process that starts with the extraction of proteins from the sample to produce an IEF-compatible sample (A). The sample is then loaded onto a pH gradient (B1) oriented with the acidic side at the anode and the basic side at the cathode. After resolution in the IPG strips, the proteins reach their pI (B2). The strip was then equilibrated in a SDS-containing buffer, so that all proteins become negatively charged (C). The IEF gel is then loaded on top of a SDS PAGE gel, and the proteins separated according to their molecular masses (D)



(Rabilloud and Lelong, 2011)

Figure 3-8: The 2-DE process

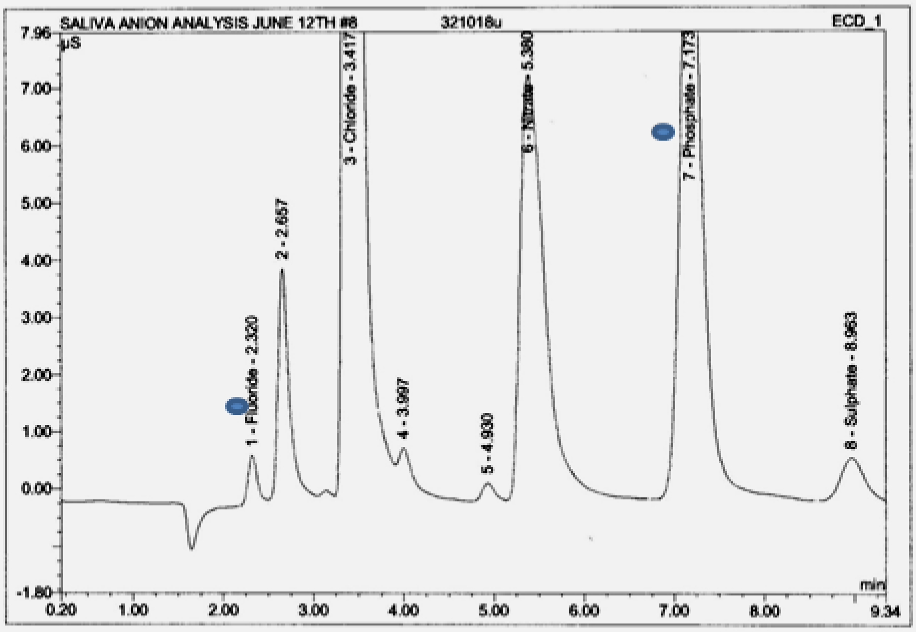
3.3.4.6.9 *Identification of protein spots by peptide mass fingerprinting using mass spectrometry*

Specific salivary protein spots, separated by 2-DE were selected for matrix assisted laser desorption/ionization (MALDI) - Time of Flight (TOF) mass spectrometry (Aldred et al., 2004). The spots of interest at; 15-17, 62-63 and 104-106 kDa were selected for in-gel tryptic digestion and sent on to the Mass Spectrometry Laboratory (*Analytical Services Unit, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Lisbon, Portugal)*. Spots were digested with trypsin and peptides were loaded onto an R2 micro-column from where they were desalted, concentrated and eluted directly onto a MALDI plate using α-cyano-4-hydroxycinnamic acid (α-CHCA) as the matrix solution in 50% acetonitrile and 5% formic acid. Mass spectra of the peptides were acquired in the positive linear and reflectron MS modes using a 4800 plus MALDI-TOF/TOF MS analyzer PO 01MS (with an exclusion list of the trypsin autolysis peaks (842.51, 1045.56, 2211.11 and 2225.12). The collected MS and MS/MS (conventional tandem mass spectrometry method) spectra were analysed in combined mode using the MASCOT search engine (http://www.matrixscience.com) and the National Centre for Biotechnology Information (NCBI (http://www.ncbi.nlm.nih.gov/pubmed) database restricted to 50 ppm peptide mass tolerance with human taxonomy.

3.3.4.6.10 *Salivary analytes and ion chromatography, isocratic method*

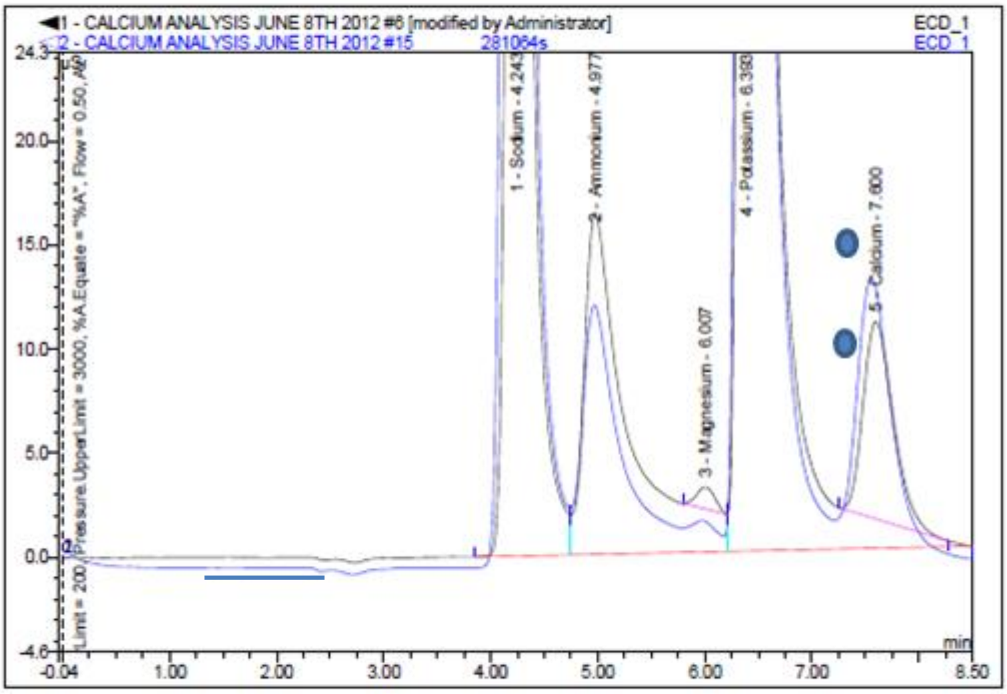
The isocratic method for ion chromatography (Dionex ICS 1000 with guard column, *Thermo Fisher Scientific*, United States) was carried out at the Department of Chemistry, UCC. The salivary anions were determined using an IonPac AS14A column, the column is designed for the separation of inorganic anions, including fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulphate, using a carbonate/bicarbonate eluent coupled with suppressed conductivity detection. Salivary cations were determined using the CS16 column. Sixty-one samples were selected for analysis of fluoride, phosphate and calcium ions. The selection of participants for IC was based on the the participants tooth wear score (no wear and moderate wear (moderate tooth wear had tooth wear with dentine exposed on both anterior and posterior teeth) and the availability of a saliva sample. Fluoride and phosphate anions were separated and quantified in a single injection using an IonPac AS14A analytical column with IonPac AG14A guard column at room temperature.

The run time was ten minutes with an 8.0 mmol l^- Na_2CO_3 (sodium carbonate) / 1.0 mmol l^- $NaHCO_3$ (sodium bicarbonate) eluent and an eluent flow rate of 1.5 ml/min. With a carbonate / bicarbonate eluent at a pH of approximately 8, phosphate elutes as HPO_4^{2-} before sulphate (Figure 3-7). Calcium cations were separated and quantified using an IonPac CS16 Analytical Column with guard column (Figure 3-10). The run time was ten minutes using 30 mmol l^- methanesulfonic acid.



No.	Ret.Time min	Peak Name	Height µS	Area µS*min	Rel.Area %	Amount mg/L	Type
1	2.32	Fluoride	0.843	0.101	0.36	0.484	BM
2	2.66	n.a.	4.041	0.579	2.09	n.a.	MB
3	3.42	Chloride	175.515	20.967	75.85	129.264	BM
4	4.00	n.a.	0.876	0.184	0.66	n.a.	MB
5	4.93	n.a.	0.296	0.047	0.17	n.a.	BM
6	5.38	Nitrate	7.391	2.345	8.48	11.286	MB
7	7.17	Phosphate	11.609	3.203	11.59	72.498	BMB
8	8.96	Sulphate	0.722	0.218	0.79	1.852	BMB
Total:			201.292	27.643	100.00	215.385	

Figure 3-9: Chromatogram of a typical saliva sample using isocratic elution for anions



No.	Ret.Time min	Peak Name	Height µS	Area µS*min	Rel.Area %	Amount mg/L	Type
1	4.24	Sodium	180.285	31.979	41.78	2.919	BM
2	4.98	Ammonium	16.389	7.778	10.16	15.254	M
3	6.01	Magnesium	1.049	0.209	0.27	0.287	Rd
4	6.39	Potassium	110.320	33.448	43.70	75.824	MB*
5	7.60	Calcium	9.486	3.124	4.08	4.237	Rd*
Total:			317.529	76.538	100.00	98.522	

Figure 3-10: Chromatogram of a typical saliva sample using isocratic elution for cations

3.3.4.6.11 Preparation and determination of salivary analytes in saliva

Five anion and cation standards were prepared. The Dionex® five anion standard mixture containing, 20 ppm F^-, 30 ppm Cl^-, 100 ppm NO^-_3, 150 ppm HPO_4^{2-} and 150 ppm SO_4^{2-} in the range of 0.2 ppm to 2 ppm fluoride were used to prepare standards. To determine the high phosphate anion concentration a 75 ppm additional phosphate standard was prepared.

Both unstimulated and stimulated saliva samples (n=61 of each) were thawed and vortex mixed to ensure homogeneity. Two mmol l^{-1} sodium azide (NaN_3) was added to prevent bacterial degradation. To eight ml of NaN_3 one ml of saliva was added with a micropipette, the micropipette was rinsed using the eight ml of NaN_3 to ensure that none of the saliva sample remained in the micropipette. The volumetric flask was then filled to the 10 ml mark.

The sample in the volumetric flask was vortex mixed to homogeneity. Prior to injection, to rinse the syringe with the sample, a one ml syringe was filled with sample and emptied twice to waste. The syringe with a 0.45µm pore size filter to remove insoluble particles was filled with sample and the sample injected into the instrument. The majority of the one ml of sample washed the sample loop in the instrument, only the final 25 µl of the injected sample were held in the sample loop for the actual injection on to the column.

Analyte concentrations were identified by their retention time and the location of the peak on the chromatogram.

3.3.5 Data Management

In line with the tested methods, direct data entry was used for the clinical examination, and each subject completed the oral hygiene, dietary habits and behaviours e-questionnaire directly to a hand-held PC. A unique reference number linked the clinical record, the consent form, medical questionnaire, inclusion and exclusion criteria, demographic data and the oral hygiene, dietary habits and behaviours questionnaire and saliva samples.

3.3.6 Means of documenting subject and examiner information during fieldwork

General Information: The participants' initials, represented by the initial of the first name, middle name and then surname, were included on all electronic and paper forms:

1^{st}	2^{nd}	Surname

Subject Identification Codes: Each child was given a discrete six-digit number. This number was included on all electronic and paper forms. The following numbering system was adopted:

School		Class	Child number within		

The 1^{st} and 2^{nd} digit correspond to the number given for each participating school. Schools in fluoridated areas were coded 1 to 30 and schools in non-fluoridated areas coded 31 to 60.

The 3^{rd} digit corresponds to the class in school the subject is in: Code 1 = Transition year (TY).

The 4^{th}, 5^{th} and 6^{th} digits denote the subject examined. The first child examined in each school was identified as 001.

Examiner Number: The examiner [M.H.] had a two-digit code number: 01

Reproducibility: Five per cent of participants were re-examined; each first examination was coded 1. Duplicate examinations were designated code 2. Intra-examiner reproducibility was determined using the K. Code 2 scores were only included for the calculation of K (Landis and Koch, 1977).

Gender: Male was recorded as Code 1; Female was recorded as Code 2.

Fluoride History: The demographic data form indicated the fluoride history of the subject and the following codes assigned:

No exposure to a fluoridated water supply – Code 1

Continuous exposure to a fluoridated water supply – Code 2

Partial exposure to fluoridated water supplies or fluoride supplements – Code 3.

Oral hygiene, dietary habits and behaviours questionnaire: Each subject completed the e-questionnaire.

Confidential medical history questionnaire: Completed by the parent or guardian for each participant.

Social Class: Whether or not the family had, a medical card was used as a dichotomous proxy for disadvantage.

3.3.7 Statistical Analyses

Statistical analyses were conducted using IBM SPSS Ver. 20 (USA) and SAS Ver.9.0. Data were analysed at the participant level; each individual was characterised by their maximum score. Descriptive statistics were reported for the proportions with no tooth wear, tooth wear in enamel and tooth wear in dentine. Prevalence was expressed as the proportion of subjects with one or more positive scores (categories; no wear, mild tooth wear or moderate tooth wear).

Tooth wear is a collective term associated with the different aetiologies of erosion, abrasion and attrition and the risk factors for tooth wear can affect enamel and dentine differently. For that reason a large number of outcome variables were selected as shown in Table 3-5 and Table 3-6. A large number of explanatory variables were selected, these variables were informed by the review of the literature and the results of the pilot questionnaire completed by the participants (Table 3-7). Multivariable binary logistic regression models were used with the outcome variables set out in Table 3-5. The number of buccal/labial and palatal/lingual surfaces affected by any tooth wear and the number of surfaces affected by tooth wear into dentine were analysed using multivariable Poisson regression models (Table 3-6). The proportion of surfaces affected by 'any tooth wear' was analysed using a linear model with the percentage of surfaces affected as the outcome variable. A square root ($\sqrt{}$) transformation was applied to the percentages prior to analysis. The potential explanatory variables (Table 3-7) were screened for inclusion in these models by considering the individual binary logistic/Poisson regression models for each. The variables that were significant at a level of 10% were then included together in the multivariable logistic or Poisson regression model. Backward elimination was manually applied and insignificant variables at the 5% level of significance removed. The final model contained only significant variables.

Table 3-5: The selected outcome variables for tooth wear at the subject level

Outcome variable	Score
Any surface	Maximum score for the individual from any of the surfaces: occlusal/incisal, buccal/labial or palatal/lingual surfaces
The buccal surface	Maximum score for the individual on the buccal/labial surface
The lingual surface	Maximum score for the individual on the palatal/lingual surface
The occlusal surface	Maximum score for the individual on the occlusal surface

Table 3-6: The selected outcome variables for tooth wear at the tooth level

Outcome variable	Score
Number of buccal/labial surfaces affected	Count
Number of palatal/lingual surfaces affected	Count

Table 3-7: The explanatory variables considered for the cross-sectional study

Explanatory variables considered	Option	Option	Option	Option
Demographics				
Gender	Male	Female		
Fluoridation status	Always resident with a fully fluoridated water supply	Never resident with a fully fluoridated water supply	Had some exposure to a fluoridated water supply (part)	
Medical card	Family have a medical card	Family do not have a medical card		
Tooth brushing habits				
Frequency of tooth brushing	Brushes twice a day and more	Brushes once a day and less	Never	Other
Timing of tooth brushing	Before breakfast	After breakfast		
	Before other meals	After other meals		
	Before going to bed	Not before going to bed		
Dietary choices and habits				
Types of drinks consumed	Fizzy drinks (cola), fruit juices, squashes, tea, coffee, water, cider and alcopops			

Explanatory variables considered	Option	Option	Option	Option
How do you typically take the fizzy or squash (acidic) drink	From a bottle	From a cup or glass	From a can	From a straw
Frequency of consumption of (fizzy drinks (cola), fruit juices, squashes) acidic drinks	Once a day and more	Less than once a day	Less than once a week	More than once a week
Frequency of consumption of citrus fruits	Once a day and more	Less than once a day		
Frequency of consumption of apples	Once a day and more	Less than once a day		

Health promoting and lifestyle activities

Do you bite your nails	Yes	No		
Tooth sensitivity	Yes	No		
Stomach upsets and vomiting	Yes	No		
Are you vegetarian	Yes	No		
Reported frequency of physical and strenuous exercise	Three to four times a week or more	Twice a week		
Dental attendance	Three or more times	Never		

Parametric and non-parametric statistical tests were used to compare the various salivary parameters. The Shapiro-Wilk test was used to test for normality and Box plots constructed to visually display quartiles and the distribution. For the comparison of the difference in the means with a normal distribution, Student's t-test was used. The Mann Whitney U test was used with non-normal distributions. The statistical significance was 5% (Table 3-8).

Table 3-8: Comparison of the difference between two means

Requirement	Statistical tests	Means to be compared
Compare the means for groups with or without tooth wear	Student's t-test	Salivary flow rate, unstimulated and stimulated
	Student's t-test	Total protein concentration
	Student's t-test	Protein carbonyl concentration
	Student's t-test	The ratio of protein carbonyl concentration to protein concentration
	Mann Whitney U test	Total fluoride ion concentration (stimulated)
	Mann Whitney U test	Total phosphate ion concentration (stimulated)
	Mann Whitney U test	Total calcium ion concentration (stimulated)

3.4 Results of training, calibration and piloting for the cross sectional study

The results of training and calibration and the intra- examiner values are presented in the this section (3.4). In section 3.5 the materials and methods for the longitudinal study are then presented. The results of the cross sectional study are then presented in chapter 4.

With the assistance of the transition year co-ordinator, forms were distributed to invite students to participate in a training and calibration programme to train MH . Eighty forms were distributed, 36 forms were returned and 32 children participated. Four students were excluded due to illness and unavailability on the days of the training and calibration programme. The software developed for the study was piloted and the conditions under which testing occurred were similar to those used for the main study. Participants were provided with a set of printed instructions indicating how to complete the e-questionnaire and no further adult support was required.

3.4.1 Kappa statistic prior to the main study

An inter- and intra-examiner unweighted kappa score of 0.7 was achieved during calibration. During the fieldwork, every 10th subject was re-examined and an intra-examiner kappa value determined. Cohen's kappa measures the agreement between two raters who each classify N items into C mutually exclusive categories. If the raters are in complete agreement then $\kappa = 1$. If there is no agreement among the raters other than what would be expected by chance $\kappa = 0$ (Landis and Koch, 1977).

3.4.2 Kappa inter-examiner calibration

The inter-examiner kappa value calculated between MH and AM (the gold standard) was 0.74. This value demonstrated substantial agreement, taking into account that which would have occurred by chance (Landis and Koch, 1977). The results of the calibration indicate that for 262 surfaces, both examiners were in agreement that no tooth wear was present, for 60 surfaces both examiners were in perfect agreement for score 1, and on 49 surfaces for score 2. For 32 surfaces, the author (MH) scored the surfaces as score 2 and the gold standard (AM) scored them as 1. The over-scoring was discussed further and improved prior to the main study (Table 3-9).

Table 3-9: Inter-examiner agreement between AM and MH (Bardsley et al., 2004)

	AM				
MH	0	1	2	9	Total
0	262	14	0	0	276
1	19	60	1	0	80
2	7	32	49	0	88
9	0	0	0	13	13
Total	288	106	50	13	457

3.4.3 *Kappa intra-examiner calibration in the main study*

Throughout the study, the Recorder selected participants to return for a second examination, this second examination was to determine the intra-examiner reliability of MH. The unweighted kappa score was 0.8 with 95% percentage agreement. For this exercise MH scored 60 surfaces score 1 on both occasions and 15 surfaces score 2 on both occasions. For 8 surfaces, MH scored 0 the first time and 1 the second time; for six surfaces, MH scored 2 for the first examination and 1 at the second examination (Table 3-10).

Table 3-10: Intra-examiner agreement for MH (Bardsley et al., 2004)

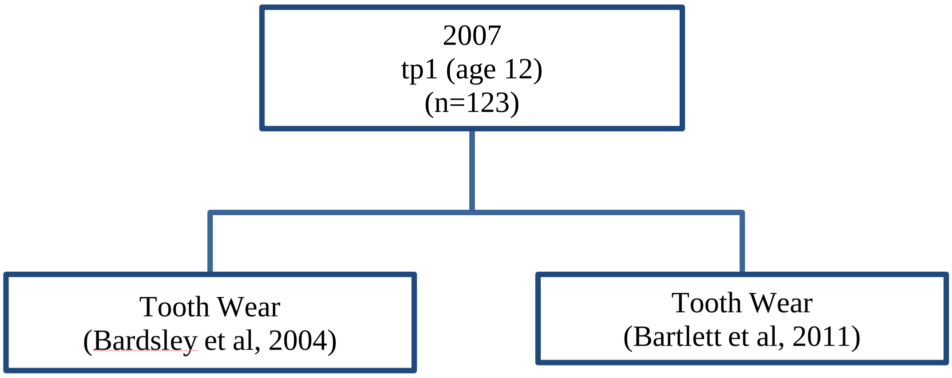
	MH (1)			
MH (2)	0	1	2	Total
0	228	2	0	230
1	8	60	6	74
2	1	0	15	16
Total	237	62	21	320

The intra-examiner kappa statistic of 0.8 indicated substantial to excellent agreement.

Once ethical approval for the study was obtained, the software designed and the examiner and recorder trained in the survey methods, the study commenced.

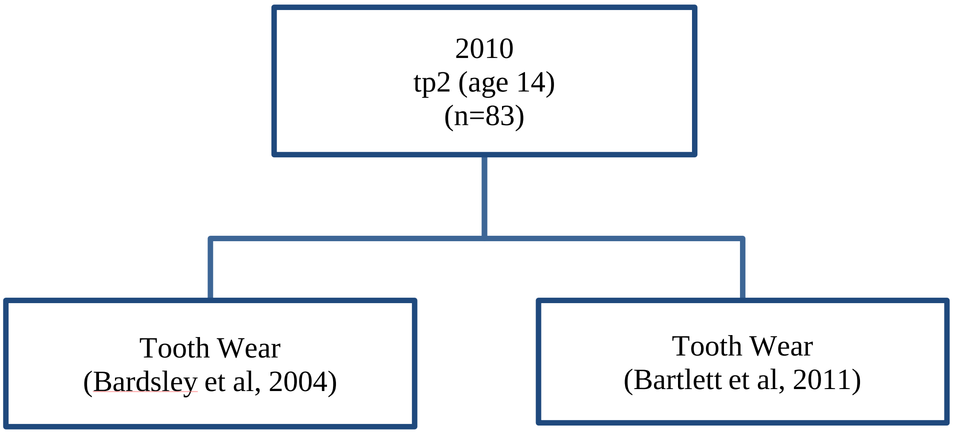
3.5 Longitudinal study: dental examination, questionnaire responses at tp1 (age 12) and tp2 (age 14)

The materials and methods for the longitudinal study are presented in this section. Schematics in Figure 3-11 and Figure 3-12 indicate the flow of subjects through the study. The children available at tp1 were children who provided consent for future contact when they participated in a study of dental erosion at age 5; the study is described as tp0 (age 5) (Harding et al., 2003).



121 children consented to follow-up

Figure 3-11: The year and indices used at tp1, and flow of participants through the study



At the second time point (tp2) the children were 14-years-old.

Figure 3-12: The indices used at tp2, and flow of participants through the study

In Table 3-11 the number and mean age of children available at each time point is set out.

Table 3-11: The mean age and number of children examined at each tp

Mean Age (SD)	Time point (tp)	Number who consented	Number examined
5.49 (0.28)	tp 0	286	202 (Harding et al., 2003)
12.01 (0.32)	tp1	168 consented to future contact	123 (Harding et al., 2010)
14.7 (0.3)	tp2	121 consented to future contact, 96 forms returned	83

3.5.1 Training and calibration in the measurement of tooth wear for the longitudinal study

The modified TWI index (Mod TWI) (Bardsley et al., 2004) was used at tp1 and tp2, similar to the cross-sectional study with 16-year-old students (Table 3-12). In addition, the Exact Tooth wear Index (ETI) (Bartlett et al., 2011b) (Table 3-13) was introduced to record tooth wear at an increased number of levels in enamel. Dr Soha Dattani (SD) provided training in the ETI (Bartlett et al., 2011b). Training took place at the Oral Health Services Research Centre (OHSRC), Cork on 23rd April 2007 and at St. Catherine's primary school, Bishopstown, Cork, with both school and parental permission, on 24th April 2007. Both SD and MH examined photographs and models for tooth wear at the OHSRC, then with six participants at St. Catherine's primary school. During training detailed discussion occurred between SD and MH until MH confidently and reproducibly categorised the level of tooth wear present. Calibration then took place with a further five participants. The gold standard (SD) and the author (MH) examined the same participants and calculated an inter-examiner kappa value for the ETI (Bartlett et al., 2011b). An individual score was assigned for each individual tooth, for tooth wear in enamel and tooth wear in dentine on the surfaces, buccal/labial including cervical, palatal/lingual and occlusal/incisal.

Table 3-12: Scoring criteria – Mod TWI (Bardsley et al., 2004)

Score	Criteria
0	Loss of enamel surface characteristics but NO dentine visible
1	Dentine is visible <1/3rd. Cupping on molar cusp tips. Change in colour
2	>1/3rd dentine exposed
3	Pulp or secondary dentine exposed
R	Missing/restored/, could not be assessed at a given examination

Table 3-13: Scoring criteria – ETI (Bartlett et al., 2011b)

Score	Enamel	Dentine	Cervical Contour
0	No loss of enamel characteristics	No dentinal tooth wear: No loss of dentine	No tooth wear: No loss of tooth contour
1	Loss of enamel affecting $<{}^{1}/_{10}{}^{\text{th}}$ of the scored surface.	Loss of dentine affecting $<{}^{1}/_{10}{}^{\text{th}}$ of the scored surface	<1mm loss of tooth surface depth
2	Loss of enamel affecting $<{}^{1}/_{3}{}^{\text{rd}}$ of the scored surface.	Loss of dentine affecting $<{}^{1}/_{3}{}^{\text{rd}}$ of the scored surface	Tooth surface loss in depth measuring ≥ 1mm but <2mm
3	Loss of enamel affecting at least ${}^{1}/_{3}{}^{\text{rd}}$ but $<{}^{2}/_{3}{}^{\text{rds}}$ of the scored surface	Loss of dentine affecting at least ${}^{1}/_{3}{}^{\text{rd}}$ but $<{}^{2}/_{3}{}^{\text{rd}}$ of the scored surface	Tooth surface loss in depth measuring ≥ 2mm
4	Loss of enamel affecting $\geq {}^{2}/_{3}{}^{\text{rds}}$ of the scored surface	Loss of dentine affecting $\geq {}^{2}/_{3}{}^{\text{rd}}$ of the scored surface, no pulpal exposure	
5		Secondary dentine formation or pulpal exposure	

The presentation format of the ETI (Bartlett et al., 2011b) above is different to the earlier depiction in table 2.6 , this is to facilitate comparison between the Mod TWI (Bardsley et al., 2004) and the ETI (Bartlett et al., 2011b), one after the other.

3.5.2 Fieldwork

3.5.2.1 Measurement and recording

The recording of the parent/guardian informed consent, subject assent, personal medical history, demographic data, inclusion and exclusion criteria were all conducted in a manner similar to that used in the cross-sectional study with 16-year-old students (Section 0) [Appendix 8].

3.5.2.2 Data entry software

Software for the direct data entry of the clinical dental examination and subject questionnaire was developed, building on the platform already developed for the cross-sectional study with the 16-year-old students (section 3.3.2). The questionnaire on oral hygiene habits, dietary habits general health and knowledge was completed on paper at tp1 and directly to the hand held device at tp2 [Appendix 8].

3.5.2.3 Parameters recorded

The Mod TWI (Bardsley et al., 2004) and ETI (Bartlett et al., 2011b), were both used to record tooth wear. The teeth were first scored using the Mod TWI and, after a time lapse of five minutes, the teeth were then scored with the ETI. The time lapse was to allow the subject to rest and ensure that the teeth did not dry out excessively. Using the Mod TWI (Bardsley et al., 2004) tooth wear scores were assigned for the buccal/labial, palatal/lingual and the incisal surface of each anterior tooth and the occlusal surface of each first permanent molar. A maximum of 40 surfaces were scored. Scores were directly entered to the laptop. (Figure 3-13).

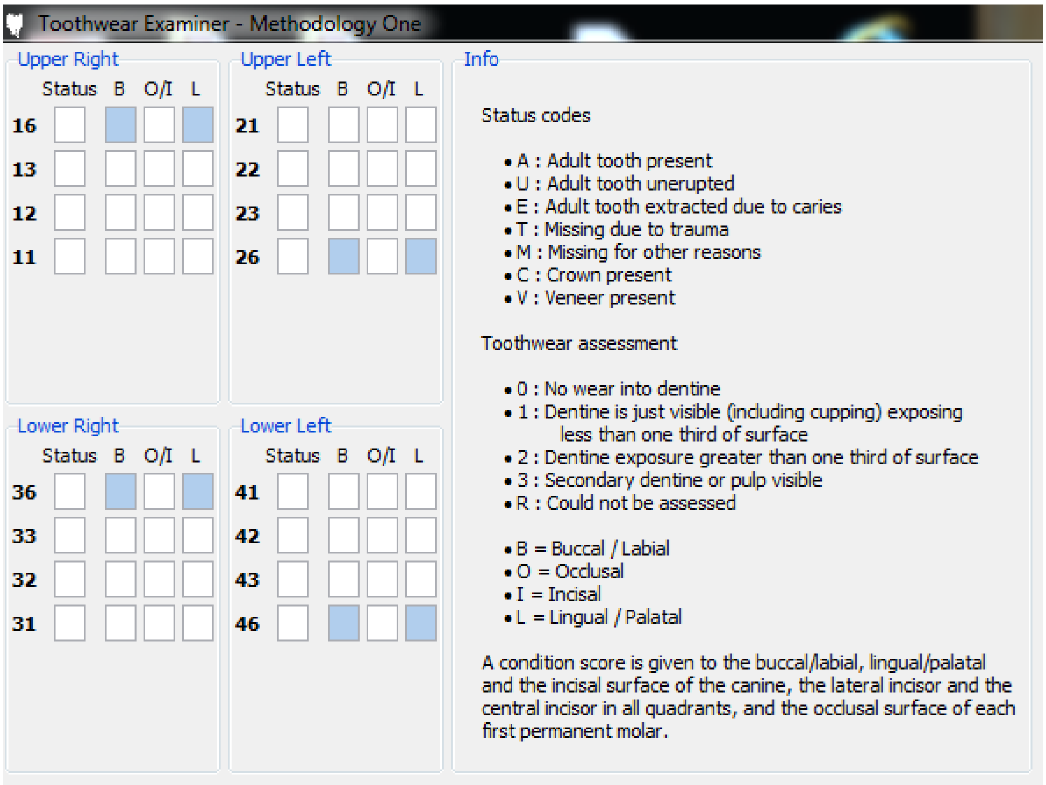


Figure 3-13: Screen interface for tooth wear Mod TWI (Bardsley et al., 2004)

With the second index, the ETI (Bartlett et al., 2011b), a score is assigned to all of the erupted permanent teeth with the exception of third molars. Dental enamel was graded for five stages (0 to 4), indicating the loss of enamel over the tooth surface without dentine being affected. Dentine was graded for six stages (0 to 5). For each tooth, the index scored four individual surfaces: buccal/labial (the assessment included the buccal-cervical portion); buccal-cervical (cervical $^{1}/_{3}{}^{rd}$ of the buccal surface); palatal/lingual; and the occlusal or incisal surface of all teeth. Thus, separate scores existed for tooth wear in enamel and dentine. Enamel was scored first, then dentine, then the cervical area. For the buccal-cervical portion, tooth wear was assessed in terms of enamel, dentine and depth and entered to the laptop (Figure 3-14). A maximum of 112 surfaces were scored for each subject. Categorisation at the subject level was according to severity and was based on the participant's maximum score.

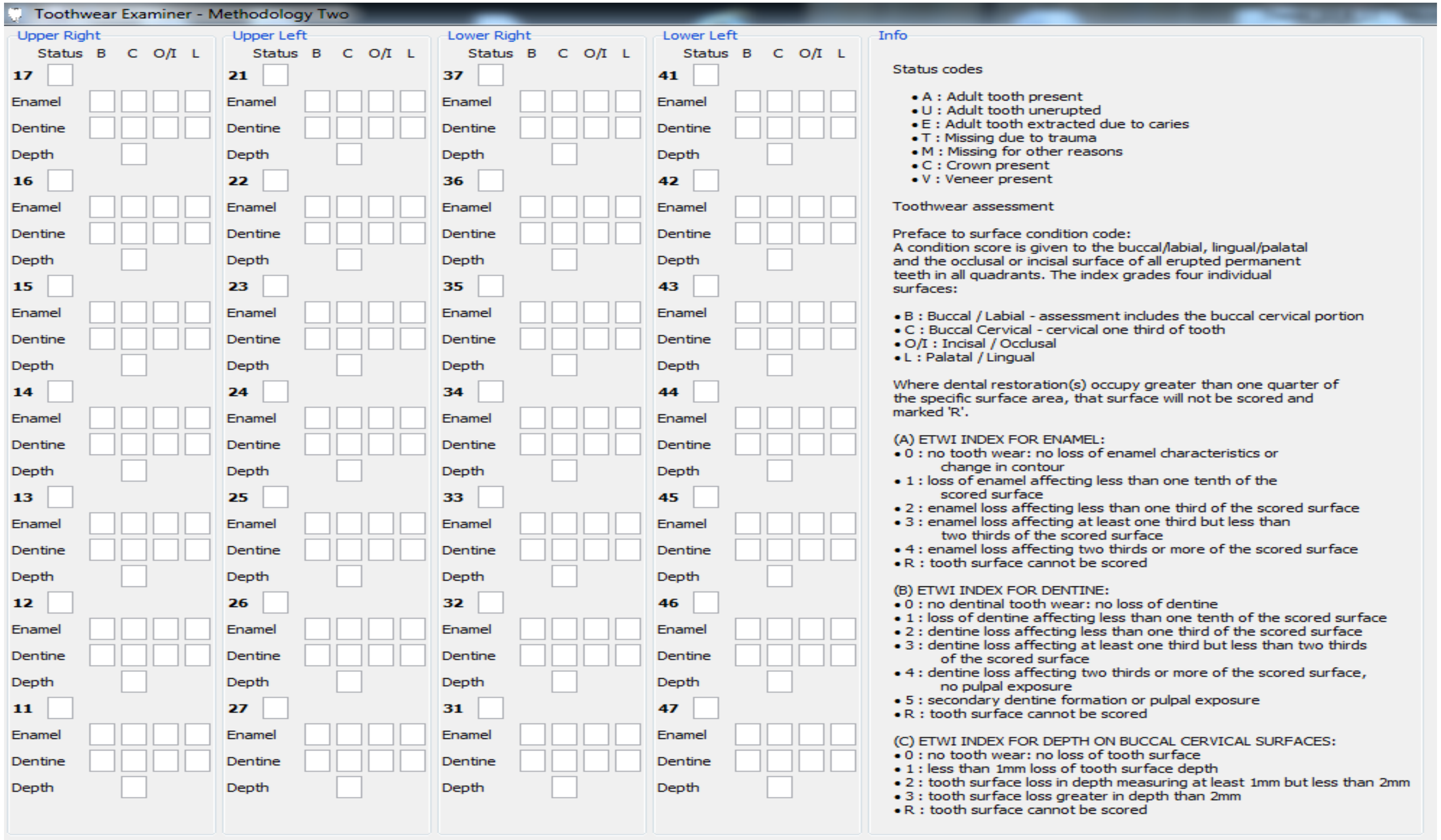


Figure 3-14: Screen interface for tooth wear ETI (Bartlett et al., 2011b)

3.5.3 *Means of documenting subject and examiner information during fieldwork*

The means of documenting subject and examiner information during fieldwork was almost identical to process in the cross sectional study (section 3.3.6). The differences were:

1. Subject ID was that assigned at tp0 (age 5), and maintained for tp1 (age 12) and tp2 (age 14)
2. At age 12 the participants completed the questionnaire themselves on paper

3.5.4 *Statistical Analyses*

Statistical analyses were conducted using IBM SPSS Version 22 (USA), and SAS Version 9.4. Previous analysis of the data at age 5-years and 12- years indicated that tooth wear on the first permanent molars at age 12 was associated with the previous experience of tooth wear in the primary dentition (Harding et al., 2010), (Figure 3-15).

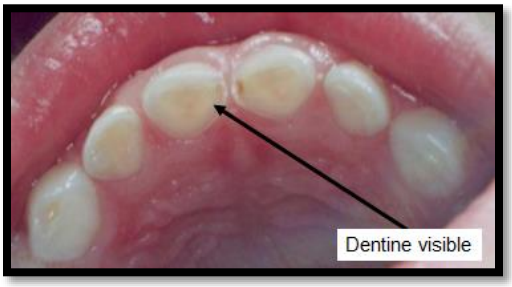


Figure 3-15: Tooth wear with dentine visible at age 5

Outcome and explanatory variables were selected based on the review of the literature, information acquired from previous analysis at tp0 (age 5) and tp1 (age 12). Explanatory variables collected via questionnaires at previous time points [Appendix 2a, 6 and 6] and tooth wear outcome variables from time points tp0 (age 5) and tp1 (age 12) were all considered as explanatory variables at age 14 (Table 3-14).

Table 3-14: Longitudinal study – the explanatory variables considered

Explanatory variables considered	Option	Option
Gender	Male	Female
Fluoridation status	Always resident with a fully fluoridated water supply	Never resident with a fully fluoridated water supply
Medical card	Family have a medical card	Family do not have a medical card
Previous erosive tooth wear at age 5	Had erosive tooth wear at age 5	Had no erosive tooth wear at age 5
Previous erosive tooth wear in enamel only at age 5	Had erosive tooth wear in enamel only at age 5	No erosive tooth wear in enamel only at age 5
Previous erosive tooth wear in dentine/pulp at age 5	Had erosive tooth wear in dentine/pulp at age 5	No erosive tooth wear in dentine/pulp at age 5
Previous tooth wear on smooth surfaces at age 12	Tooth wear on smooth surfaces at age 12	No tooth wear on smooth surfaces at age 12
Previous tooth wear on the incisal surfaces of incisors exposing dentine at age 12	Had tooth wear into dentine at age 12, on the incisal surface of one of the maxillary or mandibular incisors	Had no tooth wear into dentine at age 12, on the O/I surface of one of the maxillary or mandibular incisors
Frequency of tooth brushing at both age 12 and age 14	Brushes twice a day and more	Brushes once a day and less
Timing of tooth brushing age 12 and 14	Before breakfast	After breakfast

Explanatory variables considered	Option	Option
	Before other meals	After other meals
	Before going to bed	Not before going to bed
Age 5 questionnaire: age at which use of baby bottle stopped		
Types of drinks consumed at age 12 and 14	Fizzy drinks (cola), fruit juices, squashes, tea, coffee, water	
How do you typically take the fizzy or squash (acidic) drink age 12 and 14	From a bottle	From a cup or glass
Frequency of consumption of (fizzy drinks (cola), fruit juices, squashes) acidic drinks age 12 and 14	Once a day and more	Less than once a day
Frequency of consumption of citrus fruits age 12 and 14	Once a day and more	Less than once a day
Frequency of consumption of apples age 12 and 14	Once a day and more	Less than once a day
Frequency of consumption of yoghurt age 12 and 14	Once a day and more	Less than once a day
Taking vitamins or medicines age 12 and at age 14	Yes	No
Use of an inhaler for asthma at age 14	Yes	No
Suffer with a dry mouth at age 14	Yes	No
Do you bite your nails at age 14	Yes	No

Explanatory variables considered	Option	Option
Tooth sensitivity at age 12 and at age 14	Yes	No
Stomach upsets and vomiting at age 12 and at age 14	Yes	No
Are you vegetarian at age 14	Yes	No
Reported frequency of physical and strenuous exercise at age 14	Once a week	Twice a week

In addition an increment between tp1 (age 12-years) and tp2 (age 14-years) was calculated. The increment scores were a maximum increment score and a mean increment. The maximum increment score was the difference between the tp1 (age 12) and the tp2 (age 14) maximum scores for each individual. The mean increment was the difference between tp1 and tp2 at the surface level summated and divided by the number of surfaces scored. There were two assumptions made for the calculation of an increment, reversals were considered zero and enamel and dentine were assigned a score from 0 through to 9. Enamel wear scores were 0-4, and dentine wear scores were assigned a numeric value of 5-9. Only surfaces present at both time points were included in the calculation of an increment.

The presence of any tooth wear on the buccal/labial and palatal/lingual surfaces, the presence of any tooth wear on the first permanent molar teeth and the presence of tooth wear with dentine visible were analysed using multivariable binary logistic regression models (Table 3-15).

Table 3-15: Selected outcome variables for tooth wear at age 14 – 'presence of'

Outcome variable	Score
The smooth surfaces (buccal/labial and palatal/lingual)	The presence of tooth wear on the buccal/labial and palatal/lingual surfaces of maxillary and mandibular incisors (smooth surfaces)
The first permanent molar teeth	The presence of any tooth wear (either within enamel, or with dentine visible) on the first permanent molar teeth
Any surface	Presence of tooth wear that has extended in to dentine on 'any' surface

The number of buccal/labial and palatal/lingual surfaces affected by any tooth wear and the number of surfaces affected by tooth wear with dentine visible were analysed using multivariable Poisson regression models. The maximum increment score was analysed using a multivariable Poisson regression model. The mean increment was analysed using a normal regression model. A natural logarithmic transformation was applied after adding one to each mean increment (Table 3-16).

Table 3-16: Selected outcome variables for tooth wear, at age 14

Outcome variable	Score
The number of buccal/labial and palatal/lingual (smooth) surfaces affected by any tooth wears	Count
The number of surfaces affected by tooth wear with dentine visible	Count
The maximum increment score between (tp1) and (tp2).	Number
The mean increment between tp1 and tp2	Number

The potential explanatory variables were screened for inclusion in the models by considering individual binary logistic/Poisson regression or normal regression models for each. Those variables that were significant at a level of 10% were then included together in the models. Backward elimination was then manually applied to remove insignificant variables, at the 5% level of significance, until the final model contained only significant variables.

To maximise the power of the study, a participant was not included in the analysis if their data did not contain the variable(s) being investigated. However if the particular variable(s) being investigated were not significant then the participant could be reconsidered for inclusion in future analysis.

3.6 Results of training and calibration for the longitudinal study

The results of training and calibration and the intra- examiner values are presented here, before moving on to chapter 4 and the results of the main study.

3.6.1 Kappa statistic longitudinal study- inter examiner prior to the study

Once training was completed the gold standard (SD) and MH examined the same participants and calculated an inter-examiner kappa value for the ETI (Bartlett et al., 2011b) and achieved a kappa value of 0.71, indicating substantial agreement. Comparison of scores suggested that MH tended to underscore rather than over score (Table 3-17).

Table 3-17: Inter-examiner kappa calculation prior to the use of the ETI

	SD				
MH	0	1	2	3	Total
0	299	4	2	0	305
1	5	13	3	0	21
2	0	0	2	0	2
3	0	0	0	2	2
Total	304	17	7	2	330

3.6.2 Kappa statistic longitudinal study - intra-examiner kappa each tp

At each time point tp 1(age 12) and tp2 (age14), the intra-examiner kappa statistic was calculated for both of the indices that were used, the Mod TWI (Bardsley et al., 2004) and the ETI (Bartlett et al., 2011b).

3.6.2.1 Intra-examiner kappa at tp1

At tp1 (age12), the intra-examiner kappa for the Mod TWI (Bardsley et al., 2004) score was one, denoting perfect agreement and consistency from the examiner. The intra-examiner kappa score for the ETI at tp1 was 0.74, indicating substantial agreement (Table 3-18).

Table 3-18: Intra-examiner agreement at tp1

	MH (1)				
MH (2)	0	1	2	3	Total
0	664	5	4	0	673
1	2	7	0	0	9
2	0	0	7	2	9
3	0	0	0	4	4
Total	666	12	11	6	695

3.6.2.2 Intra-examiner kappa at tp2

During the examinations at tp2 (age14), the intra-examiner kappa value calculated for the Mod TWI (Bardsley et al., 2004) was 0.78 (Table 3-19) and 0.82 for the ETI (Bartlett et al., 2011b) (Table 3-20).

Table 3-19: Intra-examiner kappa for the Mod TWI (Bardsley et al., 2004) at tp2

	MH (1)		
MH (2)	0	1	Total
0	102	2	104
1	2	8	10
Total	104	10	114

Table 3-20: Intra-examiner kappa for the ETI (Bartlett et al., 2011b) at tp2

	M H (1)					
MH (2)	0	1	2	3	4	Total
0	703	3	0	1	0	707
1	4	13	1	0	0	18
2	0	1	1	1	0	3
3	0	0	0	8	1	9
4	0	0	0	1	6	7
Total	707	17	2	11	7	744

K values achieved at each time point all demonstrated substantial agreement (Landis and Koch, 1977).

The values considered by Landis and Koch, (1977) for the strength of agreement are provided in Table 3-21.

Table 3-21: Guidelines for the strength of agreement between two examiners

Kappa value		Description
0		Poor
0	0.2	Slight
0.21	0.40	Fair
0.41	0.60	Moderate
0.61	0.80	Substantial
0.81	1.00	Almost perfect

Chapter 4 **Results**

4.1 Introduction

The results of the cross-sectional study at age 16 are presented first and are followed by the longitudinal study for participants at age 12 and 14 years, referred to as tp1 and tp2 respectively. In chapter 5, the results presented in this chapter are discussed and comparisons made with previous research.

4.2 Cross sectional study, participants age 16: dental examination, questionnaire responses and salivary analyses

In the cross sectional study at age 16, the prevalence of tooth wear is reported according to the proportion of individuals with at least one lesion present (subject level).

The results of the cross sectional study addressed the following research objectives:

1. Estimate the prevalence and levels of tooth wear in 16-year-old students.
2. Investigate tooth wear levels and associations with demographic factors.
3. Investigate tooth wear levels and associations with oral hygiene practices and dietary factors.
4. Investigate tooth wear levels and associations with the properties of resting and stimulated saliva.

The characteristics of the sample, the tooth wear scores and the questionnaire responses for demographics, oral hygiene practices, dietary practices, lifestyle and general health are presented. The results of the analysis of both unstimulated and stimulated saliva are also presented.

The statistical analyses for the cross-sectional study are presented in the order:

- Salivary proteins
- Salivary inorganic ions
- Analysis of the data on oral hygiene practices, dietary practices, general health and demographic factors collected via questionnaire.

Neither the unstimulated nor the stimulated saliva were included in the logistic regression models. No difference was determined when the mean flow rate for those without tooth wear and those with tooth wear at the various levels were compared.

4.2.1 Sample characteristics for the cross-sectional study

In total 394 teenagers were examined, all of whom were in transition year in schools in counties Cork and Kerry. The mean age of the participants recruited from transition year classes in Cork and Kerry post primary schools was 16.28 (0.43) years for males and 16.30 (0.47) years for females. The fluoridated group comprised, 61% male and 39% female; in the non-fluoridated group, there were 39% male and 61% female. The part- fluoridated group contained a small number of participants and comprised 52% male and 48% female. The sample proportion with a medical card was 17%, and 83% had no medical card. Six participants did not provide information on their medical card status (Table 4-1).

Table 4-1: Sample Characteristics of 16-year-old students

Gender	(n)	Mean Age (SD)	Fluoridated		Non Fluoridated		Part Fluoridated		Medical Card	
			n	%	n	%	n	%	Yes (n)	No (n)
Male	223	16.28 (0.43	176	61	26	39	21	53	37	180
Female	171	16.30 (0.47)	112	39	40	61	19	48	28	143
Total	394		288		66		40		65 (17%)	323
Each Strata Totalled	394				394				388 and (6, NR)*394	

*NR= not reported

4.2.2 The prevalence and demographic factors of tooth wear in transition year students

4.2.2.1 Objectives addressed in Section 4.2.2:

Objective 1: Estimate the prevalence and pattern of tooth wear in 16-year-old students.

Objective 2: Investigate tooth wear levels and demographic factors for 16-year-old students at a point in time.

Tooth wear was recorded according to the modified TWI (Mod TWI) (Bardsley et al., 2004). Scores for tooth wear in enamel (mild) or dentine (moderate or severe) were included (Table 4-2) (Bardsley et al., 2004). Tooth wear is expressed in terms of the proportion of participants with one or more positive scores. Participants are grouped according to their highest level of tooth wear.

Table 4-2: Tooth wear scores for 16-year-old students

Score	Description	Analysis Group	Dental tissues affected
0	No wear evident	No wear	No wear
1	Loss of surface characteristics	Mild	Enamel
2	Wear in enamel but dentine is not visible	Mild	Enamel
3	Wear in dentine ≤1/3rd dentine visible	Moderate	Dentine
4	Wear in dentine ≥1/3rd dentine visible	Moderate	Dentine
R	Missing/restored/could not be assessed	Not analysed	Not recorded

The frequency distribution for individuals exhibiting tooth wear at each individual score by fluoridation status and medical card (n and %) is set out in Table 4-3 and in Table 4.4. For clarity, the six subjects without a medical card status recorded are not included in the breakdown of fluoridation status by medical card status. Three belonged to the fluoridated group, one to the non-fluoridated group and two to the partially fluoridated group. Moderate tooth wear was present in 43% of participants who were lifetime residents of a fluoridated area (40% score 3~ and 3% score 4~). In the non-fluoridated group, the proportion was similar at 41% (41% with score 3~ and none with score 4~) Figure 4-1 and Figure 4-2

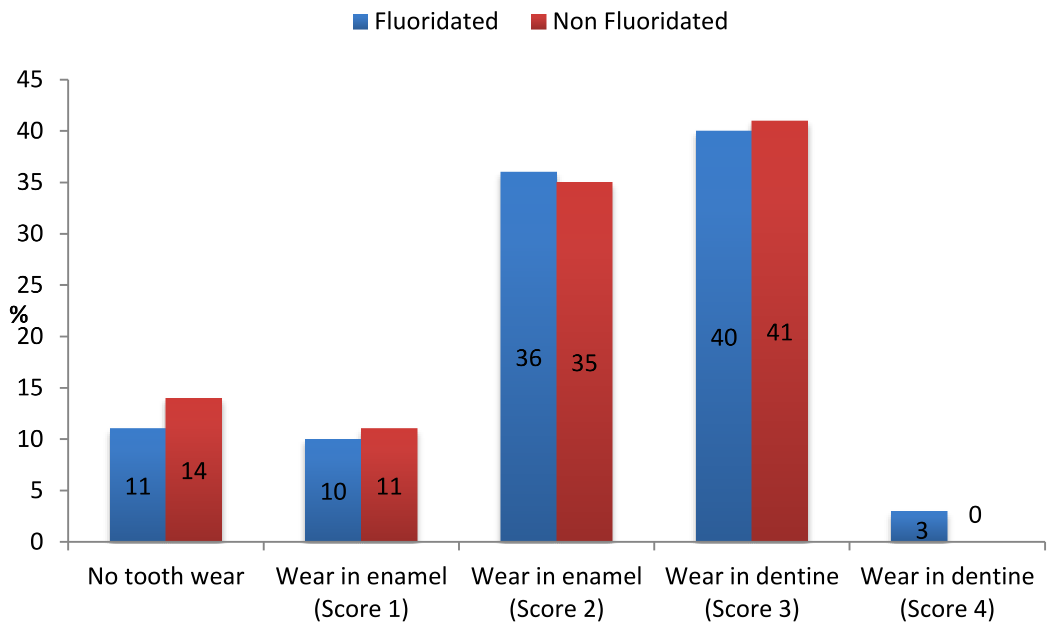


Figure 4-1: The percentage with tooth wear by their highest score for fluoridation status

The tooth wear scores as presented in Table 4-3 were grouped into ‘no’, ‘mild’ or ‘moderate’ tooth wear and the results are presented in

Table 4-5 . The proportion with tooth wear was similar irrespective of fluoridation or medical card status, p>0.05. In lifetime residents of a fluoridated area with no medical card 42% (n=101) had moderate tooth wear, and 40% (n=23) without a fluoridated water supply or medical card had moderate tooth wear.

Table 4-3: Frequency distribution of maximum tooth wear scores in enamel or dentine by fluoridation status and medical card

Medical Card	Fluoride (F) Status	No wear		Mild				Moderate				Total	
		0*		1(Enamel)+		2(Enamel)#		3(Dentine)~		4(Dentine)~			
		n	%	n	%	n	%	n	%	n	%	n	
Yes	Yes	3	7	5	11	16	36	20	44	1	2	45	65
	No	3	38	1	13	1	13	3	38	0	0	8	
	Partial	0	0	1	8	4	33	7	58	0	0	12	
No	Yes	29	12	23	10	87	36	93	39	8	3	240	323
	No	6	11	6	11	22	39	23	40	0	0	57	
	Partial	3	12	2	8	6	23	15	58	0	0	26	
Total (F)													
	Yes	33	11	28	10	103	36	115	40	9	3	288	394
	No	9	14	7	11	23	35	27	41	0	0	66	
	Partial	3	8	3	8	12	30	22	55	0	0	40	

* No Tooth Wear into Enamel, + # Tooth Wear in Enamel, ~ Tooth Wear with Dentine exposed

Table 4-4: Frequency distribution of maximum tooth wear scores in enamel or dentine by fluoridation status

Fluoride (F) Status	No wear		Mild				Moderate				Total
	0*		1(Enamel)+		2(Enamel)#		3(Dentine)˜		4(Dentine)˜		
	n	%	n	%	n	%	n	%	n	%	N
Yes	33	11	28	10	103	36	115	40	9	3	288
No	9	14	7	11	23	35	27	41	0	0	66

Table 4-5 and Figure 4-2 indicate that the levels of tooth wear in this sample are similar for both medical card status and fluoridation status.

Table 4-5: Frequency distribution of maximum tooth wear scores by fluoridation status and medical card expressed as no tooth wear, mild tooth wear and moderate tooth wear

Medical Card	Fluoride (F) Status	No Wear		Mild		Moderate		Total	
		n	%	n	%	n	%	N	
	Yes	3	7	21	57	21	46	45	
Yes	No	3	38	2	26	3	38	8	65
	Partial	0	0	5	41	7	58	12	
	Yes	29	12	110	46	101	42	240	
No	No	6	11	28	50	23	40	57	323
	Partial	3	12	8	31	15	58	26	
	Total (F)								
	Yes	33	11	131	46	124	43	288	
	No	9	14	30	46	27	41	66	394
	Partial	3	8	15	38	22	55	40	

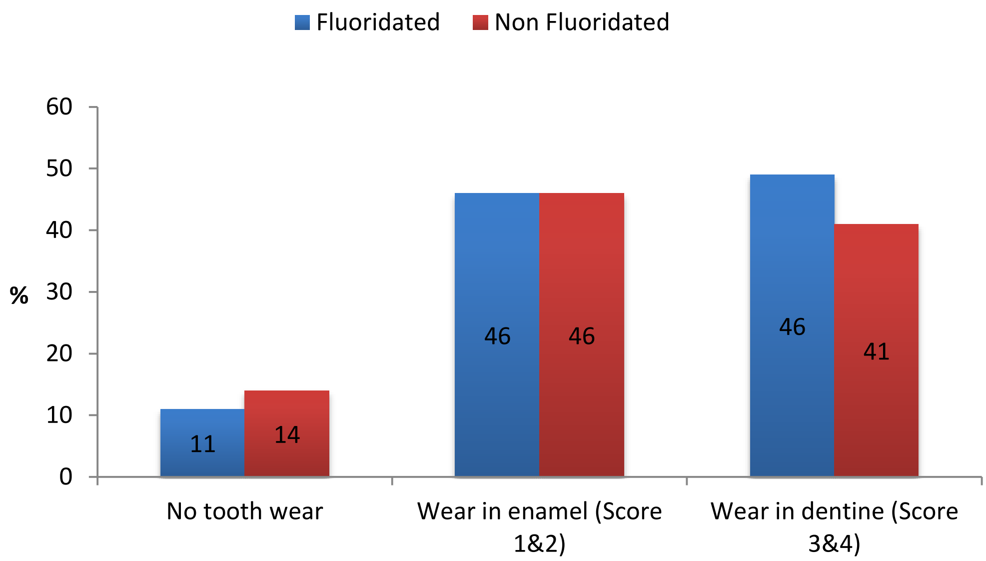


Figure 4-2: The percentage with tooth wear, none, mild moderate score for fluoridation status

4.2.2.2 Summary of results Section 4.2.2

Objective 1:

The prevalence of mild tooth wear (score 1 and 2) was 45% and moderate tooth wear (scores 3 or score 4**)** was 44% (n=173) in the sample**.**

Objective 2:

Tooth wear levels in the sample were similar for 16-year-old students irrespective of their fluoridation status or their medical card status.

4.2.3 Saliva samples

4.2.3.1 Objective addressed in Section 4.2.3:

Objective 3: Investigate tooth wear levels and associations with the properties of unstimulated and stimulated saliva.

Samples of unstimulated (resting) and stimulated saliva were collected from consenting individuals. In order to allow equilibration of fluoride in saliva, participants were asked not to brush from 21.00 hrs. the evening prior to the visit of the survey team [Appendix 6]. Saliva samples were collected prior to the dental examination and 349 participants provided samples

4.2.3.2 Tooth wear scores and mean saliva flow rate

The mean saliva flow rate per minute for each individual saliva sample was calculated gravimetrically.

4.2.3.2.1 Unstimulated saliva flow rate

The mean unstimulated saliva flow rate for the sample was 0.51±0.49 ml/min, which was within the accepted range of normal. When the mean flow rate for the different tooth wear levels was calculated no difference existed in the mean unstimulated saliva flow rate according to the levels of tooth wear (Table 4-6 and Figure 4-3).

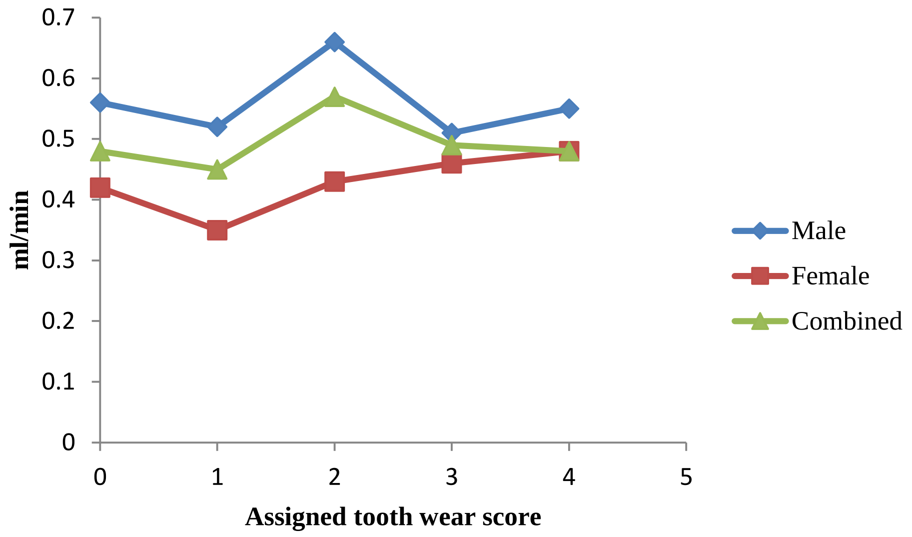


Figure 4-3: Mean unstimulated flow rate for males, females and combined for tooth wear levels

Table 4-6: Mean saliva flow rate for participants who produced an unstimulated saliva sample, grouped according to their highest level of tooth wear

	Gender	(n)	Tooth wear status	Mean flow rate (SD) ml/min
No wear			No Wear: Score 0	
	Male	16		0.56 (0.30)
	Female	23		0.42 (0.24)
	Total	39	No Wear	0.48 (0.27)
Mild			Mild: Enamel, Score 1	
	Male	20		0.52 (0.34)
	Female	14		0.35 (0.18)
	Total	34		0.45 (0.30)
			Mild: Enamel, Score 2	
	Male	70		0.66 (0.93)
	Female	49		0.43 (0.25)
	Total	119		0.57 (0.74)
	Total	153	Mild	0.51 (0.66)
Moderate			Moderate: Dentine, Score 3	
	Male	79		0.51 (0.32)
	Female	70		0.4 (0.26)
	Total	149		0.49 (0.29)
			Moderate: Dentine, Score 4	
	Male	5		0.55 (0.36)
	Female	2		0.48 (0.21)
	Total	7		0.48 (0.33)
	Total	156	Moderate	0.49 (0.29)
	Mean for all samples	348		0.51 (0.49)

4.2.3.2.2 Stimulated saliva flow rate

The mean stimulated saliva flow rate for the sample was 2.05 (0.86) ml/min. The mean stimulated saliva flow rate did not vary according to the levels of tooth wear as classified. For gender, however, there was a trend for the mean stimulated flow rate of males with no tooth wear to be higher than that of females with no tooth wear, $p<0.1$. For mild enamel wear (scores 1 and 2), the means were significantly higher in males compared to females, $p<0.04$ and $p<0.01$ respectively. Again, for moderate tooth wear the mean stimulated saliva flow rate was higher for males, $p<0.01$ (Table 4-7 and Figure 4-4).

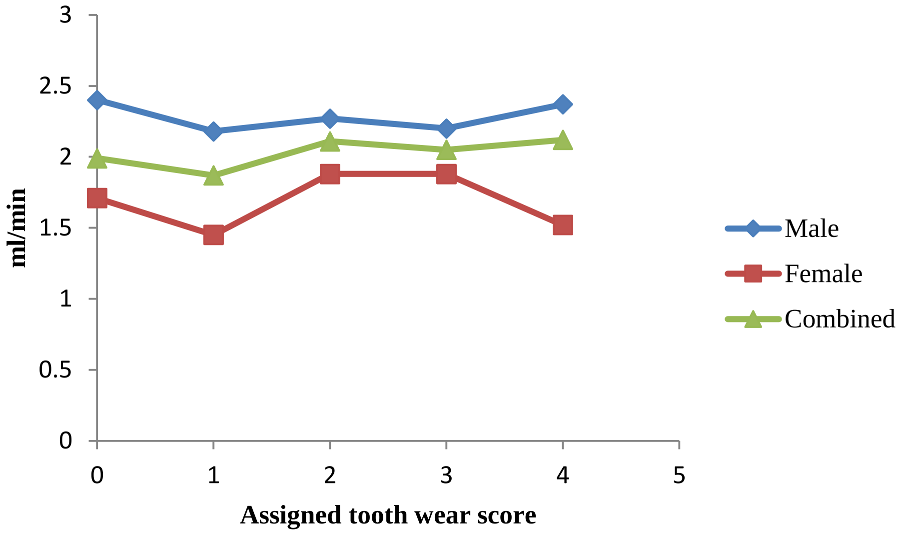


Figure 4-4: Mean stimulated flow rates, males, females and combined for tooth wear levels

Table 4-7: Mean saliva flow rate for participants who produced a stimulated saliva sample, grouped according to their highest level of tooth wear

	Gender	(n)	Tooth wear status	Mean flow rate (SD) ml/min
No Wear			No Wear: Score 0	
	Male	16		2.40 (1.06)#
	Female	23		1.71 (1.08)
	Total	39	No Wear	1.99 (1.11)
Mild			Mild: Enamel, Score 1	
	Male	20		2.18 (1.11)*
	Female	14		1.45 (0.71)
	Total	34		1.87 (1.02)
			Mild: Enamel, Score 2	
	Male	70		2.27 (0.87)*
	Female	49		1.88 (0.70)
	Total	119		2.11 (0.82)
	Total	153	Mild	1.99 (0.87)
Moderate			Moderate: Dentine, Score 3	
	Male	79		2.20 (0.7)*
	Female	70		1.88 (0.80)
	Total	149		2.05 (0.80)
			Moderate: Dentine, Score 4	
	Male	5		2.37 (0.45)
	Female	2		1.52 (0.02)
	Total	7		2.12 (0.56)
	Total	156	Moderate	2.06 (0.79)
	Mean for all samples	348		2.05 (0.86)

Trend p value p<0.1
* Statistically significant all values, p<0.05

4.2.3.3 Proteomics – Determination of protein and protein carbonyl concentrations

To follow the proteomics results presented in this chapter, the work flow diagram presented in Chapter 3 (Figure 3-5) is reproduced below as Figure 4-5.

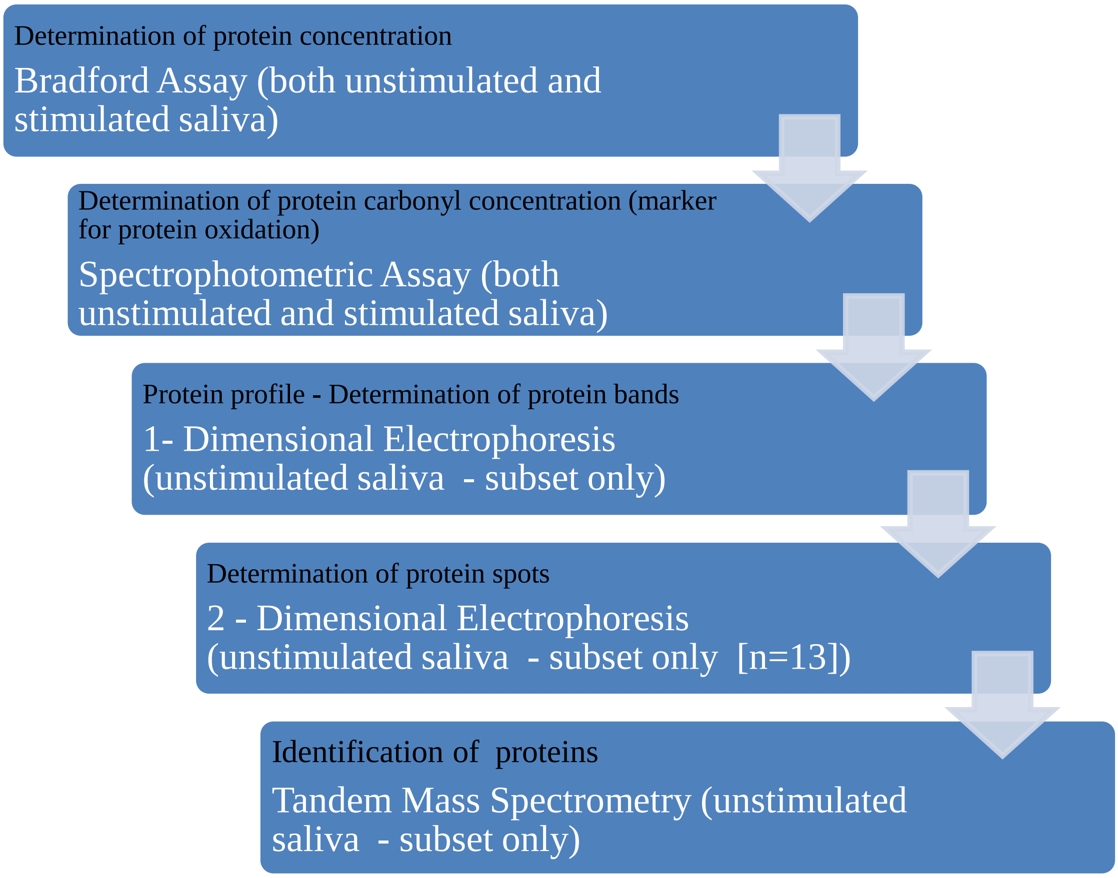


Figure 4-5: Work flow leading to protein identification

In this section of the research, a subset of unstimulated and stimulated saliva samples were selected. One subset contained the saliva samples provided by participants rated as having no tooth wear: score 0 (n=29), and the other subset contained the saliva samples provided by participants with moderate tooth wear (dentine exposed on the occlusal surface of a first permanent molar): score 4 and some score 3 (n=43) (Figure 4-6). In order to determine the total protein concentration for the unstimulated and stimulated saliva samples, the Bradford assay (Bradford, 1976) was used. The protein carbonyl concentrations for the unstimulated and stimulated saliva samples were then determined (Levine et al., 1994).

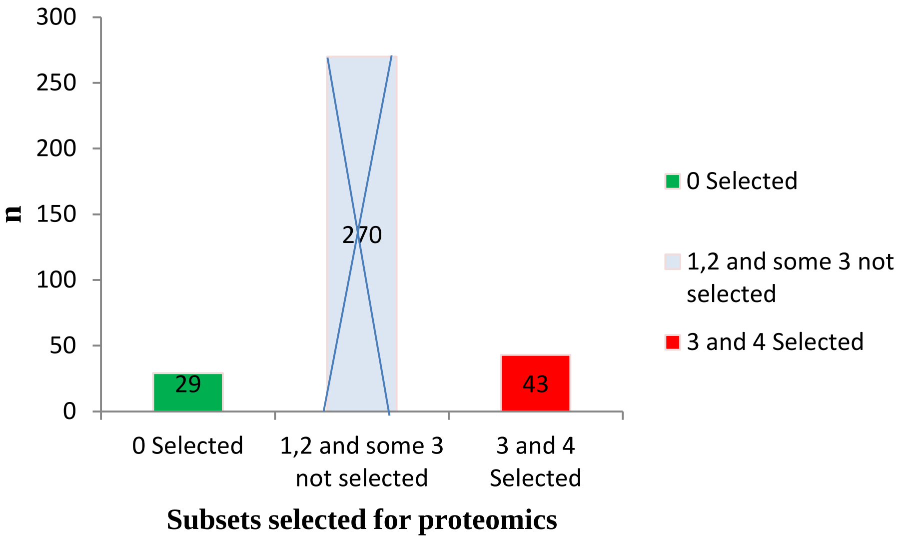


Figure 4-6: Unstimulated and stimulated saliva sample subsets selected for proteomics

4.2.3.3.1 Mean protein and protein carbonyl concentrations - unstimulated saliva

The mean protein concentration determined for unstimulated saliva was similar for both the group with no tooth wear and the group with moderate tooth wear (Table 4-8). The mean protein carbonyl concentration was significantly higher in the group with moderate tooth wear, $p<0.0001$ (Table 4-8). The presence of carbonylated proteins were used as an indication of oxidative stress and reactive oxygen species. For the protein carbonyl/protein ratio, no difference existed between the groups (Table 4-8).

Table 4-8: The mean total protein and protein carbonyl concentration in unstimulated saliva from the selected subsets: no tooth wear and moderate tooth wear

Tooth wear score	n	Protein concentration (SD) µg µml^{-1}	Carbonyl concentration (SD) mmol l^{-1}	Protein carbonyl/Protein ratio (SD)
No wear	29	0.97 (0.42)	0.014 (0.002)*	0.018 (0.011)
Moderate	43	1.07 (0.22)	0.018 (0.002)	0.018 (0.006)

*Statistically significant values, p<0.0001

4.2.3.3.2 Mean protein and protein carbonyl concentrations - stimulated saliva

In stimulated saliva, participants with moderate tooth wear had a significantly lower protein concentration than the group with no tooth wear, $p<0.01$. This important factor may compromise the ability of saliva to buffer acids and lubrication in the oral cavity. The ratio of protein carbonyl concentration to protein concentration similarly was higher for the participants with moderate tooth wear, p<0.01, (Table 4-9).

Table 4-9: The mean total protein and protein carbonyl concentration in stimulated saliva from the selected subsets: no tooth wear and moderate tooth wear

Tooth wear score	n	Protein concentration (SD) µg/µml	Carbonyl concentration (SD) mmol l^{-1}	Protein carbonyl/Protein ratio (SD)
No wear	29	0.81 (0.36)*	0.013 (0.002)	0.019 (0.008)*
Moderate	43	0.64 (0.21)	0.014 (0.003)	0.025 (0.012)

*Statistically significant values, p<0.0001

4.2.3.4 Proteomics - Protein identification in unstimulated saliva

A subset (n=13) was selected for 1-DE and 2-DE analysis that was based on the individual protein carbonyl concentrations. There were six participants with no tooth wear and seven participants with moderate tooth wear selected. The tooth wear groups were sub-divided such that each sub-group contained participants with the lowest and the highest protein carbonyl concentration (Figure 4-7).

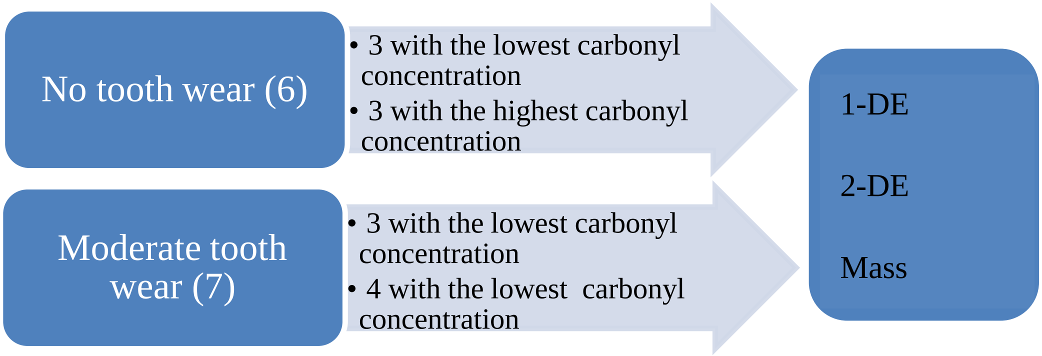


Figure 4-7: Subset selected for 1-DE and 2-DE

In Table 4-10 the gender, ID number, protein concentration, protein carbonyl concentration and the ratio of protein carbonyl to protein for the participants with no tooth wear, and who were selected for 1-DE and 2-DE are provided. Similarly, in Table 4-11 the details are provided for the participants with moderate tooth wear who were selected. Both tables are referred to throughout the results.

Table 4-10: The protein, protein carbonyl concentration and the carbonyl protein ratio in unstimulated saliva of the participants with no tooth wear selected for electrophoresis

Gender	ID Number no tooth wear	Protein concentration µg/µml	Protein carbonyl concentration mmol l^{-1}	Protein carbonyl/Protein ratio
Male	241060	0.88	0.01*	0.012
Male	281011	0.66	0.011*	0.016
Female	321001	0.26	0.011*	0.043
Male	281018	1.99	0.017~	0.009
Female	211017	0.99	0.019~	0.019
Female	301022	0.97	0.019~	0.019

* Low protein carbonyl concentration ~ High protein carbonyl concentration

Table 4-11: The protein, protein carbonyl concentration and the carbonyl protein ratio in unstimulated saliva of the participants with moderate tooth wear selected for electrophoresis

Gender	ID Number moderate tooth wear	Protein concentration µg/µml	Protein carbonyl concentration mmol l^{-1}	Protein carbonyl/Protein ratio
Male	281020	1.39	0.012*	0.008
Male	301003	1.29	0.012*	0.009
Female	331012	0.79	0.011*	0.014
Male	221022	0.65	0.02~	0.031
Female	231008	1.00	0.024~	0.024
Male	251009	1.35	0.024~	0.018
Male	271009	0.83	0.024~	0.029

* Low Carbonyl Concentration ~High Carbonyl Concentration

4.2.3.4.1 1-DE and 2-DE SDS PAGE

For 1-DE, all selected samples were analysed in triplicate. Visually the 1-DE separations displayed protein bands that were obvious and/or present in some samples but absent from other samples (arrows) (Figure 4-8, Figure 4-9).

The molecular mass ranges finally focussed on were in the 15–17 kDa, 62–63 kDa and 104-106 kDa mass ranges, because of the visibility of the protein spots present in those positions in the 2-DE images. A colour key to assist with the display of the molecular mass ranges – 15–17 kDa, 62–63 kDa and 104-106 kDa, is presented in Table 4-12; this colour key will be referred to for both 1-DE and 2-DE images.

Table 4-12: Colours assigned to molecular mass ranges

Molecular mass range (kDa)	Colour
15-17	
62-63	
104-106	

The 1-DE images prepared for participants 281020, 301003 and 271009 (Table 4-11) are displayed in Figure 4-8. Participant 281020 had the highest protein concentration of the samples selected but a low protein carbonyl concentration (Table 4-11). Subject 271009 had the lowest protein concentration of the samples selected, and the highest protein carbonyl concentration. In the 1-DE image Figure 4-8, protein bands are visible for participant 271009 and these bands are not seen in the images from the two other participants.

The 1-DE images prepared for participants 231008, 251009 331012 also with moderate tooth wear (Table 4-11) are seen in Figure 4-9. Arrows indicate the areas of interest that were considered after visual inspection and the coloured dots (Table 4-12) the molecular mass ranges finally focussed on.

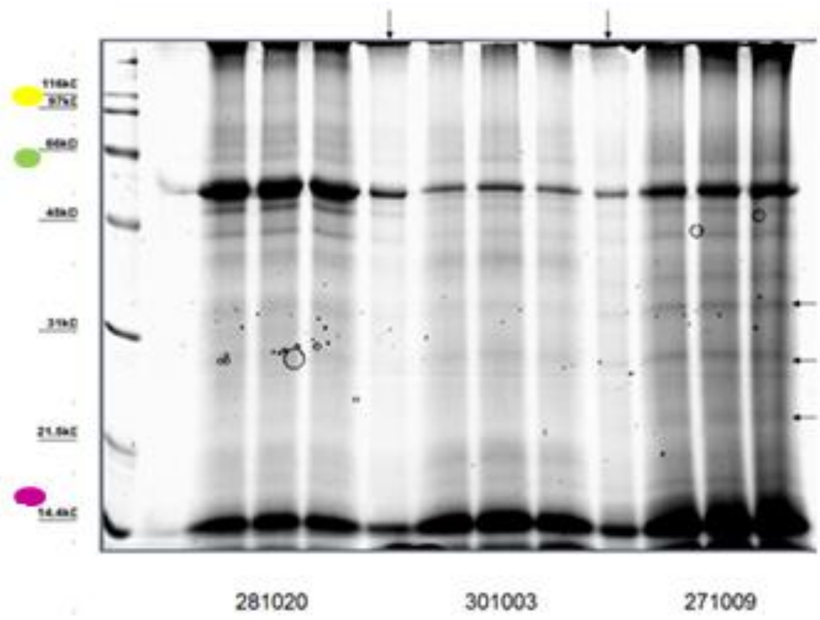


Figure 4-8: Silver stained 1-DE electrophoresis of three participants with moderate tooth wear

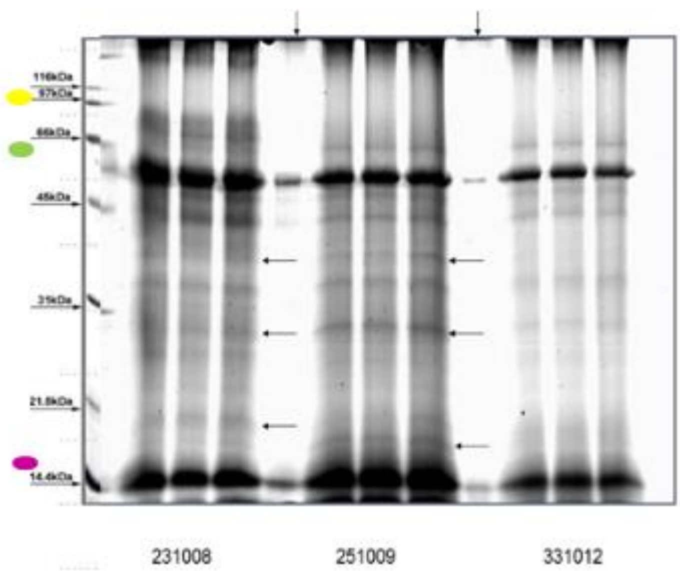
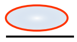


Figure 4-9: Silver stained 1-DE electrophoresis of three participants with moderate tooth wear

4.2.3.4.2 Silver stained 2-DE images

Once 1-DE was completed, a more elaborate 2-DE separation was performed, where individual proteins were separated using their isoelectric points and molecular weights. Visual differences existed between the images produced with 2-DE for participants with no tooth wear and participants with moderate tooth wear.

For ease of reading, the protein spots with a red ring around them  are the protein spots of interest. The coloured dots indicate the molecular mass range (Table 4-12).

Participant ID 301022 had no tooth wear, the prepared 2-DE image is displayed in Figure 4-10.

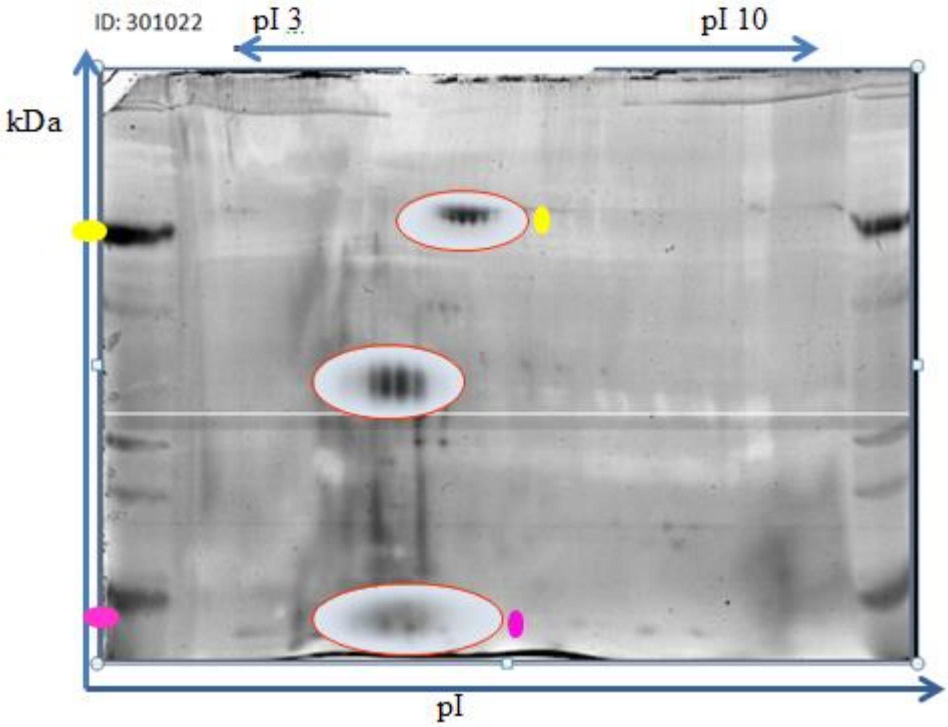


Figure 4-10: 2-DE image from unstimulated saliva for ID 301022, female, with no tooth wear and a 'high' protein carbonyl concentration

In the 2-DE images prepared from the unstimulated saliva sample of participants with moderate tooth wear, additional protein spots were present in the 62-63 kDa mass range, shown with the green dot beside them. The 2-DE images in

Figure 4-11 and Figure 4-12 show the presence of protein spots for participants 331012 and 231008, respectively. Appendix [9] provides the protein concentration, protein carbonyl concentration and inorganic ion concentration where available in the unstimulated whole saliva of participants selected for 2-DE who had either no or moderate tooth wear.

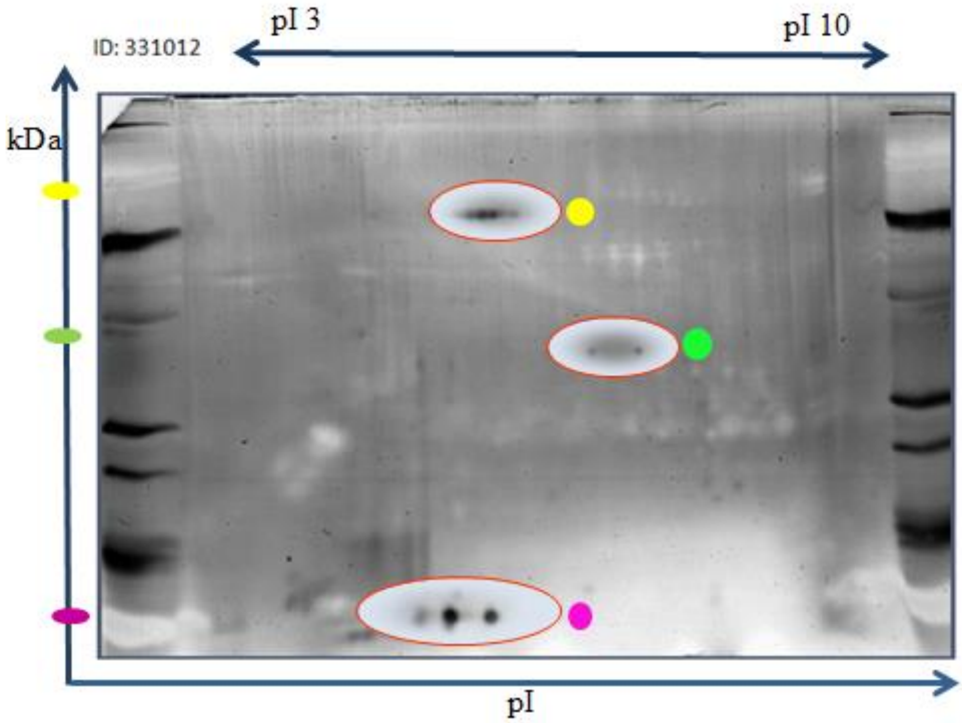


Figure 4-11: 2-DE Image prepared from unstimulated whole mouth saliva for ID 331012, female, with moderate tooth wear and a low carbonyl concentration

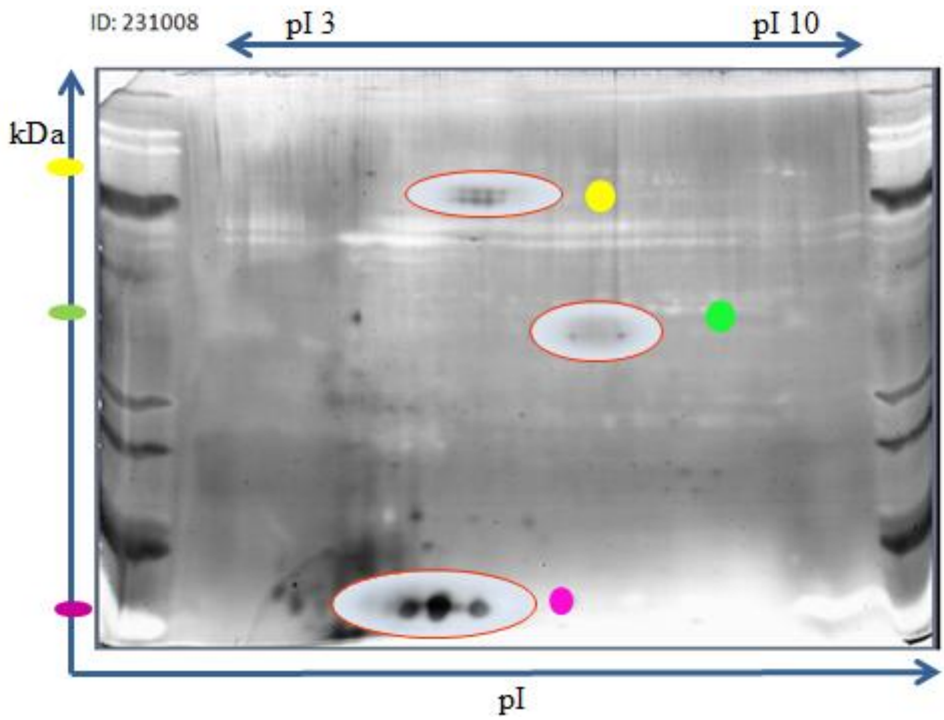


Figure 4-12: 2-DE Image prepared from unstimulated whole mouth saliva for ID 231008, female, with moderate tooth wear and a high carbonyl concentration.

4.2.3.4.3 Protein identification by mass spectrometry for labelled spots

Protein spots identified in 2-DE from participants with no tooth wear and with moderate tooth wear were excised for in gel tryptic cleavage and mass spectrometry. The protein spots that were identified are labelled with a letter and digit or digit only (L3, 5, 6, 7) (Figure 4-13), and sent for analysis using MALDI-TOF mass spectrometry (MS) Reflectron MS spectrum. MS/MS spectra information were used for protein identification. The five highest Mascot scores that matched the protein spots were reported. The information returned included the protein name, accession identifier number, the sequence coverage and the number of patterns assigned to the peptides. The protein spots present at 104-106 kDa were not identified.

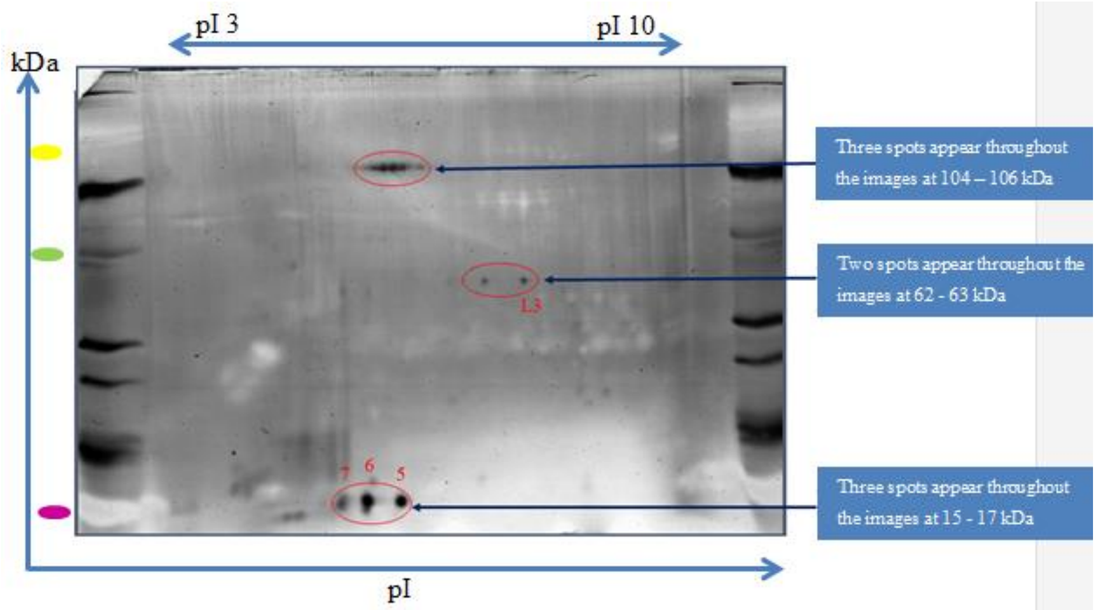


Figure 4-13: Spots selected for identification by mass spectrometry

4.2.3.4.4 Database searching using the National Center for Biotechnology Information resource (NCBI) and Mascot

The BLAST search tool was used to identify regions of similarity

4.2.3.4.4.1 Protein spot labelled "L3"

Protein spot **L3** was one of the protein spots present in the 2-DE images from the participants with moderate tooth wear. The five highest Mascot scores for the spot marked **L3** were:

1. Chain A, the crystal structure of human Fcari bound to IgA1-Fc, a human Fc alpha RI (CD89) is the receptor specific for IgA. This is an immunoglobulin that is abundant in mucosa and is also found in high concentrations in serum (Ding et al., 2003); (Herr et al., 2003)
2. Chain A, the crystal structure of a *Staphylococcus aureus* protein (Ss17) in complex with Fc of human IgA1
3. An unnamed protein product (*Homo sapiens*)
4. An immunoglobulin heavy chain constant region alpha 1 (*Homo sapiens*)
5. Protein Tro alpha 1 H, myeloma.

4.2.3.4.4.2 Protein spot labelled "5"

Protein spot **5** was one of the protein spots seen in all images in the 15-kDa range. The highest Mascot scores for the protein spot marked **5** were:

1. Chain B, Crystal structure of the complex formed between Mhc-like zinc alpha2-glycoprotein and prolactin inducible protein (PIP)
2. Prolactin induced protein precursor (PIP)[*Homo sapiens*]
3. Prolactin induced protein [*Homo sapiens*].

4.2.3.4.4.3 Protein spot labelled "6"

The highest Mascot scores for the protein spot marked **6,** which was present in all 2-DE images in the 15-kDa range, irrespective of tooth wear score were:

1. Protein SAP1
2. Cystatin SA-III, a potential precursor of acquired enamel pellicle [human peptide 121 aa]
3. Recommended name = Cystatin-SN; Alternative name = Cystatin 1; Alternative full name=salivary cystatin-SA-1; AltName: full=Cystatin-SA-1; Flags: Precursor
4. Chain A, Human thioredoxin double mutant with cysteine 32 replaced by serine and cys 35 replaced by serine
5. Thioredoxin [*Homo sapiens*].

4.2.3.4.4.4 Protein spot labelled "7"

The highest Mascot scores for the protein spot marked **7,** present in all 2-DE images irrespective of tooth wear score were:

1. Protein SAP1
2. Cystatin SA-III = potential precursor of acquired enamel pellicle [human peptide 121 aa]
3. Recommended name: Full = Cystatin-SN; AltName: Full = Cystatin-1; RecName:Full=Cystatin-SN; AltName:Full=Cystatin-1;AltName:Full=Salivary cystatin-SA-1; AltName: full=Cystatin-SA-1;Flags:Precursor.
4. Cystatin-SA precursor [*Homo sapiens*]
5. Cystatin SN [human submandibular-sublingual saliva, peptide partial, 35 aa], (Table 4-13). In Table 4-14 the peptide sequences identified in the protein spots sent for MS identification, and the protein sequences of the known proteins are indicated with coloured letters.

Table 4-13: Proteins identified for the different 2-DE protein spots using MS and peptide mass fingerprinting

Protein	Accession Number /Mw (Da)	Protein Score*[a] /Protein Confidence Interval (%)	Total Ion Score* /Total Ion Confidence Interval (%)	Sequence Coverage (%)	Number of MS/MS patterns assigned to peptides[b]
Identification for protein spot labelled 'L3'					
Chain A, Crystal structure of human Fcari bound to Iga1-Fc	gi/31615935/23186	308/100	269/100	29	2
Chain A, Crystal structure of a staphylococcus aureus protein (Ss17) in complex with Fc of human Iga1	gi/58430162/23428	307/100	269/100	29	2
Unnamed protein product[*Homo sapiens*]	gi/34535864/52632	301/100	269/100	22	2
Immunoglobulin heavy chain constant region alpha 1 [*Homo sapiens*]	gi/12054070/37623	297/100	269/100	17	2
Protein Tro alpha 1 H, myeloma	gi/223069/51040	296/100	296/100	19	2
Identification for protein spot labelled '5'					
Chain B, crystal structure of the complex formed between Mhc-like zinc alpha2-glycoprotein and prolactin inducible protein	gi/145579641/13514	236/100	219/100	46	3

Protein	Accession Number /Mw (Da)	Protein Score*[a] /Protein Confidence Interval (%)	Total Ion Score* /Total Ion Confidence Interval (%)	Sequence Coverage (%)	Number of MS/MS patterns assigned to peptides[b]
Prolactin induced protein precursor [*Homo sapiens*]	gi/4505821/16562	234/100	219/100	37	3
Prolactin induced protein [*Homo sapiens*]	gi/116642259/9061	181/100	165/100	45	2
Identification for protein spot labelled '6'					
Protein SAP1	gi/352334/13263	337/100	295/100	78	3
Cystatin SA-III=potential precursor of acquired enamel pellicle [human, Peptide, 121 aa]	gi/235948/14181 (protease inhibitor)	332/100	295/100	73	3
RecName:Full=Cystatin-SN; AltName:Full=Cystatin-1;AltName:Full=Salivary cystatin-SA-1; AltName: full=Cystatin-SA-1;Flags:Precursor	gi/118188/16351 Cystatin-SN (Protease inhibitor)	201/100	181/100	46	2
Chain A, Human thioredoxin double mutant with Cys 32 replaced by Ser And Cys 35 replaced By Ser	gi/157831000/11698	122/100	106/100	54	1
Thioredoxin [*Homo sapiens*]	gi/50592994/11730 Sulphydrl protein	120/100	106/100	54	1

Protein	Accession Number /Mw (Da)	Protein Score*[a] /Protein Confidence Interval (%)	Total Ion Score* /Total Ion Confidence Interval (%)	Sequence Coverage (%)	Number of MS/MS patterns assigned to peptides[b]
Identification for protein spot labelled '7'					
Protein SAP1	gi/352334/13263	540/100	466/100	85	5
Cystatin SA-III=potential precursor of acquired enamel pellicle [human, peptide, 121 aa]	gi/235948/14181	534/100	466/100	80	5
RecName:Full=Cystatin-SN; AltName:Full=Cystatin-1; AltName:Full=Salivary cystatin-SA-1; AltName: full=Cystatin-SA-1;Flags:Precursor	gi/118188/16351	501/100	465/100	58	5
Cystatin-SA precursor [*Homo sapiens*]	gi/4503105/16434	250/100	232/100	37	3
Cystatin SN [human submandibular-sublingual saliva, Peptide Partial, 35 aa]	gi/240102/4131	134/100	128/100	51	1

[a] The protein score probability limit (where p <0.05) is 66.

[b] Peptides with confidence interval above 95% were considered.

Key to the 20 amino acids abbreviations

Amino acid	3 letter abbreviation	Single letter abbreviation
alanine	ala	A
arginine	arg	R
asparagine	asn	N
aspartic acid	asp	D
cysteine	cys	C
glutamine	gln	Q
glutamic acid	glu	E
glycine	gly	G
histidine	his	H
isoleucine	ile	I
leucine	leu	L
lysine	lys	K
methionine	met	M
phenylalanine	phe	F
proline	pro	P
serine	ser	S
threonine	thr	T
tryptophan	trp	W
tyrosine	tyr	Y

Table 4-14: Matches to sequence for protein spots

Accession	Peptide sequences	Matches to sequences (top match and exemplar of protein functional type)
("L3") gi|31615935|	YLTWASR TFTCTAAYPESK WLQGSQELPREK DASGVTFTWTPSSGK QEPSQGTTTFAVTSILR	gi|31615935|pdb|1OW0|A Chain A, Crystal Structure Of Human Fcari Bound To Iga1-fc ACHPRLSLHRPALEDLLLGSEANLTCTLTGLR**DASGVTFTWTPSSGK**SAVQGPP ERDLCGCYSVSSVLPGCAEPWNHGK**TFTCTAAYPESK**TPLTATLSKSGNTFRPE VHLLPPPSEELALNELVTLTCLARGFSPKDVLVR**WLQGSQELPREKYLTWASR** **QEPSQGTTTFAVTSILR**VAAEDWKKGDTFSCMVGHEALPLAFTQKTI ***GenBank accession AY647978.1 Constant region of IgA*** ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPS QDASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCPVPSTPPTPS PSTPPTPSPSCCHPRLSLHRPALEDLLLGSEANLTCTLTGLR**DASGVTFTWTPSS** **GK**SAVQGPPDRDLCGCYSVSSVLSGCAEPWNHGK**TFTCTAAYPESK**TPLTATL SKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPKDVLVR**WLQGSQELPR** **EKYLTWASRQEPSQGTTTFAVTSILR**VAAEDWKKGDTFSCMVGHEALPLAFT QKTIDRLAGKPTHVNVSVVMAEVDG TCY Protein match: Constant region of IgA

Accession	Peptide sequences	Matches to sequences (top match and exemplar of protein functional type)
("5") gi|145579641/13514	FYTIEILKVE YTACLCDDNPK ELGICPDDAAVIPIK SVRPNDEVTAVLAVQTELK	gi|145579641/13514|Chain B, Crystal Structure Of The Complex Formed Between Mhc-Like Zinc Alpha2-Glycoprotein And Prolactin Inducible Protein At 3Å Resolution QDNTRKIIIKNFDIPK**SVRPNDEVTAVLAVQTELK**ECMVVKTYLISSIPLGAFNYK**YTACLCDDNPK**TFYWDFYTNRTVQIAAVVDVIR**ELGICPDDAAVIPIK**NNR**FYTIEILKVE** Prolactin-induced protein accession gi|4505821 MRLLQLLFRASPATLLLVLCLQLGANKAQDNTRKIIIKNFDIPK**SVRPDEVTAVLAVQTELK**ECMVVKTYLISSIPLQGAFNYK**YTACLCDDNPK**TFYWDFYTNRTVQIAAVVDVIR**ELGICPDDAAVIPIK**NNR**FYTIEILKVE** Protein match: Prolactin-induced protein

Accession	Peptide sequences	Matches to sequences (top match and exemplar of protein functional type)
(“6”) gi|352334/13263	RPLQVLR ALHFAISEYNK QLCSFEIYEVPWEDR EQTFGGVNYFFDVEVGR IIPGGIYDADLNDEWVQ R KQLCSFEIYEVPWEDR SQPNLDTCAFHEQPELQ K AREQTFGGVNYFFDVEV GR	gi|352334|prf||1008184A protein SAP1 IIPGGIYDADLNDEWVQRALHFAISEYNKATEDEYYRRPLQVLR AREQTFGGVNYFFDVEVGRTICTKSQPNLDTCAFHEQPELQKKQLCSFEIYEVPW EDRMSLVDSRCQEA gi|235948|cystatin SA-III=potential precursor of acquired enamel pellicle SSSKEENRIIPGGIYDADLNDEWVQRALHFAISEYNKATEDEYYRRPLQVLRARE QTFGGVNYFFDVEVGRTICTKSQPNLDTCAFHEQPELQKKQLCSFEIYEVPWEDR MSLVDSRCQEA **Protein match:** Cystatin SA-III (it is unique among cystatins in that it possesses phosphate residues at its amino terminus as well as at its carboxyl terminus)

Accession	Peptide sequences	Matches to sequences (top match and exemplar of protein functional type)
(“7”) gi|352334/13263	RPLQVLR ALHFAISEYNK QLCSFEIYEVPWEDR EQTFGGVNYFFDVEVGR IIPGGIYDADLNDEWVQR KQLCSFEIYEVPWEDR SQPNLDTCAFHEQPELQK AREQTFGGVNYFFDVEVGR	gi|352334|prf||1008184A protein SAP1 IIPGGIYDADLNDEWVQRALHFAISEYNKATEDEYYRRPLQVLRAREQTFGGVNYFFDVEVGRTICTKSQPNLDTCAFHEQPELQKKQLCSFEIYEVPWEDRMSLVDSRCQEA gi|235948|cystatin SA-III=potential precursor of acquired enamel pellicle SSSKEENRIIPGGIYDADLNDEWVQRALHFAISEYNKATEDEYYRRPLQVLRAREQTFGGVNYFFDVEVGRTICTKSQPNLDTCAFHEQPELQKKQLCSFEIYEVPWEDRMSLVDSRCQEA Protein match: Cystatin SA-III (it is unique among cystatins in that it possesses phosphate residues at its amino terminus as well as at its carboxyl terminus)

Of the protein spots identified through MS spectrometry the protein spot marked **'L3'** was the only protein spot associated with the moderate tooth wear group only. The most likely protein type that was identified was a glycoprotein immunoglobulin.

4.2.3.5 Mean concentration of inorganic ions in unstimulated and stimulated saliva

In line with objective 3, the mean concentrations of total fluoride, total phosphate and total calcium were determined. Ion chromatography was conducted at the Department of Chemistry, UCC.

The unit of measurement used to report the concentration of inorganic ions in the body of the text is 'mmol l^{-1}'; in the tables, both 'mmol l^{-1}' and 'ppm' measurements are reported.

The mean concentrations of total fluoride, phosphate and calcium ions in saliva are first presented (n=61). Mean concentrations were then organised according to tooth wear scores, no tooth wear (n=26) and moderate tooth wear (n=19). The results for the mean total fluoride, phosphate and calcium ions for both unstimulated and stimulated saliva were calculated. In unstimulated saliva, the mean concentration of total fluoride ion was 0.12 (0.06) mmol l^{-1}, of phosphate ion 4.9 (1.46) mmol l^{-1} and of calcium ion 0.99 (0.43) mmol l^{-1} (Table 4-15). The mean concentration of total fluoride present in the sample was higher than the value reported, whilst the total calcium and phosphate concentrations were similar to reported values. The total fluoride, phosphate and calcium ion concentrations in unstimulated saliva for individual participants are provided in the Appendix [10].

Table 4-15: The mean, standard deviation (SD), minimum (min) and maximum (max) values of total fluoride (F^-), phosphate (HPO_4^{2-}) and calcium (Ca^{2+}) ions from all available unstimulated saliva samples (n=61) expressed in mmol l^{-1} and ppm

Unstimulated saliva	F^- mmol l^{-1}	F^- ppm	HPO_4^{2-} mmol l^{-1}	HPO_4^{2-} ppm	Ca^{2+} mmol l^{-1}	Ca^{2+} ppm
mean	0.12	2.29	4.90	465.51	0.99	39.77
(SD)	0.06	1.12	1.46	138.96	0.43	17.28
min	0.02	0.38	2.42	229.88	0.25	9.9
max	0.28	5.31	8.13	772.15	1.99	79.72

For the samples of stimulated saliva (n=60), as expected the mean total fluoride concentration of 0.07 (0.04) mmol l^{-1} and total phosphate ion concentration of 3.93 (1.4) mmol l^{-1} were lower than their respective mean concentrations in unstimulated saliva. The mean total calcium ion concentration, 1.06 (0.35) mmol l^{-1} was higher. The mean concentration of the ions determined from the saliva samples followed the normal trend (Table 4-16).

Table 4-16: The mean, standard deviation (SD), minimum (min) and maximum (max) values of total fluoride anion (F^-), phosphate anion (HPO_4^{2-}) and calcium cation (Ca^{2+}) from all stimulated saliva samples (n=60) expressed in mmol l^{-1} and ppm

Stimulated saliva	F^- mmol l^{-1}	F^- ppm	HPO_4^{2-} mmol l^{-1}	HPO_4^{2-} ppm	Ca^{2+} mmol l^{-1}	Ca^{2+} ppm
mean	0.07	1.31	3.93	373.50	1.06	42.45
(SD)	0.04	0.73	1.41	133.73	0.35	13.90
Min	0.004	0.08	1.81	171.67	0.46	18.23
Max	0.19	3.67	7.82	743.02	1.94	77.80

The mean concentration of total fluoride, phosphate and calcium ions in unstimulated and stimulated saliva were subsequently determined for participants grouped according to their tooth wear score (no tooth wear, moderate tooth wear).

The mean total fluoride concentration in unstimulated saliva for the group with no tooth wear was 0.12 (0.06) mmol l^{-1}; mean total phosphate ion concentration was 4.57 (1.5) mmol l^{-1} and mean total calcium 0.87 (0.39) mmol l^{-1}. The participants with tooth wear had a mean total fluoride ion concentration of 0.15 (0.1) mmol l^{-1}; mean total phosphate ion concentration of 5.44 (1.73) mmol $l^{-1}$and a mean total calcium ion concentration of 1.26 (0.4) mmol l^{-1} . Comparing the two groups, the mean concentration of total fluoride and mean concentration of total phosphate ion were similar for both those with no tooth wear and those with moderate tooth wear. For the calcium cation, the concentration was significantly higher in the group with moderate tooth wear $p<0.002$ (Table 4-17), Figure 4-14 Figure 4-15

Table 4-17: The mean total fluoride, phosphate and calcium ion concentrations and (SD) in unstimulated saliva for selected participants with no tooth wear (n=26) and those with moderate tooth wear (n=19)

Tooth wear score	F^- mmol l^{-1}	F^- ppm	HPO_4^{2-} mmol l^{-1}	HPO_4^{2-} ppm	Ca^{2+} mmol l^{-1}	Ca^{2+} ppm
No wear						
mean	0.12	2.23	4.57	434.12	0.87	34.79
(SD)	0.06	1.20	1.50	142.02	0.39	15.52
Moderate						
mean	0.15	2.92	5.44	516.89	1.26*	50.58*
(SD)	0.1	1.86	1.73	164.46	0.40	16.21

* Statistically significant value, p<0.002

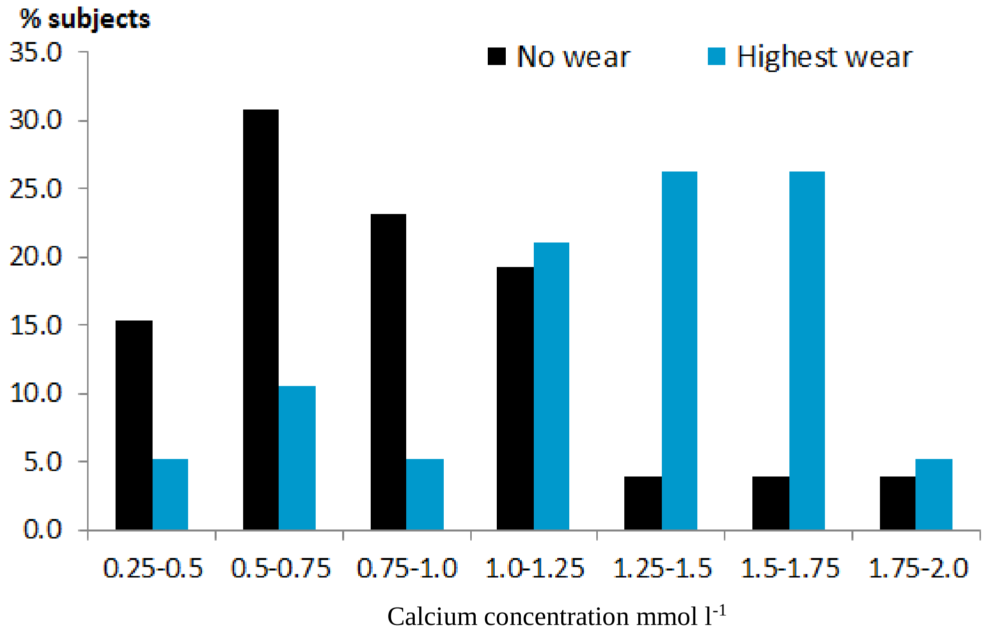


Figure 4-14: Total calcium ion concentration (mmol l^{-1}) in unstimulated saliva in the subsample of participants with no tooth wear (n=26) and those with moderate tooth wear (n=19)

Figure 4-15: Boxplot of total calcium ion concentration in unstimulated saliva in participants with no tooth wear and participants with moderate tooth wear

The stimulated saliva samples were also divided into the two groups based on tooth wear scores: those with no tooth wear and those with moderate tooth wear. No difference existed between the groups for mean concentrations of total fluoride, phosphate or calcium (Table 4-18), [Appendix 10].

Table 4-18: The mean total fluoride, phosphate and calcium ion concentrations and (SD) in stimulated saliva of those with no tooth wear (n=24)# and those with moderate tooth wear (n=19)

Tooth wear score	F^- mmol l^{-1}	F^- ppm	HPO_4^{2-} mmol l^{-1}	HPO_4^{2-} ppm	Ca^{2+} mmol l^{-1}	Ca^{2+} ppm
No wear						
mean	0.06	1.20	3.58	339.87	1.05	41.93
(SD)	0.03	0.62	1.41	134.09	0.30	12.10
Moderate						
mean	0.06	1.17	3.95	375.02	1.07	42.81
(SD)	0.05	0.94	1.32	125.87	0.40	16.07

#For two participants there was insufficient saliva available to determine the salivary ions concentrations (n=24)

4.2.3.6 Summary of results section 4.2.3

Objective 3: Investigate tooth wear levels and associations with properties of resting and stimulated saliva.

- Participants with moderate tooth wear had a higher mean protein carbonyl concentration in unstimulated saliva than participants with no tooth wear.
- Participants with moderate tooth wear had a lower mean protein concentration in stimulated saliva and a higher mean protein carbonyl concentration to protein ratio in stimulated saliva than participants with no tooth wear.
- Proteomic analysis with 1-DE, 2-DE and mass spectrometry in a subset indicated that the participants with moderate tooth wear may have a different protein profile.
- Participants with moderate tooth wear had a higher mean total calcium ion concentration in unstimulated saliva than participants with no tooth wear.

4.2.4 Questionnaire results in the cross sectional study

Participants completed a self-administered *e*-questionnaire. The responses to each of the questions posed in the questionnaire are set out for the number of responses associated with the students maximum tooth wear score. Students may not have answered every question and some questions had more than one answer so responses do not always sum to 394. The results are presented as numbers (n) and percentages (%). Percentages are in **bold print** in the table and without brackets ( ). Percentage whole numbers are presented in the tables but in the analysis they are calculated to at least three decimal places and rounded up to the nearest whole number. Column headers indicate the different levels of tooth wear and the questionnaire responses are presented in rows. Statistical tests for significant associations between the explanatory variables and tooth wear will be reported with the multivariable logistic regressions in section 4.2.5.

4.2.4.1 Objectives addressed in Section 4.2.4:

Objective 4: Investigate tooth wear levels and associations with oral hygiene practices, dietary factors, health behaviours and knowledge.

4.2.4.2 Questionnaire responses

4.2.4.2.1 Oral hygiene practices

The majority of respondents indicated that they brushed their teeth twice each day 65% (256). With respect to the proportions with tooth wear, 12% (31) had no tooth wear, 46% (117) had mild tooth wear and 42% (108) had moderate tooth wear. From the table one can see two trends: an increased prevalence in mild wear with increased frequency of tooth brushing and at the same time a decreased prevalence of moderate tooth wear with an increased frequency of tooth brushing (Table 4-19).

Table 4-19: How often do you brush your teeth?

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	%	(n)	%	(n)	%	(n)
Three or more times a day	(2)	6	(18)	55	(13)	39	(33)
Twice a day	(31)	12	(117)	46	(108)	42	(256)
Once a day	(11)	12	(39)	43	(40)	44	(90)
Less than once a day	-		(4)	29	(10)	71	(14)
Total (n)	(44)		(178)		(171)		(393)

The co-existence of the aetiologies of erosion abrasion and attrition is synergistic (Davis and Winter, 1980); for this reason, specific oral hygiene advice on the timing of tooth brushing has been provided (Addy and Shellis, 2006). In the questionnaire, participants indicated all of the times at which they brushed their teeth. Participants that responded that they brushed after breakfast had a lower proportion with moderate tooth wear (40%) than those brushing before going to bed or brushing before breakfast. The responses to this question are not mutually exclusive (Table 4-20).

Table 4-20: Time of tooth brushing

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total *
	(n)	%	(n)	%	(n)	%	(n)
Brush after breakfast	(36)	12	(145)	48	(122)	40	(303)
Before going to bed	(40)	12	(152)	45	(143)	42	(335)
Before breakfast	(4)	8	(23)	46	(23)	46	(50)

* Participants could indicate more than one answer and the total summated is greater than the number of participants answering the questions

4.2.4.2.2 Dietary practices

Questions on dietary practices were to establish the type and frequency of acidic intakes, and included a range of questions on the choice, preferences, habits and availability of foods and drinks. These questions on drinks and foods were followed by questions on alcohol consumption. The first question on dietary practices related to the intake of fizzy (carbonated) drinks. In the responses, 16% (63) of participants responded that they had a carbonated beverage daily, 52% (33) had mild tooth wear and 42% had moderate tooth wear. Twelve per cent (47) of the responses indicated they never had fizzy drinks. Table 4-21 shows that the lowest prevalence of moderate tooth wear at 32% was amongst the respondents who reported that they never had a fizzy drink, and this group also had the highest proportion with no tooth wear (26%), albeit this cell was small (n = 12).

Table 4-21: How often do you drink fizzy (carbonated) drinks?

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	%	(n)	%	(n)	%	(n)
Everyday	(4)	6	(33)	52	(26)	42	(63)
More than once a week	(12)	8	(64)	44	(69)	48	(145)
Less than once a week	(16)	12	(62)	45	(61)	44	(139)
Never	(12)	26	(20)	43	(15)	32	(47)
Total (n)	(44)		(179)		(171)		(394)

Participants were asked *What is the typical drink you usually have*? Thirty-five per cent indicated water as their typical drink; for these respondents, the proportion with mild or moderate tooth wear was similar, 43% (n = 58) and 42% (n = 57). For participants who indicated that fruit squash (*Ribena/Miwadi*) was their typical drink, the proportion with moderate tooth wear was lower than the proportion with mild tooth wear, 39% (n = 20) and 49% (n = 25) respectively. Where participants indicated that a carbonated beverage (*Cola* or *other fizzy*) was the typical drink, only one participant had no tooth wear; the remaining 98% of participants had tooth wear equally divided between mild or moderate wear, 49% (n = 24) (Table 4-22).

Table 4-22: During your typical day what kind of drink would you usually have?

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	**%**	**(n)**	**%**	**(n)**	**%**	**(n)**
Water	(20)	15	(58)	43	(57)	42	(135)
Milk	(4)	11	(16)	43	(17)	46	(37)
Milk or water	(7)	15	(20)	42	(21)	44	(48)
Mineral water	-		(2)	25	(6)	75	(8)
Fruit squash	(6)	12	(25)	49	(20)	39	(51)
Fruit juice	(2)	11	(7)	39	(9)	50	(18)
Smoothie	-		(5)		(1)		(6)
Cola / other fizzy	(1)		(24)	49	(24)	49	(49)
Coffee	-		-		(2)	100	(2)
Tea or water	(4)		(13)	48	(10)	37	(27)
Total (n)	(44)		(178)		(170)		(392)

Students were also asked about the availability of vending machines in their schools, 45% (n = 178) of participants responded that they attended a school with the availability of a vending machine, 51% (n = 198) did not have access and 4% (n = 15) did not know. The proportion of participants with no tooth wear or mild tooth wear was higher in the schools without a vending machine.

Table 4-23: Availability of vending machines in school

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	%	(n)	%	(n)	%	(n)
Yes vending machine	(15)	8	(89)	50	(74)	42	(178)
No vending machine	(27)	14	(82)	41	(89)	45	(198)
Don't know	(15)	2					(15)
Total (n)	(44)		(178)		(168)		(391)

Milk was available to 34% of respondents and not available to 55% while 11% did not know whether milk was available or not in the school. The respondents were distributed evenly between those with mild or moderate tooth wear, irrespective of whether or not they had school milk (Table 4-24).

Table 4-24: Is milk available in you school for example 'the school milk scheme'?

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	%	(n)	%	(n)	%	(n)
Yes	(13)	9	(63)	47	(58)	44	(134)
No	(26)	12	(95)	44	(95)	44	(216)
Don't know	(5)		(20)	46	(18)	44	(43)
Total (n)	(44)		(178)		(171)		(393)

Once the series of questions on beverages and access to beverages at school was completed, the participants moved onto questions on food choices. Participants were first asked about their consumption of citrus fruits (citric acid). The highest proportion of participants with moderate tooth wear was the proportion that never ate citrus fruits, 52% (28). Where citrus fruits were eaten more than once per week (n=110), 12% (13) had no tooth wear, 46% (50) had mild wear and 43% (47) had moderate tooth wear. It is also interesting to observe that the participants who consumed citrus fruits less than once a week had the highest proportion with no tooth wear, 17% (n=14), and the lowest proportion with moderate tooth wear, 39% (n=32) (Table 4-25).

Table 4-25: How often do you eat citrus fruits?

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Four or more times a day	-		(2)	100	-		(2)
Three times a day	-		(1)	50	(1)	50	(2)
Twice a day	(1)	5	(10)	50	(9)	45	(20)
Once a day	(5)	9	(26)	46	(25)	45	(56)
More than once a week	(13)	12	(50)	46	(47)	43	(110)
Once a week	(7)	11	(29)	45	(28)	44	(64)
Less than once a week	(14)	17	(37)	45	(32)	39	(83)
Never	(4)	7	(22)	41	(28)	52	(54)
Total (n)	(44)		(177)		(170)		(391)

Participants were next asked how often they ate apples (malic acid). Eleven per cent (n = 43) responded that they never ate apples; in this group 33% (n = 14) had moderate tooth wear and 58% (n = 25) had mild wear. Of the participants who responded that they had an apple every day, 48% (42) had moderate tooth wear, 43% (37) had mild tooth wear and 9% (10) had no tooth wear. For participants who ate apples less than one per week, 47% (46) had moderate tooth wear, 50% (32) had mild tooth wear and 3% (2) had no tooth wear. The answer to this question suggested a trend towards an increasing proportion with moderate tooth wear as the frequency of eating apples increased (Table 4-26).

Table 4-26: How often do you eat apples?

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Three times a day	(1)	14	(3)	43	(3)	43	(7)
Twice a day	(3)	13	(5)	23	(14)	64	(22)
Once a day	(10)	9	(37)	43	(42)	48	(89)
More than once a week	(11)	10	(50)	47	(46)	43	(107)
Once a week	(13)	21	(26)	42	(23)	37	(62)
Less than once a week	(2)	3	(32)	50	(30)	47	(64)
Never	(4)	9	(25)	58	(14)	33	(43)
Total (n)	(44)		(178)		(172)		(394)

Questions on the consumption of cider and alcopops were included in this research because of their low pH and popularity with young adults. The first question in this section concerned the consumption of cider. The responses indicated that 78% (306) of participants did not drink cider. Of the respondents who replied that they drank cider at weekends, 5% (2) had no wear, 45% (17) had mild tooth wear and 50% (19) had moderate tooth wear. It is interesting to note that 50% (n=19) of the respondents who consumed cider at the weekend had moderate tooth wear, whereas 43% (n=130) of those who did not drink cider had moderate tooth wear (Table 4-27).

Table 4-27: How often do you drink cider?

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Weekends	(2)	5	(17)	45	(19)	50	(38)
Special Occasions	(1)	9	(4)	36	(6)	55	(11)
Out with friends	(1)	17	(3)	50	(2)	33	(6)
Parties	(2)	50	(2)	50	-		(4)
Twice a month	(1)	11	(3)	33	(5)	56	(9)
Once a month	-		(1)	33	(2)	67	(3)

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Rarely	-		(7)	50	(7)	50	(14)
Never	(37)	12	(139)	45	(130)	43	(306)
Total (n)	(44)		(176)		(171)		(391)

The consumption of alcopops indicated that 80% (313) never consumed alcopops. The 20% of respondents who consumed alcopops on special occasions, the proportion with mild and moderate tooth wear was identical 48% (n = 23) in each group (Table 4-28).

Table 4-28: How often do you drink alcopops?

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Weekends	(3)	10	(15)	48	(13)	42	(31)
Special Occasions	(2)	4	(23)	48	(23)	48	(48)
Never	(39)	13	(140)	45	(134)	43	(313)
Total (n)	(44)		(178)		(170)		(392)

4.2.4.2.3 Health behaviours

Particular health conditions associated with tooth wear have been identified in the literature. Questions were asked on the following: the use of vitamins or medication, sensation of a dry mouth, strenuous exercise, tummy upsets. The proportions with no wear, mild wear or moderate wear were similar for the respondents taking or not taking any vitamins or medicines (Table 4-29).

Table 4-29: Are you taking any vitamins or medicines?

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Yes	(12)	10	(55)	46	(52)	44	(119)
No	(32)	12	(122)	45	(118)	43	(272)
Total (n)	(44)		(177)		(170)		(391)

A dry mouth can have detrimental consequences to both the hard and soft tissues. In addition to determining the saliva flow rate of the participants, they were asked in the questionnaire whether they considered that they suffered from a dry mouth or not. In the sample 19% reported that they suffered from a dry mouth. The proportion with moderate wear was higher among respondents that reported a dry mouth (46%) compared to those who indicated they did not suffer from dry mouth (42%). The respondents who reported a dry mouth had a lower proportion with no tooth wear, 9% as opposed to 12% (Table 4-30).

Table 4-30: Do you often suffer from a dry mouth?

	No Wear (n)	%	Mild *Enamel Wear* (n)	%	Moderate *Dentine Wear* (n)	%	Total (n)
Yes	(7)	9	(33)	44	(35)	46	(75)
No	(37)	12	(145)	46	(134)	42	(316)
Total (n)	(44)		(178)		(169)		(391)

From the responses to the question *How often are you involved in physical and strenuous exercise*, the same proportion of participants at 46% had moderate tooth wear whether they exercised every day (n = 27) or less than once per week (n = 22). The proportion with mild wear who exercised daily was 49% (n = 29). For participants that exercised less than once per week, the proportion with mild tooth wear was 35% (n = 17). The respondents with the highest proportion of moderate tooth wear increased according to frequency of exercise (Table 4-31).

Table 4-31: How often are you involved in physical and strenuous exercise?

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	**%**	**(n)**	**%**	**(n)**	**%**	**(n)**
Everyday	(3)	5	(29)	49	(27)	46	(59)
Three or four times each week	(10)	8	(57)	47	(54)	45	(121)
Twice a week	(16)	16	(41)	41	(43)	43	(100)
Once a week	(6)	9	(34)	53	(24)	38	(64)
Less than once a week	(9)	19	(17)	35	(22)	46	(48)
Total (n)	(44)		(178)		(170)		(392)

When subjects were asked *Do you ever vomit or have tummy upsets?*, 88% (n=334) responded hardly ever and 14 participants did not answer the question. The high proportion of the sample not having the characteristic of interest makes it difficult to comment (Table 4-32).

Table 4-32: Do you ever vomit or have tummy upsets?

	No Wear		Mild *Enamel Wear*		Moderate *Dentine Wear*		Total
	(n)	**%**	**(n)**	**%**	**(n)**	**%**	**(n)**
Never or hardly ever	(37)	11	(156)	47	(141)	42	(334)
Less than once a month	(4)	11	(16)	46	(15)	43	(35)
More than once a month	-		(4)		(1)		(5)
More than once a week	(1)		(3)				(4)
Total (n)	(42)		(179)		(157)		(378)

The prevalence of asthma in the sample at 12% was less than the prevalence nationally of 19% in 13–15 year-olds. The prevalence of asthma amongst those with moderate tooth wear was 39% and 44% in participants who were not asthmatic (Table 4-33).

Table 4-33: Are you asthmatic?

	No Wear (n)	%	Mild Enamel Wear (n)	%	Moderate Dentine Wear (n)	%	Total (n)
Yes	(9)	19	(20)	42	(19)	39	(48)
No	(35)	10	(158)	46	(151)	44	(344)
Total (n)	(44)		(178)		(170)		(392)

4.2.4.2.4 Knowledge

Eighty one per cent of participants had heard of dental erosion. We used the term dental erosion in the questionnaire, as it is the terminology frequently used in marketing and the media. The proportion with moderate tooth wear was higher in the group that had heard of dental erosion, 44% (n=142) (Table 4-34).

Table 4-34: Awareness of erosion

	No Wear (n)	%	Mild Enamel Wear (n)	%	Moderate Dentine Wear (n)	%	Total (n)
No	(6)	8	(38)	52	(29)	40	(73)
Yes	(38)	12	(140)	43	(142)	44	(320)
Total (n)	(44)		(178)		(171)		(393)

Participants were then asked the cause of dental erosion, whether it was sweet or acidic foods. The majority of students were aware that acidic foods were associated with dental erosion. The participants who knew acidic foods were associated with erosion had a higher proportion with moderate tooth wear, 44% (n = 149). Possibly, this is further evidence to suggest that knowing the cause does not necessarily lead to its avoidance, or perhaps the cause is ubiquitous or they had already been advised by their general dental practitioner (Table 4-35).

Table 4-35: What causes erosion?

	No Wear		Mild Enamel Wear		Moderate Dentine Wear		Total
	(n)	%	(n)	%	(n)	%	(n)
Acidic food	(38)	11	(150)	45	(149)	44	(337)
Sweet food	(6)	11	(28)	53	(19)	36	(53)
Total (n)	(44)		(178)		(168)		(390)

The proportions with tooth wear according to the responses to the questionnaire were presented in Table 4-19 to Table 4-35. The multifactorial aetiology presented in the literature and the proportions with tooth wear at the different levels, according to the questions asked in the questionnaire suggest a number of explanatory variables are acting together. Multivariate statistical analyses that allowed for the analysis of multiple variables simultaneously are presented in section 4.2.5. The statistical analyses will consider both the presence of tooth wear, the count of the number of surfaces and the percentage of surfaces affected.

4.2.5 Statistical analyses

When considering the logistic regression models, the unstimulated and stimulated saliva flow rates were not included as explanatory variables. Statistically significant differences did not exist when unstimulated and stimulated flow rates for the respective tooth wear outcomes were analysed with bivariate analysis.

The outcome variables considered in the multivariate analysis were: the presence of tooth wear at the subject level – the maximum score for the individual for any surface, the buccal surface, the lingual surface and the occlusal surface (Table 4-36) [reproduced from table 3-5]. And a count of, the number of teeth affected by tooth wear by surface (Table 4-37) [reproduced from table 3-6].

Table 4-36: The selected outcome variables for tooth wear at the subject level

Outcome variable	Score
Any tooth wear	Maximum score for the individual from any of the surfaces: occlusal/incisal, buccal/labial or palatal/lingual surfaces
The buccal surface	Maximum score for the individual on the buccal/labial surface
The lingual surface	Maximum score for the individual on the palatal/lingual surface
The occlusal surface	Maximum score for the individual on the occlusal surface

Table 4-37: The selected outcome variables for tooth wear at the tooth level

Outcome variable	Score
Number of buccal/labial surfaces affected	Count
Number of palatal/lingual surfaces affected	Count

The explanatory variables considered are reproduced in Table 4-38 from Table 3-7. The explanatory variables were accommodated simultaneously in the multivariate regression models. Thus we were assessing the association of each variable while adjusting for all others. Explanatory variables that were significant at $p<0.05$ are presented in the results.

Table 4-38: Cross sectional study, explanatory variables considered

Explanatory variables considered	Option	Option	Option	Option
Demographics				
Gender	Male	Female		
Fluoridation status	Always resident with a fully fluoridated water supply	Never resident with a fully fluoridated water supply	Had some exposure to a fluoridated water supply (part)	
Medical card	Family have a medical card	Family do not have a medical card		
Tooth brushing habits				
Frequency of tooth brushing	Brushes twice a day and more	Brushes once a day and less	Never	
Timing of tooth brushing	Before breakfast	After breakfast		
	Before other meals	After other meals		
	Before going to bed	Not before going to bed		
Dietary choices and habits				
Types of drinks consumed	Fizzy drinks (cola), fruit juices, squashes, tea, coffee, water, cider and alcopops			

Explanatory variables considered	Option	Option	Option	Option
How do you typically take the fizzy or squash (acidic) drink	From a bottle	From a cup or glass	From a can	From a straw
Frequency of consumption of (fizzy drinks (cola), fruit juices, squashes) acidic drinks	Once a day and more	Less than once a day		
Frequency of consumption of citrus fruits	Once a day and more	Less than once a day		
Frequency of consumption of apples	Once a day and more	Less than once a day		
Health promoting and lifestyle activities				
Do you bite your nails	Yes	No		
Tooth sensitivity	Yes	No		
Stomach upsets and vomiting	Yes	No		
Are you vegetarian	Yes	No		
Reported frequency of physical and strenuous exercise	Three to four times a week or more	Twice a week		
Dental attendance	Three or more times	Never		

4.2.5.1 Outcome of interest – The presence of any tooth wear

In the multivariable binary logistic regression, significant variables that explained the presence of tooth wear on the surfaces (buccal/labial, palatal/lingual, occlusal/incisal) that were examined were: the time at which tooth brushing took place, and the frequency of fizzy drink consumption. Participants who brushed their teeth after breakfast, 77% (n = 303), had lower tooth wear scores than participants that did not brush after breakfast, p<0.03. Participants who had fizzy drinks more than once a week, 53% (n = 208), were more likely to have higher maximum scores than those that never drink fizzy drinks, p<0.03 (Table 4-39).

4.2.5.2 Outcome of interest – The presence of tooth wear on the occlusal surface

Participants that brushed after breakfast were more likely to have less wear than those that do not brush after breakfast, p<0.02, (Table 4-39).

4.2.5.3 Outcome of interest – The presence of tooth wear on the buccal surface

In the sample, 197 participants responded that they were in the habit of biting their nails; a higher proportion of this group, 13% (n=27), were more likely to have buccal/labial tooth wear scores than those who did not bite their nails (7%), p<0.03, (Table 4-39).

Table 4-39: Results of multivariate analysis, tooth wear outcome measures and associated explanatory variables

Outcome variable: Maximum tooth wear score	**Explanatory variable**	***p*-value**	**Description**
Any surface	Timing of tooth brushing	0.03	Participants who brush their teeth after breakfast are more likely to have lower tooth wear scores than participants who do not brush after breakfast.
	Frequency of consumption of fizzy drinks	0.03	Participants that drink fizzy drinks more than once a week are more likely to have higher maximum scores than those that never drink fizzy drinks.
The buccal surface	Nail biting	0.03	Participants that bite their nails are more likely to have higher maximum buccal/labial scores than those who do not bite their nails.
The occlusal surface	Timing of tooth brushing	0.02	Participants that brush after breakfast are more likely to have lower maximum occlusal scores than those that do not brush after breakfast.
	Frequency of consumption of fizzy drinks	0.03	Participants that drink fizzy drinks more than once a week are more likely to have higher maximum occlusal scores than those that never drink fizzy drinks.

4.2.5.4 Outcome of interest – The number of buccal or lingual surfaces with tooth wear

Participants who have asthma, complain of a dry mouth or drink readymade juices daily had a significantly higher number of buccal surfaces affected than those who did not have these health complaints or dietary habit, (all values, $p<0.05$). Participants exercising everyday had more lingual surfaces affected than participants who indicated they exercised less than once a week ($p<0.02$). The impact of oral hygiene, dietary and health behaviours on the number of buccal and lingual surfaces affected is set out in Table 4-40 and Table 4-41

Table 4-40: Results of multivariable Poisson regression analysis, outcome of interest; the number of buccal surfaces affected by tooth wear.

Variable	p-value	Description
Dental attendance	0.01	Participants who attended the dentist three or more times had fewer teeth affected than participants who never attended the dentist.
Frequency of drinking readymade juices	0.05	Participants who drink readymade fruit drinks every day have more teeth affected than participants who drink them less than once a week.
Frequency of drinking tea	0.01	Participants who drink tea once a week or more than once a week have more teeth affected than participants who drink tea less than once a week.
Choice of water	0.02	Participants who drink flavoured sparkling bottled water or flavoured still bottled water have fewer teeth affected than participants who drink bottled sparkling water or bought still water.
Choice of water	0.008	Participants who drink plain water from the tap or other water have fewer teeth affected than participants that drink bottled sparkling water or bought still water.
Availability of milk scheme at school	0.003	Participants who have access to milk at school have more teeth affected than participants who do not have access to milk at school.
Frequency of eating apples	0.002	Participants who eat apples more than once a week to once a day have fewer buccal surfaces affected than participants who eat apples less than once a week or never.
Suffer with asthma	0.04	Participants who are asthmatic have more buccal surfaces affected than participants who are not asthmatic.
Complaining of a dry mouth	0.03	Participants who reported having a dry mouth have more buccal surfaces affected than participants who do not.

Table 4-41: Results of multivariable Poisson regression analysis, outcome of interest; the number of lingual surfaces affected.

Variable	p-value	Description
Frequency of dental attendance	0.01	Participants who attended the dentist three or more times had fewer lingual surfaces affected than participants who never attended the dentist.
Number of fizzy drinks consumed in a week	0.03	Participants who drank 1–3 cans/week had more lingual surfaces affected than participants who drank < 1 can/week.
Frequency of eating apples	<0.0001	Participants who eat apples more than once a week to once a day have fewer lingual surfaces affected than participants who eat apples less than once a week or never.
Frequency of exercise	0.02	Participants who exercise everyday have more lingual surfaces affected than participants who exercise less than once a week.

4.2.5.5 Proportion of surfaces affected

Analysis using a linear model and square root (√) transformation of percentages considered the explanatory variables that were associated with the proportion of surfaces affected. The analysis indicated that being male and drinking cider were positively associated with a higher proportion of surfaces being affected, $p<0.001$ and $p<0.04$ respectively. Participants who replied that they ate apples daily or more than once per week (n = 258) had a lower percentage of surfaces affected than participants that were infrequent consumers of apples (n = 107), $p<0.04$, (Table 4-42).

Table 4-42: Results of multivariate analysis, outcome for tooth wear; the percentage of surfaces affected

Variable	p-value	Description
Gender	0.001	Male participants had a higher percentage of surfaces affected by wear than females.
Frequency of dental attendance	0.03	Participants who attended the dentist once had a lower percentage of surfaces affected by wear than participants who never attended the dentist.
Whether they drink cider	0.04	Participants who drink cider had a higher percentage of surfaces affected by wear than participants who do not drink cider.
Frequency of eating apples	0.04	Participants who eat apples more than once a week to once a day had a lower percentage of surface affected than participants who eat apples less than once a week or never.

4.2.6 Objectives addressed in the cross sectional study Section 4.2.4

Oral hygiene practices, dietary factors, health behaviours and gender are associated with tooth wear, variously impacting on the different surfaces and on the number and proportion of surfaces affected.

4.2.7 Summary of results

- Male participants had a **higher** percentage of surfaces affected by wear than females.
- Participants who brush after their breakfast are more likely to have **lower** tooth wear scores than participants who do not brush after breakfast.
- Participants who drink carbonated drinks more than once a week are more likely to have **higher** tooth wear scores than participants who never drink carbonated drinks. The participants who drink 1–3 cans/week had **more** lingual surfaces affected than participants who drink <1 can/week.
- Participants who drink readymade fruit drinks every day had **more** buccal surfaces affected than participants who drink them less than once a week.
- Participants who drink cider had a **higher** percentage of surfaces affected by wear than participants who do not drink cider.
- Participants who eat apples more than once a week to once a day have **fewer** buccal and lingual surfaces affected than participants who eat apples less than once a week or never. They also had a **lower** percentage of surfaces affected.
- Participants who bite their nails are more likely to have **higher** maximum buccal/labial scores than participants who do not bite their nails.
- Participants who are asthmatic have **more** buccal surfaces affected than participants who are not asthmatic.
- Participants who reported having a dry mouth have **more** buccal surfaces affected than participants who do not.
- Participants who exercise everyday have **more** lingual surfaces affected than participants who exercise less than once a week (Table 4-43).

Table 4-43: Summary table for tooth wear outcomes; and explanatory variables (all p <0.05)

Outcome variable for tooth wear	Explanatory variable: less tooth wear	Explanatory variable: more tooth wear
Percentage of surfaces affected	Tooth brushing after breakfast Eating apples more than once a week	Male Drink cider
Lower maximum scores	Tooth brushing after breakfast	
Higher maximum scores		Drinking carbonated drinks once a week and more
Lingual tooth wear	Eating apples more than once a week	Drinking 1 to 3 cans each week of carbonated drinks Strenuous exercise everyday
Buccal tooth wear	Eating apples more than once a week	Ready-made fruit drinks daily Indicated that they bit their nails Indicated that they were asthmatic Indicated that they had a dry mouth

4.3 Longitudinal study

The results of the longitudinal study are presented in this section, each time point is described as time point 1 or 2 and abbreviated to tp1 or tp2 (Table 4-44). Participants each had a clinical examination and completed a questionnaire on their oral hygiene practices, dietary factors, health behaviours and knowledge of dental erosion. MH first completed the examination using the Mod TWI (Bardsley et al., 2004); once completed, the subject had a five-minute rest. The examination continued with the ETI (Bartlett et al., 2011b). Scores were assigned to the buccal/buccal-cervical and labial surfaces, the palatal/lingual surfaces and the occlusal or incisal surfaces of all erupted teeth. First the enamel was scored, indicating the spread of enamel loss from the tooth surface, and then dentine, indicating the amount of dentine visible.

4.3.1 Objectives addressed in the longitudinal study

1. Investigate whether tooth wear at age 14 and the tooth wear increment between age 12 (tp1) and 14 (tp2) are associated with demographic factors, oral hygiene practices, dietary factors, health behaviours using data collected at three time points in a longitudinal study.
2. Investigate whether tooth wear at age 14 and the tooth wear increment between age 12 (tp1) and 14 (tp2) are associated with tooth wear recorded when the participants were examined at earlier time points.

At age 12 (tp1), 123 children were examined. Some participants examined at tp1 were not examined at age 5 as they were absent on the day of the examination but had consented to future contact (Table 4-44).

Table 4-44: Record of participants at tp1

Record of participants at tp1 age 12	
Participants with complete data that were examined	123
Participants that had left the school	22
Participants absent on the day of the exam	5
Participants excluded	9
Participants that refused	9
Total number of forms distributed	168

At age 14 (tp2), 121 participants were invited to attend and from that group 83 participants were examined (Table 4-45).

Table 4-45: Record of participants at tp2

Record of participants at tp2 age 14	
Participants with complete data that were examined	83
Participants that had left the school	2
Participants absent on day of exam	4
Participants excluded	2
Participants for whom forms were not returned	30
Total number of forms distributed	121

4.3.2 Characteristics of the longitudinal sample

The mean age and the number of children examined at each time point are set out in Table 4-46. The five-year-olds who initiated the cohort are included for clarity (Harding et al., 2003; Harding et al., 2010).

Table 4-46: The mean age and the number of children examined at tp1 and tp2

Mean age (years)	Time point (tp)	Number that consented	Number examined
5.49 (± 0.28)	tp0	286	202
12.01 (± 0.32)	tp1	168 consented to future contact	123
14.70 (± 0.3)	tp2	121 consented to future contact 96 forms returned	83

No reminders or incentives were provided to retain the cohort between the study examinations. Some children who consented at age five but were absent on the day of the examination at age five were examined at age 12, and some children seen at age 14 were absent on the day of the examination at age 12. At tp(2), 83 participants had complete data. Seventy nine of these participants were also examined at tp(1) and 88 of them were examined at tp(0) (Table 4-47). As children had moved on to secondary school at age 14, some were examined at the OHSRC, under identical conditions, to the examinations conducted in the schools.

Table 4-47: The participants contributing to the final cohort at tp(2)

Age 5 cohort (tp0)	Age 12 cohort (tp1)	Age 14 cohort (tp2)
88	79	83

4.3.3 Tp1

Fieldwork at tp1 was conducted at the participants' primary schools; 55% of 12 year-olds had a lifetime exposure to a home-fluoridated water supply (Table 4-48).

Table 4-48: Summary of fluoride status at tp1

Water Supply / Lifetime Exposure	(n)	%
Fluoridated	68	55
Non-Fluoridated	45	37
Part	10	8
Total	123	100

At tp1, for both indices, Mod TWI (Bardsley et al., 2004) and ETI (Bartlett et al., 2011b), 62% (n=76) of the participants had a score of 0 and 38% (n=47) had tooth wear with dentine visible (Table 4-49).

Table 4-49: Prevalence of tooth wear at tp1 with dentine visible (Bardsley et al., 2004) and (Bartlett et al., 2011b)

Tooth Wear	(n)	%
No tooth wear with dentine visible	76	62
Tooth wear with dentine visible	47	38
Total	123	100

4.3.4 Tp2

The fieldwork for tp2 commenced in February 2010 and was completed in August 2010. At tp2, the proportion of the sample with a fluoridated water supply was similar to that at tp1, 54% (n=44) and 55% (n=68) respectively; likewise, the proportions that were non-fluoridated at tp2 and tp1 were similar, 34% (n=28) and 37% (n=45) respectively (Table 4-50).

Table 4-50: Summary of fluoride status at tp2

Water Supply / Lifetime Exposure	(n)	%
Fluoridated	44	54
Non-Fluoridated	31	34
Part	8	12
Total	83	100

At tp2, 46% (n=38) had tooth wear with dentine visible (Table 4-51). Between tp(1) age 12 and tp(2) age 14, the prevalence of tooth wear had increased.

Table 4-51: Prevalence of tooth wear with dentine visible at tp2 (Bardsley et al., 2004) and (Bartlett et al., 2011b)

Tooth Wear dentine	(n)	%
No tooth wear into dentine	45	54
Tooth wear with dentine visible	38	46
Total	83	100

At tp(2), tooth wear was recorded on the buccal/labial and palatal/lingual surfaces and the occlusal surfaces of first permanent molar teeth using the ETI (Bartlett et al., 2011b) (Table 4-52 and Table 4-53).

Table 4-52: Prevalence of tooth wear at tp2 on buccal/labial and palatal/lingual surfaces (Bartlett et al., 2011b)

Tooth wear buccal/labial and palatal/lingual (smooth) surfaces	(n)	%
No tooth wear present on smooth surfaces	51	61
Tooth wear present on smooth surfaces	32	39
Total	83	100

Table 4-53: Prevalence of any tooth wear on the first permanent molar at tp2 (Bartlett et al., 2011b)

Tooth wear on first permanent molar teeth	(n)	%
No tooth wear present on first permanent molar teeth	55	66
Tooth wear present on first permanent molar teeth	28	34
Total	83	100

Table 4-54: Prevalence of tooth wear that has extended into dentine at tp2 (Bartlett et al., 2011b)

Tooth wear that has extended into dentine	(n)	%
No tooth wear that has extended into dentine	45	54
Tooth wear present that has extended into dentine	38	46
Total	83	100

At tp2, a count of the number of buccal/labial and/or palatal/lingual surfaces (Bartlett et al., 2011b) with tooth wear indicated that 61% of the sample had no tooth wear on buccal/labial and/or palatal/lingual surfaces. Thirteen per cent had two surfaces affected and 8% had one surface affected (Table 4-55 and Figure 4-16).

Table 4-55: The number and percentage of buccal/labial and/or palatal/lingual surfaces affected by tooth wear at age 14

Count	0	1	2	3	4	5	6	7	8	9	10	11	Total
n	51	7	11	4	4	2	0	3	0	0	0	1	83
%	61	8	13	5	5	2	0	4	0	0	0	1	100

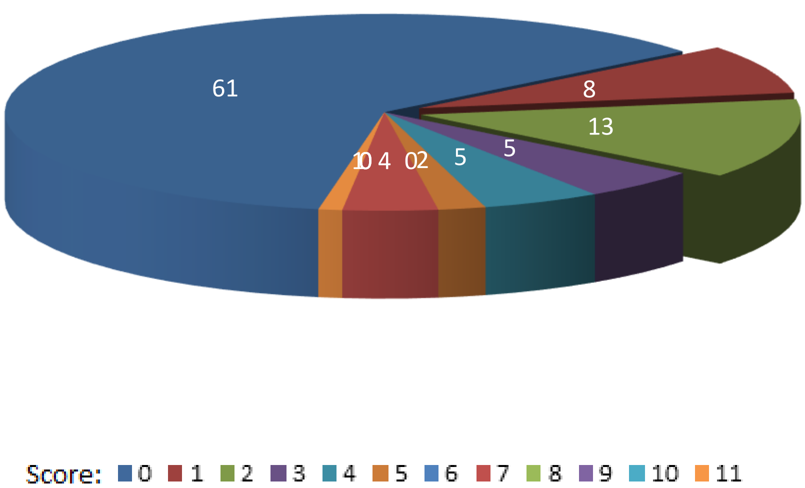


Figure 4-16: Frequency distribution of the percentage with tooth wear on the smooth surfaces

At tp2, just over half of participants had no tooth wear with dentine visible, 10% had a single surface affected, while 18% had two surfaces affected and 11% had three surfaces affected (Table 4-5 and Figure 4-17).

Table 4-56: The number and percentage of surfaces affected by tooth wear with dentine visible at tp2 age 14

Count	0	1	2	3	4	5	6	7	8	9	Total
n	45	8	15	9	1	1	2	1	0	1	83
%	54	10	18	11	1	1	2	1	0	1	100

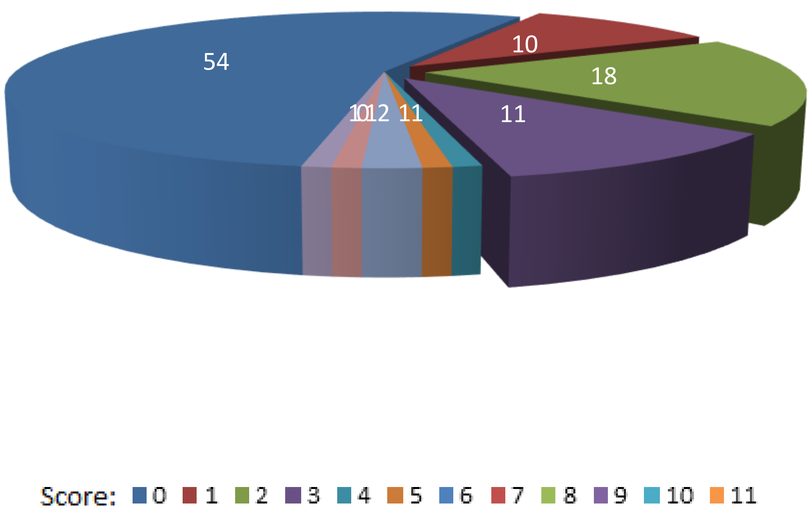


Figure 4-17: Frequency distribution of the percentage with tooth wear with dentine visible

A maximum increment score was calculated for 66 individuals between tp1 and tp2 (Table 4-57).

Table 4-57: Maximum increment score between tp1 and tp2 (n=66)

Maximum increment score	0	1	2	3	4	5	6	7	Total
n	16	12	10	9	8	8	2	1	66

%	24	18	15	14	12	12	3	2	100

The mean increment was calculated for 66 individuals between tp1 and tp2.

Table 4-58: Mean increment between tp1 and tp2 (n=66)

n	Mean	(SD)	Median	Minimum	Maximum
66	0.1	0.13	0.06	0.0	0.73

4.3.5 Statistical analyses

Using multivariable binary logistic regression models, statistically significant variables were identified for the outcome variables: tooth wear on buccal/labial and palatal/lingual (smooth) surfaces, tooth wear with dentine visible and tooth wear (either within enamel, or with dentine visible) on the first permanent molar teeth at age 14 (tp2). The *count of the number of* buccal/labial and palatal/lingual surfaces affected by any tooth wear, the number of surfaces affected by tooth wear into dentine and the maximum increment score between tp1 and tp2 were analysed using multivariable Poisson regression models. The mean increment between tp1 and tp2 was analysed using a normal regression model.

The explanatory variables used in the models are set out in Table 4-59 reproduced from Table 3-14.

Table 4-59: Explanatory variables included in the logistic regression

Explanatory variables considered	Option	Option	Option	Option
Demographics				
Gender	Male	Female		
Fluoridation status	Always resident with a fully fluoridated water supply	Never resident with a fully fluoridated water supply		
Medical card	Family have a medical card	Family do not have a medical card		
Previous tooth wear				
Previous erosive tooth wear at age 5	Had erosive tooth wear at age 5	Had no erosive tooth wear at age 5		
Previous erosive tooth wear in enamel only at age 5	Had erosive tooth wear in enamel only at age 5	No erosive tooth wear in enamel only at age 5		
Previous erosive tooth wear in dentine/pulp at age 5	Had erosive tooth wear in dentine/pulp at age 5	No erosive tooth wear in dentine/pulp at age 5		
Previous tooth wear on smooth surfaces at age 12	Tooth wear on smooth surfaces at age 12	No tooth wear on smooth surfaces at age 12		

Explanatory variables considered	Option	Option	Option	Option
Previous tooth wear on the incisal surfaces of incisors exposing dentine at age 12	Had tooth wear into dentine at age 12, on the incisal surface of one of the maxillary or mandibular incisors	Had no tooth wear into dentine at age 12, on the O/I surface of one of the maxillary or mandibular incisors		
Tooth brushing habits				
Frequency of tooth brushing at both age 12 and age 14	Brushes twice a day and more	Brushes once a day and less		
Timing of tooth brushing age 12 and 14	Before breakfast	After breakfast		
	Before other meals	After other meals		
	Before going to bed	Not before going to bed		
Dietary choices and habits				
Age 5 questionnaire: age at which use of baby bottle stopped				
Types of drinks consumed at age 12 and 14	Fizzy drinks (cola), fruit juices, squashes, tea, coffee, water			
How do you typically take the fizzy or squash (acidic) drink age 12 and 14	From a bottle	From a cup or glass	From a can	From a straw
Frequency of consumption of (fizzy drinks (cola), fruit juices, squashes) acidic drinks age 12 and 14	Once a day and more	Less than once a day		

Explanatory variables considered	Option	Option	Option	Option
Frequency of consumption of citrus fruits age 12 and 14	Once a day and more	Less than once a day		
Frequency of consumption of apples age 12 and 14	Once a day and more	Less than once a day		
Frequency of consumption of yoghurt age 12 and 14	Once a day and more	Less than once a day		
Health promoting and lifestyle activities				
Taking vitamins or medicines age 12 and at age 14	Yes	No		
Use of an inhaler for asthma at age 14	Yes	No		
Suffer with a dry mouth at age 14	Yes	No		
Do you bite your nails at age 14	Yes	No		
Tooth sensitivity at age 12 and at age 14	Yes	No		
Stomach upsets and vomiting at age 12 and at age 14	Yes	No		
Are you vegetarian at age 14	Yes	No		
Reported frequency of physical and strenuous exercise at age 14	Three to four times a week or more	Twice a week		

4.3.5.1 Logistic regression

The results of multivariable binary logistic regression models are presented, followed by the results of the Poisson regression models and finally the mean increment using a normal regression model. The outcome variables considered in the multivariable binary logistic regression models were:

(i) The presence of tooth wear at age 14 on the buccal/labial and palatal/lingual surfaces of maxillary and mandibular incisors (smooth surfaces)

(ii) The presence of tooth wear at age 14 where dentine is visible

(iii) The presence of any tooth wear (either within enamel, or with dentine visible) at age 14 on the first permanent molar teeth.

4.3.5.1.1 Outcome of interest - (i) the presence of tooth wear at age 14 on the buccal/labial and palatal/lingual surfaces of maxillary and mandibular permanent incisors (smooth surfaces) (n=72)

Data from 72 participants were available for the analysis. The significant explanatory variables, the direction of the effect and the odds ratio (OR) are presented in Table 4-61 . The analysis indicated individuals with tooth wear on the smooth surfaces of permanent teeth at age 14 were 26% more likely to have had at age 5 tooth wear with dentine or pulp exposed on the maxillary primary incisors (OR 1.26, 95% CI (1.08–1.48), $p<0.004$. The participants who ate apples once a day or more at age 14 were 90% less likely to have tooth wear on smooth surfaces at age 14 (OR 0.10, 95% CI (0.01– 0.96), $p<0.04$) (Table 4-60).

Table 4-60: Results of multivariate analysis, outcome tooth wear on the buccal/labial and/or palatal/lingual (smooth) surfaces (n=72)

Variable	Age	p-value	OR 95% and (CI)	Description
Previous erosive tooth wear in dentine/pulp at age 5	5	0.004	1.26 (1.08–1.48)	Participants with erosive tooth wear into the dentine or pulp at age 5 were **more** likely to have tooth wear on smooth surfaces at age 14
Frequency of consumption of apples	14	0.05	0.10 (0.01, 0.96)	Participants who eat apples once a day or more at age 14 were **less** likely to have tooth wear on smooth surfaces at age 14 than those that eat apples less than once a day.

4.3.5.1.2 Outcome of interest - (ii) the presence of tooth wear that has extended into the dentine (n=63).

The likelihood of having tooth wear with dentine visible at age 14 was again significantly associated with tooth wear exposing dentine or pulp on the maxillary primary incisors at age 5 (OR 1.23, 95% CI (1.04–1.46), p<0.02). Somewhat thought-provoking, the results indicated that participants eating apples daily or more frequently at age 12 were more likely to have tooth wear at age 14 with dentine visible (OR 4.51, 95% CI (1.21–16.81), p<0.03) (Table 4-62).

4.3.5.1.3 Outcome of interest - (iii) the presence of any tooth wear (either within enamel, or with dentine visible) on the first permanent molar teeth (n=83).

Participants who always had a fluoridated water supply were more likely to have tooth wear on the first permanent molars, (OR 9.28, 95% CI (2.38 – 36.12), p<0.001). Tooth wear in particular that associated with abrasion and tooth to tooth contact is a recognised post eruptive phenomenon when dental fluorosis is present. In this research, we cannot say, whether fluorosis was present or not. The participants with a lifetime exposure to fluoridated water were the participants who had more first permanent molar teeth present that could be scored (Table 4-62).

Table 4-61: Results of the multivariate analysis, outcome tooth wear that has extended into dentine (n=63)

Variable	Age	p-value	OR 95% and (CI)	Description
Previous erosive tooth wear in dentine/pulp at age 5	5	0.02	1.23(1.04–1.46)	Participants with erosive tooth wear into the dentine or pulp at age 5 were **more** likely to have wear extending into dentine at age 14.
Frequency of consumption of apples	12	0.03	4.51(1.21–16.81)	Participants that eat apples once a day or more at age 12 were **more** likely to have tooth wear extending into dentine at age 14, than those that eat apples less than once a day.

Table 4-62: Results of multivariate analysis, outcome tooth wear on the first permanent molar teeth (n=83)

Variable	Age	p-value	OR and 95% (CI)	Description
Fluoridation status	14	0.001	9.28 (2.38–36.12)	Participants with a lifetime exposure to fluoridated water were **more** likely to have wear than those with non-fluoridated water.
Brushing after breakfast	12	0.03	0.31 (0.11–0.91)	Participants who brush after breakfast at age 12 were **less** likely to have wear at age 14 than those who did not.

To assess the association between *the count of the number of* surfaces affected at age 14-years and to also assess the association between *the maximum increment score* between tp1 and tp2 with the explanatory variables, multivariable Poisson regression models were used. The outcome variables considered in the multivariable Poisson regression models were:

(i) The number of buccal/labial and palatal/lingual (smooth) surfaces affected by any tooth wear at age 14-years
(ii) The number of surfaces affected by tooth wear with dentine visible.
(iii) The maximum increment score between (tp1) and (tp2).

4.3.5.1.4 Outcome of interest - (i) a count of the number of buccal/labial and palatal/lingual (smooth) surfaces affected by any tooth wear

Similarities existed between the explanatory variables associated with the presence of and the count of the number of smooth surfaces affected. Interestingly, participants that self-reported a dry mouth at age 14 had more smooth surfaces affected, (OR 2.51, 95% CI (1.31, 4.79), $p<0.006$). Tooth brushing twice each day conferred a benefit, (OR 0.23, 95% CI (0.12, 0.43), $p<0.0001$). Counter intuitively, participants brushing before bed at age 14 had a higher number of smooth surfaces affected, (OR 2.52, 95% CI (1.17, 5.41), $p<0.014$), (Table 4-63).

Table 4-63: Results of the Poisson regression, outcome the number of smooth surfaces affected by any tooth wear (n = 56)

Variable	Age	p-value	Description	Ratio of means and (95% CI)
Previous erosive tooth wear in dentine/pulp at age 5	5	0.0007	Participants with erosive tooth wear into the dentine or pulp at age 5 had **more** surfaces affected at age 14	1.13 (1.06, 1.22)
Taking vitamins or medicines	12	0.005	Participants who took vitamin tablets at age 12 had **fewer** surfaces affected.	0.31 (0.13, 0.70)
Brushing before bed	14	0.0177	Participants who brush before bed at age 14 had **more** surfaces affected than participants who do not brush before bed	2.52 (1.17, 5.41)
How do you typically take the fizzy or squash (acidic) drink	14	<0.0001	Participants that drink from a can, bottle, or straw at age 14 had **more** surfaces affected than participants that drink from a glass/cup.	3.36 (1.99, 5.70)
Suffer with a dry mouth	14	0.006	Participants who reported a dry mouth at age 14 had **more** surfaces affected.	2.51 (1.31, 4.79)
Frequency of brushing	14	<0.0001	Participants who brush twice a day or more at age 14 had **fewer** surfaces affected than participants who brush once a day or less	0.23 (0.12, 0.43)
Frequency of consumption of apples	14	0.02	Participants who eat apples once a day or more at age 14 had **fewer** surfaces affected than participants that eat apples less than once a day.	0.18 (0.04, 0.76)

4.3.5.1.5 Outcome of interest - (ii) a count of the number of surfaces affected by tooth wear with dentine visible.

In this analysis, erosive tooth wear into the dentine or pulp at age 5 was again a risk indicator for tooth wear at age 14. Participants who had erosive tooth wear into the dentine or pulp at age 5 had tooth wear with dentine visible and had a higher number of surfaces affected by tooth with dentine visible at age 14, $p<0.0001$ (Table 4-65). The outcome *the count of the number of* surfaces affected by tooth wear added additional explanatory variables to the presence of tooth wear with dentine visible. Participants with tooth wear at age 12 (tp1) with dentine visible on the incisal surface of incisors had more surfaces affected at age 14, $p<0.005$, (Table 4-64). Participants who were tooth brushing after breakfast at age 12 had fewer surfaces affected ($p<0.002$), but at age 14 participants brushing after breakfast had more surfaces with dentine visible affected ($p<0.04$). Whether this is the impact of explanatory variables acting synergistically when dentine is exposed requires further investigation. Other explanatory variables associated with an increased number of surfaces affected with dentine visible were using a straw to consume acidic drinks at age 12 ($p<0.03$) and using a can, bottle or straw rather than a glass or cup at age 14 to consume acidic drinks ($p<0.02$) (Table 4-64). Yoghurt when consumed once a day and more at age 12-years was associated with a higher number of surfaces affected at age 14, $p<0.05$ (Table 4-64).

Table 4-64: Results of the Poisson regression, outcome the count of the number of tooth surfaces affected by tooth wear into dentine (n = 58)

Variable	Age	p-value	Description	Ratio of Means and (95% CI)
Previous erosive tooth wear in dentine/pulp at age 5	5	0.0001	Participants with erosive tooth wear into the dentine or pulp at age 5 had **more** surfaces affected by tooth wear into dentine at age 14	1.15 (1.07, 1.24)
Previous tooth wear on the incisal surface of incisors exposing dentine at age 12	12	0.02	Participants with tooth wear into the dentine on the incisal surface of incisors at age 12 had **more** surfaces affected by tooth wear into dentine at age 14	2.88 (1.20, 6.88)
Typically take acidic drinks through a straw	12	0.03	Participants who drink from a straw at age 12 had **more** surfaces affected than participants who do not.	2.20 (1.10, 4.39)
Yoghurt	12	0.01	Participants who eat yoghurt once a day or more at age 12 had **more** surfaces affected than participants who eat yoghurt less than once a day.	2.08 (1.19, 3.62)
Brushing after breakfast	12	0.01	Participants who brush after breakfast at age 12 had **fewer** surfaces affected than participants who do not.	0.50 (0.29, 0.87)
Brushing after breakfast	14	0.01	Participants who indicated that they brush after breakfast at age 14 had **more** surfaces affected than participants who do not.	2.60 (1.21, 5.56)
How do you typically take the fizzy or squash (acidic) drink	14	0.02	Participants who drink from a can, bottle, or straw at age 14 had **more** surfaces affected than participants who drink from a glass or cup.	2.34 (1.36, 4.02)

4.3.5.1.6 *Outcome of interest – (iii) the maximum increment score between tp1 and tp2*

The presence of wear with dentine visible on the primary maxillary incisors at age 5, drinking acidic drinks through a straw at age 12, eating apples once a day and more at age 12 and eating yoghurt once a day and more were each associated with a higher maximum increment score (Table 4-65) . Some of the explanatory variables were associated with a lower maximum increment score. Brushing before bed at age 12 and eating citrus fruit once a day and more at age 14 were both associated with a lower mean increment (Table 4-65).

Table 4-65: Results of the Poisson regression, outcome the maximum increment score between tp1 and tp2 (n=66)

Variable	Age	p-value	Description	Ratio of means and (95% CI)
Erosion into dentine or pulp	5	0.004	Participants with erosive tooth wear into the dentine or pulp at age 5 had had a **higher** maximum increment	1.07 (1.02, 1.12)
Brushing before bed	12	0.003	Participants who brush before bed at age 12 had a **lower** maximum increment	0.51 (0.32, 0.80)
Drinking from a straw	12	0.039	Participants who drink from a straw at age 12 had a **higher** maximum increment	1.56 (1.02, 2.37)
Eating citrus fruit	14	0.007	Participants who eat citrus fruit once a day or more at age 14 had a **lower** maximum increment than participants who eat citrus fruits less than once a day.	0.49 (0.29, 0.83)
Eating apples	12	0.013	Participants who ate apples once a day or more at age 12 had a **higher** maximum increment than participants who ate apples less than once a day.	1.75 (1.13, 2.71)
Eating yoghurt	12	<0.0001	Participants who ate yoghurt once a day or more at age 12 had a **higher** maximum increment than participants who ate yoghurt less than once a day.	2.29 (1.55, 3.37)

4.3.5.1.7 Outcome of interest – mean increment between tp1 and tp2

Explanatory variables that were associated with a higher mean increment were participants who reported that they took their acidic drink through a straw and participants who reported a dry mouth at age 14. The variables associated with a lower mean increment were participants who reported twice a day brushing at age 14 and participants who reported taking vitamins at age 14 (Table 4-66).

Table 4-66: Results of linear regression analysis for the mean increment between tp1 and tp2 (n=66)

Variable	Age	p-value	Description	Ratio of means and (95% CI)
Frequency of tooth brushing	14	0.039	Participants who brush twice a day or more at age 14 had a **lower** mean increment than participants who brush once day or less.	0.95 (0.90, 1.00)
Drinking from a straw	12	0.0005	Participants who drink from a straw at age 12 had a **higher** mean increment	1.13 (1.06, 1.20)
Eating apples	14	0.016	Participants who eat apples at age 14 once a day or more at age 14 had a **lower** mean increment than those that eat apples less than once a day	0.93 (0.89, 0.99)
Dry mouth	14	0.039	Participants who reported a dry mouth at age 14 had a **higher** mean increment	1.07 (1.01, 1.14)
Vitamin tablets	14	0.007	Those that take vitamin tablets at age 14 had a **lower** mean increment	0.92 (0.87, 0.98)

4.3.6 Objectives addressed in Section 4.3

Objective 1: Investigate oral hygiene habits and dietary factors and their association with tooth wear at a point in time using cross-sectional data and over time using data collected in a longitudinal study.

Objective 2: Investigate the associations with tooth wear at age 14 and the increment between age 12(tp1) and 14(tp2) in a cohort of children examined at age 5, age 12-years and age 14-years.

4.3.7 Summary of results in Section 4.3:

- Tooth wear at age 14 was associated with a previous history of tooth wear in the permanent dentition at age 12 and in the primary dentition at age five. Tooth wear was more likely on, the smooth buccal/labial and palatal/lingual surfaces; at age 14 of participants who had tooth wear on the maxillary primary incisors with dentine visible at age 5. Tooth wear that had extended into dentine and tooth wear that had extended into dentine and a higher number of surfaces affected surfaces affected were also associated with tooth wear on the maxillary primary incisors with dentine visible at age 5.
- Tooth wear was more likely on the first permanent molar teeth at age 14 of participants who were life time residents of a fluoridated area.
- At age 14 more surfaces had tooth wear extending into dentine in participants who at age 12 had tooth wear extending into dentine on the incisal surface of maxillary or mandibular incisors.
- Oral hygiene practices, dietary factors and health behaviours were associated with tooth wear, variously impacting on the different surfaces and on the number of teeth at age 14.
- Tooth wear at age 14 was less likely on the smooth buccal/labial and palatal/lingual surfaces of participants who brushed twice or more each day than participants who brushed less frequently. Somewhat counter intuitively participants who brushed before bed at age 14 had more smooth buccal/labial and palatal/lingual surfaces affected than participants who did not. At age 14 fewer surfaces were affected with tooth wear into dentine in participants who at age 12 brushed after

breakfast than those who did not. However at age 14 participants who brushed after breakfast had more surfaces affected at age 14.

- Participants who ate apples once a day or more at age 14, were less likely to have tooth wear on the smooth buccal/labial and palatal/lingual surfaces and they also had fewer of these surfaces affected. However, participants who ate apples once a day or more at age 12 were more likely to have tooth wear that extended into dentine than those who ate apples less than once per day.
- Participants who typically took their acidic drink through a can or bottle or straw rather than a glass or cup at age 14 were more likely to have tooth wear on smooth surfaces, to have more smooth surfaces affected and to have a higher number of surfaces with dentine visible.
- At age 14 there were more surfaces affected by tooth wear with dentine visible in participants who indicated that at age 12 they had yoghurt once a day and more often than participants who ate yoghurt less than once a day.
- Participants who reported taking vitamins at age 12 and 14 were less likely to have tooth wear on smooth surfaces and had fewer smooth surfaces affected. Those who reported taking vitamins at age 12 also had fewer surfaces affected.
- At age 14 more smooth surfaces were affected by tooth wear in participants who reported at age 14 that they had a ‘dry mouth’.
- A higher maximum increment score was associated with tooth wear with dentine visible on the upper primary incisors at age 5, participants using a straw to drink acidic drinks at age 12, participants who ate apples once a day and more at age 12 and participants who ate yoghurt once a day and more at age 12.
- A lower maximum increment score was associated with participants brushing before bed at age 12 and participants who ate citrus fruit once a day and more at age 14
- The mean increment was lower in participants who brushed twice a day or more at age 14 than those who brushed less frequently and in participants who reported tasking vitamins at age 14.
- The mean increment was higher in participants who reported using a

straw to drink acidic drinks at age 12 and participants who reported a dry mouth (Table 4-67).

Table 4-67: Summary table for tooth wear outcomes; and the explanatory variables, associated with tooth wear at age 14 (p <0.05)

Tooth wear Outcome variable	Explanatory variable – less tooth wear at age 14	Explanatory variable – more tooth wear at age 14
Tooth wear on smooth surfaces	Taking vitamins at age 12 or 14	Tooth wear with dentine visible at age 5
	Participants who brush twice a day or more at age 14	Typically using a can bottle or straw for acidic drinks at age 14
	Eating apples once a day or more at age 14	Strenuous exercise 3 to 4 times per week at age 14
Tooth wear that has extended into the dentine		Tooth wear with dentine visible at age 5
		Eating apples once a day or more at age 12
Any tooth wear (either within enamel, or extending to dentine) on the first permanent molar teeth		Lifetime exposure to fluoridated water up to age 14
The number of smooth surfaces affected by any tooth wear	Participants who brush twice a day or more at age 14	Tooth wear with dentine visible at age 5
	Eating apples once a day or more at age 14	Brushing before bed at age 14
	Taking vitamins at age 14	Typically using a can bottle or straw for acidic drinks at age 14
		Indicated that they had a dry mouth at age 14
The number of surfaces affected by tooth wear into dentine	Participants who brush after breakfast at age 12	Tooth wear with dentine visible at age 5
		Tooth wear with dentine exposed on permanent incisors at age 12
		Brushing after breakfast at age 14
		Typically using a straw for acidic drinks at age 12

Tooth wear Outcome variable	Explanatory variable – <u>less</u> tooth wear at age 14	Explanatory variable – <u>more</u> tooth wear at age 14
		Typically using a can bottle or straw for acidic drinks at age 14 Yoghurt once a day or more at age 12
Maximum increment score	Participants who brush before bed at age 12 Eating citrus fruit once a day or more at age 12	Tooth wear with dentine visible at age 5 Using a straw for acidic drinks at age 12 Eating apples once a day or more at age 12 Yoghurt once a day or more at age 12
Mean increment	Participants who brush twice a day or more at age 14 Eating apples once a day or more at age 14 Taking vitamins at age 12 or 14	Using a straw for acidic drinks at age 12 Indicated that they had a dry mouth at age 14

Chapter 5 **Discussion**

5.1 Introduction

The results of the research project presented in this thesis contribute to the body of knowledge on tooth wear in both the arena of dental public health and in salivary research. Standard methods in chemistry and biochemistry were applied to whole mouth saliva samples, collected as part of an epidemiological study. The epidemiological study involved a clinical examination and the collection of information on oral hygiene practices, dietary practices, general health and lifestyle practices and knowledge of erosive tooth wear. The results of both the cross sectional study and the longitudinal study provide useful insights into oral hygiene and dietary associations with tooth wear. The longitudinal study identifies an important significant association between tooth wear present at age 14 and tooth wear previously identified at both age 12 and age 5.

The aim of the research project was to investigate the characteristics, development and determinants of tooth wear among Irish school children. The specific study objectives were addressed through either the cross sectional study, the longitudinal study or both.

The first study was a cross sectional study with participants aged 16 years in the main and the second study a prospective longitudinal study with data available for children from age five years to 14-years.

The prevalence of tooth wear at age 16 in transition year school going children was determined in the cross sectional study. In addition the associations between the quantity and the quality of whole mouth saliva including salivary proteins and the ions calcium, phosphate and fluoride and tooth wear were investigated. The protein carbonyl concentration was studied for a selected sub group. The subgroup selected had either no tooth wear or moderate tooth wear. Data collected in both the cross sectional and longitudinal study demonstrated associations between demographic factors, oral hygiene practices, dietary practices, general health and lifestyle activities and tooth wear. Some of these associations were with the likelihood of having more tooth wear and some with the possibility of less tooth wear being present. The associations are tabulated in Table 4-43 and Table 4-67.

Data from the longitudinal study demonstrated the importance of taking a life course approach to oral health. The presence of tooth wear, with dentine visible on the maxillary primary incisors at age 5-years, was significantly associated with tooth wear in the permanent dentition at age 12 (tp1), OR 5.06 (Harding et al., 2010) and at age 14 (tp2), OR 1.26 for the presence of tooth wear on smooth surfaces, the association also existed for the maximum increment score between the two time points (tp1 and tp2), ($p<0.004$).

The objectives by which the aim of the research was achieved are reproduced for convenience.

Objectives addressed in the cross-sectional study

1. Estimate the prevalence and pattern of tooth wear in 16-year-old transition year students
2. Investigate tooth wear levels and associations with demographic factors
3. Investigate tooth wear levels and associations with oral hygiene practices and dietary factors
4. Investigate tooth wear levels and associations with the properties of resting and stimulated saliva.

Objectives addressed in the longitudinal study

1. Investigate whether tooth wear at age 14 and the tooth wear increment between age 12 (tp1) and 14 (tp2) are associated with demographic factors, dietary factors, oral hygiene practices, or lifestyle practices using data collected at three time points in a longitudinal study.
2. Investigate whether tooth wear at age 14 and the tooth wear increment between age 12 (tp1) and 14 (tp2) are associated with tooth wear recorded when the participants were examined at earlier time points.

The discussion of the results is constructed under the headings:

- Measurement of tooth wear
- The correlates of tooth wear including previous tooth wear
- Salivary factors

Where it is appropriate both studies are discussed together. The possible sources of bias and risk of bias in the studies will be addressed after discussion of the responses to the questionnaire. Salivary factors are discussed after the correlates of tooth wear in this chapter; this permits an easier discussion of the correlates of tooth wear in both studies together.

5.2 Measurement

Measurement of a condition such as tooth wear requires valid and reproducible methods, the condition can be documented, prevalence established and comparisons made with previous research. The literature on tooth wear acknowledges that challenges exist in recording tooth wear and in making comparisons between different studies (Bartlett et al., 2008; Ganss et al., 2006; Ganss and Lussi, 2008; Ganss et al., 2011; Holbrook and Ganss, 2008; Young et al., 2008). Recording dental caries in epidemiological studies and defining cases at the dentinal level meets with far less challenges. Although, recording dental caries at the dentinal level is currently the source of much discussion, and is a particular challenge in caries clinical trials (Pitts, 2004). Nor is the measurement of periodontal disease in epidemiological studies without its challenges (Leroy et al., 2010). When tooth wear is recorded at the level of exposed dentine specificity is high, although at the expense of sensitivity (Ganss et al., 2006; Huysmans et al., 2011). When conducting a cross sectional epidemiological study specificity is desirable, however to monitor tooth wear in the individual greater sensitivity is desirable.

5.2.1 Measurement in the cross sectional study

In the cross sectional study, no individual had a tooth wear score that involved either secondary dentine or pulp exposure. In the cross sectional study the maximum score recorded was 'more than $^{1}/_{3}$rd of the dentine was visible'. In the cross sectional study, using the modified TWI (Bardsley et al., 2004), the prevalence of tooth wear with dentine visible was 44%. This value was lower than in the study conducted by

(Bardsley et al., 2004). Bardsley et al. (2004) reported that 53% of their entire sample had at least one tooth surface with dentine exposed at age 14. The modified TWI (Bardsley et al., 2004) was again used in Amman, Jordan and they reported a prevalence of 51% in 16-year-olds (Abu-Ghazaleh et al., 2013). The partial mouth index described by (Bardsley et al., 2004) fulfils most of the criteria for an ideal index. It is acceptable, easy to use, amenable to statistical analysis, valid and reliable (Burt and Eklund, 2005). However, because of the levels at which tooth surface loss is recorded, sensitivity with the ability to detect small changes in time is not a feature of the index. This would limit the use of the index in a study of progression or incidence.

The prevalence of tooth wear recorded in the 16-year-olds in this research was higher than that recorded in the last Irish national survey for the 15-year-old age group. The overall prevalence recorded in the national survey was 30% however, there was considerable regional variation (9% - 49%) (Whelton et al., 2006). The difference in prevalence of tooth wear between the current study and the national survey (Whelton et al., 2006) could be accounted for with three possible explanations; (i) it is a true increase in the prevalence as the national survey was conducted in 2002 (Whelton et al., 2006). (ii) The index used in this research and that used in the national survey were not identical; therefore, the studies are not directly comparable. In the national survey the index for measurement counted only tooth wear on the upper anterior teeth. (iii) The current sample was approximately 12 months older; which may or may not have an effect. The last Irish national adult oral health survey measured tooth wear using the same index as that used in the children's national survey. In the adult survey the prevalence of tooth wear in the 16 to 24-year-old age was higher at 44% (Whelton et al., 2007). The results of the Irish national surveys also demonstrated that tooth wear levels recorded increased in magnitude from the 12-year-old age group right up to the 65^{+} age group (Whelton et al., 2006; Whelton et al., 2007). Kreulen et al. (2010) published a systematic review on the prevalence of tooth wear in children and adolescents. They were unable to make a statement on tooth wear in the permanent dentition for subjects up to age 18. Van't Spijker et al. (2009) did conclude that tooth wear increased with age in adults. In addition, they identified that the prevalence in males was higher, but reasons have not been identified.

A number of epidemiological studies of tooth wear and dental erosion (Al-Dlaigan et al., 2001b; Bardolia et al., 2010; Bardsley et al., 2004; Hasselkvist et al., 2010; Milosevic et al., 1997) have shown that there is a higher prevalence of tooth wear in teenage and adult males than in females. This result was also recorded in the systematic review conducted for tooth wear in adult populations (Van't Spijker et al., 2009). The reasons for this difference are still being discussed. In the current study, the prevalence of tooth wear was similar for both males and females at the subject level. Multivariate statistical analysis though indicated that the male participants had a higher percentage of surfaces affected by tooth wear than female participants did, ($p<0.001$).

5.2.2 Measurement in the longitudinal study

In the longitudinal study, no individual had a tooth wear score that involved either secondary dentine or pulp exposure. The highest score descriptor was, dentine loss affecting $^{2}/_{3}$rds or more of the scored surface. In the longitudinal study, children were examined at age 12 and again at age 14. Between the ages of 12- and 14-years, the dentition is transitioning from primary to permanent, and not all of the permanent successors have yet erupted. In addition, it is the period when children undergo orthodontic treatment, and orthodontic bands or brackets may obscure surfaces. For this reason, some buccal/labial and palatal/lingual surfaces had to be excluded from the examination. This meant that some surfaces were assigned a score ‘R’ and excluded from measurement. At age 14 then tooth wear was recorded on some teeth such as the premolars for the first time. The prevalence of tooth wear increased between the two time points, the difference was not the incidence as some children were not present for both time points. An incremental change between tp1 (age 12) and tp2 (age 14 years) was also calculated in the research. Only teeth present at both time points were included for the calculation of an increment. Both the modified TWI (Bardsley et al., 2004) and the ETI (Bartlett et al., 2011b) were used in the longitudinal study to measure tooth wear. The ETI (Bartlett et al., 2011b) was selected in an attempt to improve the sensitivity of the measurement, and to record tooth wear in enamel and dentine in more detailed levels. For the analyses of tooth wear outcomes in the longitudinal study, the ETI (Bartlett et al., 2011b) scores were combined to provide singular information on tooth wear for enamel wear and then dentine wear. The size of the sample meant that combining the scores in this manner provided more meaningful numbers for analysis, and at the same time allowed enamel and dentine to be analysed separately. The prevalence of tooth wear measured at each time point was similar for both the modified TWI (Bardsley et al., 2004) and the ETI (Bartlett et al., 2011b) when the proportions with dentine visible were compared. The ability to combine the scores within the ETI (Bartlett et al., 2011b) permits comparison with other indices derived from the TWI (Smith and Knight, 1984), according to age group and the surfaces and teeth on which tooth wear was recorded. The ability to combine scores and to make comparisons is a distinct advantage of the index. To determine the increment between the two time points demanded much thought. The loss of tooth surface between two time points discernible to the human eye is difficult. This is evident too in the comments of Dugmore and Rock (2003) and El Aidi et al., (2010) when they mention in their papers the difficulty on managing reversals when they examined teenagers at two time points. The human eye can see to approximately 40 µm without magnification. The average loss of tooth surface per year calculated on dental casts can be as little as 15µm (Rodriguez et al., 2012) or 30 to 40 µm (Lambrechts et al., 1989). Certainly, there must be further research around the ability to measure change over time in epidemiological studies of tooth wear. Not forgetting that tooth wear occurs as part of the ageing process, somewhat akin to wrinkles on the skin. Recording small changes is difficult; a surface can be naturally smooth and shiny without tooth wear or be smooth and shiny because of tooth wear (Figure 5-1). Genetic variation exists too in tooth anatomy; well-defined mamelons may remain on some teeth into early adulthood or are quickly worn away on others. The first step to determine an incremental change using the ETI (Bartlett et al., 2011b) was to manage the scores in a continuous ordinal manner from 0 to 4 for the scores in enamel followed by the values from 5 to 9 for the scores in dentine. The research to establish an incremental score and progression will be developed further using the data that is now available for the cohort at age 16-years (tp3), thus analysis will be performed for the time points, tp1, tp2 and tp3 in the permanent dentition. At age 12 and at age 14 there were no positive scores for premolar teeth or the buccal-cervical region, despite enamel being at its thinnest in the buccal-cervical area, and the site of wear in adults (Wood et al., 2008) Further investigation of an effective means of calculating incremental change, progression and relative risk (RR) will be important future work.

From the evidence in this research, we can conclude that at age 12 and 14 years a partial index to record tooth wear is acceptable. Hasselkvist et al., (2010) used the maxillary anterior teeth on the labial and palatal surfaces, and the occlusal surface of the first permanent molars and concluded similarly, although that study referred only to dental erosion. The planned future analysis of the data will also inform whether partial recording at age 16-years, captures all of the necessary information, or whether examination of the premolar teeth provides additional information, or at what stage tooth wear becomes apparent on the premolars and the buccal-cervical site.

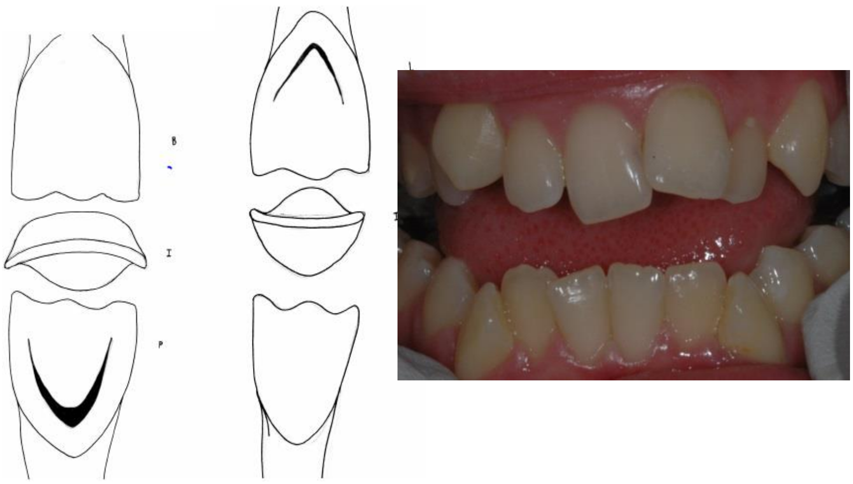


Figure 5-1: Sketch demonstrates mamelons present and the image displays varying levels of enamel wear, with mamelons present / absent on permanent incisors

> ***Important point 1*: When recording tooth wear in the permanent dentition at age 12 or 14, partial mouth recording is appropriate for epidemiological purposes. The premolar teeth do not provide additional information**

5.2.3 *Measurement consistency of examination in both the cross sectional study and the longitudinal study*

When conducting research, consistency in measurement is essential to minimise systematic error. Prior to the initiation of the cross sectional study and at each stage in the longitudinal study systematic error and drift were minimised by means of training and calibration. Re-examination of some subjects took place throughout all studies and both an inter- and intra-examiner Kappa statistic were calculated. These duplicate exams were not counted in the prevalence of the condition. The calculated Kappa statistics during the studies ranged from 1 to 0.7, values that are accepted as substantial agreement (Landis and Koch, 1977).

5.3 Study design

In this research, two study types were selected to address the aim of the research. In the cross sectional study, data on the outcome and the possible explanatory factors were collected simultaneously. This type of study is often referred to as a point prevalence study; a snap shot at a particular time. Establishing the prevalence in the sample allowed for comparison with other studies. The availability of prevalence data along with statistical analyses can suggest associations between the cumulative condition of tooth wear and explanatory variables. The statistical analyses conducted allowed for the calculation of odds ratios (OR). The odds ratios permitted inferences to be drawn from those exposed or not to the variables of interest.

The sample in the longitudinal study was originally selected from 5-year-olds who agreed to future contact by the researcher. A longitudinal study contributes to what we can infer about a population, instead of explanatory variables and outcome variables being collected only at one time data are collected at several time points that allow associations to be identified longitudinally. For example in the current research the consistent association at age 14- and 12-years with tooth wear at age 5.

In the current research the proportion with tooth wear was determined at each time point. Using statistical analyses, the OR was calculated for those exposed or not to the explanatory variables collected at age 5-, 12- and 14-years and the tooth wear outcome measure. The odds ratios (OR) were calculated for the longitudinal study and provide an estimation of relative risk (RR). A ratio of means was calculated based on the increment between tp1 and tp2. A strength of the current research that data are now available for the cohort at age 16. This will allow future analysis of the increment between tp1 and tp2 and tp3 at age 16 and the generation of further hypotheses.

5.3.1 Questionnaire responses

The collective term tooth wear was used in this research. The use of the collective term meant that explanatory variables for the aetiologies of, erosion, abrasion and attrition were included. Accordingly, a large number of possible explanatory variables were included in the statistical models that were used in both the cross sectional and the longitudinal study. The variables that were significant in bivariate analysis were included in the different models. In addition, in the longitudinal study, variables were selected based not only on the questionnaire responses at age 14 but also at tp0, and tp1. At age 14-years (tp2), the outcome variables selected were to reflect the associations that different explanatory variables could have on both different tooth surfaces and the different tooth tissues over time.

The information on oral hygiene practices, dietary practices, health behaviours and knowledge of the condition was collected via a self-completed questionnaire in both the cross sectional and the longitudinal study. Survey questionnaires are often criticised as a source of bias, where individuals may incorrectly recall what they did, or provide the answer that they feel the researcher would favour. Despite this self-completed questionnaires, are regularly used in large epidemiological studies. An e-questionnaire with direct data entry was designed for the study (Harding et al., 2006). Where appropriate the responses to the questionnaires from the cross sectional and longitudinal study are discussed together.

5.3.1.1 Oral hygiene habits

In the cross sectional study, 65% of the 16-year-olds claimed to brush their teeth twice a day, which was similar to that reported for 15-year-olds in the last Irish national survey of children's oral health (Whelton et al., 2006). The frequency of tooth brushing was not associated with tooth wear, which differed from the results in the national survey (Whelton et al., 2006). In the national survey participants who brushed once a day or less at age 15-years had more tooth wear present than those brushing more frequently (Whelton et al., 2006). The observation that there was no difference in the cross sectional study may have occurred because of differences in the sample selected or because of a type II error. When the actual responses were reported (Table 4-19), there were two trends, there was an increased prevalence in mild wear with increased frequency of tooth brushing and at the same time a decreased prevalence of moderate tooth wear with increased frequency of tooth brushing. In the Irish national survey the sample size for 15-year-olds was 3,535 (Whelton et al., 2006). In the longitudinal study, tooth-brushing habits were associated with a number of the selected tooth wear variables. Participants however, who brushed twice a day and more at age 14 had a lower number of buccal surfaces affected, they also had a lower mean increment between tp1 (age 12) and tp2 (age 14) than participants brushing once a day and less. It would be ideal if this oral health message to brush twice each day to reduce tooth wear could be supported. That message is consistent with the oral health message provided in caries prevention, which is still most important in the teenage population (Levine and Stillman-Lowe, 2009), thus the same information being disseminated but to manage two different risks.

In both the cross sectional and longitudinal study the time at which brushing took place was associated with tooth wear. In the cross sectional study brushing after breakfast appeared to confer a benefit, where those who brushed after breakfast (77%) were more likely to have lower maximum tooth wear scores on the occlusal surface than those who did not brush after breakfast ($p<0.03$). In the longitudinal study, participants brushing after breakfast at age 12 had fewer teeth affected by tooth wear into dentine. At age 14 however, the converse was the result. The complexity of tooth wear and possibly the abrasivity of toothpastes should be considered in this respect. When the explanatory variable of interest was brushing before bed, the participants who brushed before bed at age 14 had a higher number of smooth surfaces affected. However, participants who brushed before bed at age 12 had a lower maximum increment between tp1 (age 12) and tp2 (age14) (Table 5-1). It is hard to explain the differences in these outcomes, however the tooth surface and the tooth tissue that is affected is again likely to be important. Two physiological factors should be considered, it may be that once the dentine is exposed the abrasivity of the toothpaste on the less mineralised dentine is more important than the calcium, fluoride and phosphate ions available in toothpaste. As 95% of the toothpastes sold in Ireland contain fluoride as a therapeutic agent, one could tentatively suggest that while enamel is present the availability of a constant low level of fluoride may confer some benefit, however once dentine is visible/exposed the abrasivity of the toothpaste exerts a greater effect. The dental hard tissues also differ between individuals, and enamel may be hypomineralised. The positive association indicated between tooth brushing after breakfast and having less tooth wear could be a consequence of recommended twice a day brushing. Brushing at night and again in the morning, permitting a constant low level of fluoride to remain within the oral environment and available to promote remineralisation. The second physiological factor to consider along with the availability of tooth mineral in saliva is the quality of the saliva and the protein content and concentration, which permits lubrication and buffering. This point will be expanded in the section on salivary parameters (section 5.4). Personal autonomy, locus of control, social norms and health beliefs around tooth brushing could all be associated with tooth brushing in the sample. Qualitative methods were not undertaken in this research and perhaps will need to be a feature of future research.

A cross sectional study with a population of 14-year-olds in the UK reported that brushing before bed was associated with higher levels of dental erosion (Al-Dlaigan et al., 2002b). The researchers speculated that it may have been the synergistic effect of erosion with the intake of acidic drinks and abrasion due to tooth brushing (Davis and Winter, 1980). A recent cross sectional study with an older age group found no association between the timing of tooth brushing and tooth wear on the buccal or lingual surfaces (Bartlett et al., 2013). Recommending that tooth brushing take place without suggesting a time delay would certainly fit better with the dental public health messages on caries prevention. All of the research that has suggested that brushing be delayed after an acidic challenge, such as breakfast is based on *in vitro* or *in situ* studies and case reports (Addy and Hunter, 2003; Lussi et al., 2014). If tooth brushing habits are to be delayed then perhaps that should be based on an individual clinical risk assessment, where salivary ion and protein characteristics are also considered. The studies reported in this thesis indicate the complexity of the associations with tooth brushing and tooth wear. The lack of consistency of results at the different longitudinal time points, in the cross sectional study and in a large national survey provide an important marker for those trying to interpret the published literature in this field. The results indicate variables that need to be considered in the planning of future studies designed to address the issue of the timing of tooth brushing.

Important fact 2: Advice on the timing of tooth brushing in relation to breakfast should be based on individual risk assessment. It is not possible to issue generic advice on the optimum time for tooth brushing to prevent tooth wear based on the evidence currently

Table 5-1: Summary table of tooth brushing habits and the association with tooth wear variables in this research and previous epidemiological research

Variable	Study	Age	Description	p-value
Brushing twice each day	Whelton et al, 2006	15	Had **less** tooth wear on the maxillary anterior teeth	$p<0.005$
	Current cross sectional	16	No association	
	Current longitudinal	14	**Less** teeth were affected on the smooth surfaces. The mean increment between tp1and tp2 was **smaller**.	$p<0.0001$, $p<0.04$
Brushing after breakfast	Bartlett et al, 2013	Adults 18-35	No association	
	Current cross sectional	16	Participants who brush after breakfast have **lower** tooth wear scores. And l**ower** first permanent molar scores	$p<0.03$, $p<0.02$
	Current longitudinal	14	There was **less** tooth wear for surfaces affected by tooth wear into dentine if participants reported brushing after breakfast **at age 12**. There was **more** tooth wear for surfaces affected by tooth wear into dentine if participants reported brushing after breakfast **at age 14**	$P<0.01$, $p<0.01$
Brushing before bed	Al-Dlaigan et al, 2002	14	Participants brushing before bed had **higher** levels of dental erosion	$p<0.05$
	Current longitudinal	14	Participants who brush before bed **at age 14** have **more** smooth surfaces affected	$p<0.02$
		14	Participants who brush before bed **at age 12** have a **lower** maximum increment score between tp1 and tp2.	$p<0.003$

5.3.1.2 Knowledge

The results of this research would suggest that knowledge dissemination was successful. In both studies students were asked 'what causes dental erosion?' Dental erosion was the term selected, because much of the literature and marketing information around tooth wear in the child and adolescent is referred to as dental erosion. The question was correctly answered by at least 80% of respondents at all of the time points for both samples. One would have to ask however, whether further research is necessary for the translation of knowledge into changed behaviours. Alternatively, perhaps, it is because of the multifactorial aetiology that a high prevalence of tooth wear exists. The associations identified in this research would suggest the latter and a need for a multifaceted approach to the prevention and management of tooth wear.

5.3.1.3 Dietary habits and practices

5.3.1.3.1 Fizzy drinks and readymade fruit drinks

Carbonated drinks also referred to as fizzy drinks contribute to a high empty calorie intake and pose a health concern to both oral health and to general health. In this research, the acidic nature of these drinks and their association with the aetiology of erosive tooth wear was the focus, rather than the effect on either Body Mass Index (BMI) or dental caries. Readymade fruit drinks are also acidic, but are often over looked as such. Participants in the cross sectional study were asked, 'how often they consumed fizzy drinks and readymade fruit drinks'? Participants who consumed fizzy drinks, more than once a week (53%) were more likely to have tooth wear than those who consumed them less frequently. This association existed for both the maximum tooth wear score and for the presence of tooth wear on the occlusal surface of first permanent molar teeth, ($p<0.03$) for both outcomes. The daily consumption of readymade fruit drinks was associated with more tooth wear on buccal surfaces ($p<0.05$). Similar associations with acidic beverages were identified previously in populations of a similar age (Bardolia et al., 2010; Jensdottir et al., 2004; Mulic et al., 2012. Given the effect of fizzy drinks on the dentition and on general health, a concerted effort to raise awareness of their general health and oral health impacts and to reduce their intake should be supported.

Important point 3: The consumption of fizzy drinks more than once each week and the consumption of readymade fruit drinks daily is associated with higher tooth wear levels in 16-year-olds

5.3.1.3.1 The manner in which fizzy or squash (acidic) drinks are consumed

Various suggestions are made to allow the consumption of acidic drinks, and at the same time minimise the effect on the dentition. A long contact time is more detrimental than moderate consumption of even highly acidic foods (Lussi et al., 2004). The recommended advice is, to reduce the frequency of consumption and to direct the flow of acidic drinks away from the dentition (Lussi and Ganss, 2014). Participants in the longitudinal study who typically used a can, bottle or straw rather than a glass or cup to consume acidic drinks were 22 times more likely to have tooth wear on smooth surfaces than those who typically used a glass or cup, ($p<0.01$). They also had a higher number of smooth surfaces affected by tooth wear, ($p<0.0001$). Drinking through a straw was associated with both the maximum increment score and the mean increment between tp1 (age 12) and tp2 (age 14). The implications of these findings are important to help guide specific independent oral health advice that is provided subsequent to a clinical assessment. Using a can, bottle or straw affects the duration for which acidic drink is in contact with the dental hard tissues and the direction of flow of the liquid. When a straw is recommended it should be wide bore (Shellis et al., 2005) and directed to the back of the mouth, to minimise contact time (Lussi and Ganss, 2008). In the questionnaire, respondents were asked to indicate how they typically consumed their ‘acidic drink’. It is reasonably possible that individuals consuming from/through a can bottle or straw were also consuming more or with an increased frequency than the participants who indicated that they used a cup or glass where consumption was associated with more formal dining or meal times. In the logistic regression, each variable was tested while controlling for all others. The discussion around amount and frequency is somewhat similar to the dialogue on the amount and the frequency of intake of sugars with respect to dental caries (Moynihan and Kelly, 2014).

5.3.1.3.2 Usual drink

In the cross sectional study 35% of participants responded that water was their usual drink and 9.4% chose milk as their typical drink. The fact that fewer than 50% of participants chose water or milk as their typical drink would suggest that these values should be monitored for the impact on general health and the value of the oral health message - to reduce the intake of sugary drinks and to replace sugary drinks with water (Safefood, 2014a). For 34% of respondents in the cross sectional study milk was available at their school. Whether the decision to have milk available was made in conjunction with general health policies was not ascertained. Consumer advocacy groups in the US were recently successful in having carbonated beverages removed from 'Kids' menus'. The thrust of the campaign was to remove the carbonated beverage so that meals taken outside the home were not automatically associated a carbonated beverage (Science in the public interest, 2015).

5.3.1.3.3 Eating citrus fruit

The responses to the questions on citrus fruit consumption in the cross sectional study are perhaps indicative of the importance of choosing appropriately from the 'food pyramid' or 'eat well plate' (Safefood, 2014b). Of significant note is the fact that the highest proportion of participants with moderate tooth wear were participants who responded that they never ate citrus fruits. Again, in support of healthy food choices, the participants in the longitudinal study, who indicated that they ate citrus fruit once a day or more at age 14, they had a lower maximum increment score between tp1 (age 12) and tp2 (age 14), than participants who ate citrus fruit less often ($p<0.007$). Research with older populations has implicated citrus fruits in erosive tooth wear. Jarvinen et al., (1991), however indicated that the increased risk was associated with citrus fruits eaten more than twice each day. In the current research, the proportion of the 14-year-olds and 16-year-olds eating citrus fruits more than twice each day was small.

5.3.1.3.4 Eating apples

In both the cross sectional and the longitudinal study, participants were asked to choose the frequency with which they ate apples. Participants' eating apples daily or more than once a week (50%) in the cross sectional study were more likely to have less wear. They had a smaller number of smooth surfaces affected and the proportion of teeth affected was less than the participants eating apples less frequently, ($p<0.002$) and ($p<0.04$) respectively. This result is the opposite of the results of the research presented by (Bartlett et al., 2011a) where eating apples was associated with a high odds ratio for tooth wear with dentine visible. Bartlett et al., (2011a) however referred to an older population; therefore, to make a direct comparison may not be appropriate. The results from the longitudinal study also indicated a favourable correlation, at age 14 with eating apples. The frequency of eating apples; 'once a day or more' was associated with a smaller number of smooth surfaces affected and with less teeth affected. Conversely but similar to the research of (Bartlett et al., 2011a), the consumption of apples 'once a day or more' at age 12 was associated with a high OR; 4.51, $p<0.03$ and similar to (Bartlett et al., 2011a), the outcome was tooth wear with dentine visible. In the longitudinal study both the maximum increment score and the mean increment between tp1 (age 12) and tp2 (age14) provided conflicting associations. The participants who responded that they ate apples once a day and more at age 12 had a higher maximum increment ($p<0.01$) and the participants at age 14 who indicated they ate apples once a day and more had a lower mean increment ($p<0.02$), (Table 5-2). Further research examining the impact of various food types on dental enamel and dentine both *in vivo* and *in situ,* along with the dietary choices made by 12-year-olds and 14-year-olds, is necessary. The differences between maximum increment score and the mean increment between tp1 (age12) and tp2 (age14) suggest further research in the area of progression. Dentine is less mineralised and more acid soluble, as previously mentioned. In addition smaller crystals in dentine, provide a greater surface area over which acid dissolution can occur (Lussi and Ganss, 2014). Therefore, once dentine is exposed the manner in which dietary factors interact with the dental hard tissues may alter. Apples contain malic acid (pH 3.4) and malic acid has a high titrateable acidity (72 mmol OH^{-} l^{-1}), (Lussi and Ganss, 2014). This may contribute to the loss of tooth mineral when tooth wear into dentine has occurred. The responses provided to the questions on the consumption of citrus fruits and apples emphasise the importance of considering the totality of the diet. A means of ranking the acidity of the diet for future research and providing a summary score must be considered. A diet diary may be a means to better collect information on dietary choices and habits in a longitudinal study, and may be more appropriate in an effort to measure associations with incremental wear.

Table 5-2: Summary of citrus fruit and apple consumption and the associations with the tooth wear variables

Variable	Age	Description	p-value
Eating apples once a day and more	16	Participants who indicated that they ate apples once a day or more had a **lower** number of smooth surfaces affected	p<0.002
	16	Participants who indicated that they ate apples once a day or more had a **lower** percentage of smooth surfaces affected	p<0.04
	14	Participants in the longitudinal study who indicated that they ate apples once a day or more **at age 12** were **more** likely to have tooth wear with dentine visible at age 14	p<0.03
	14	Participants in the longitudinal study who indicated that they ate apples once a day or more **at age 14** had a **lower** number of smooth surfaces affected	p<0.02
	14	Participants in the longitudinal study who indicated that they ate apples once a day or more **at age 14** had a **lower** mean increment between tp1 and tp2	p<0.02
	14	Participants in the longitudinal study who indicated that they ate apples once a day or more **at age 12** had a **higher** maximum increment score between tp1 and tp2	p<0.01
Eating citrus fruit once a day and more	14	Participants in the longitudinal study who indicated that they ate citrus fruit once a day or more **at age 14** had a **lower** maximum increment score between tp1 and tp2	p<0.007

***Important point 4*:** The design of future studies to investigate risks associated with dietary choices, tooth brushing habits and, tooth wear particularly tooth wear increment should consider a summary score for the explanatory variables and/or the use diet diaries throughout the study period, to capture the consistency of habits and practices

5.3.1.3.5 Consuming cider

Despite the fact that the legal age for the purchase of alcohol in Ireland is 18, adolescents of younger years experiment with alcohol as reported in the Irish health behaviours in school age children (Kelly et al., 2012). Cider is an alcoholic beverage made from fermented apple juice, which makes it palatable as a first alcoholic drink. It contains malic acid (pH 3.4) titrateable acidity (72 mmol OH^{-} l^{-1}), (Jolicoeur, 2011; Lussi and Ganss, 2014). Twenty two per cent of the participants in the cross sectional study indicated that they consumed cider albeit infrequently. Respondents who consumed cider had more teeth affected than the non-drinkers did ($p<0.0001$). Al-Dlaigan et al., (2001a) reported an alcohol consumption of 23% in their study of dental erosion involving 14-year-old schoolchildren in Birmingham UK. They demonstrated an association between dental erosion on the buccal and lingual surfaces and the frequency and the consumption of beer. The association with cider identified in this cross sectional study does not signify causation, but may be indicative of choices that are made around future habits, lifestyles and behaviours. The fact that the consumption of alcohol was associated with more teeth affected could be problematic if restorative management of the condition is necessary.

5.3.1.3.6 Consuming alcopops

In the sample 20% of participants had consumed alcopops, results of the logistic regression for 'the presence of any tooth wear', indicated that participants consuming alcopops were more likely to have tooth wear; OR 3.24, ($p<0.02$). El Aidi et al., (2011) identified a positive association between alcoholic mixed drinks and the incidence of tooth wear in a population in the Netherlands for a population first examined at a mean age of 11.9 years and then again after three years. The association of alcopops in the present research with tooth wear presents similar concerns to those already expressed with respect to the consumption of cider.

Dental professionals have an important role in smoking cessation (Health Development Agency, 2004) because of the harmful effects of tobacco on both general health and oral health (Oral Health Services Research Centre, 2014). The association between alcohol and tooth wear identified in this study and the known detrimental effects of alcohol to both oral health and general health provides the opportunity for the dental professional to enquire specifically around alcohol consumption (Oral Health Services Research Centre, 2014). This provides a further purpose to develop client centred dental practice and oral health as integral to general health.

> ***Important point 5:*** Dental health professionals should be recognised as having a valuable role in promoting, healthy eating habits and alcohol consumption along with a role in smoking cessation

5.3.1.4 Health behaviours and lifestyles

5.3.1.4.1 Exercise

In the cross sectional study 46% of students reported that they were engaged in exercise either 'daily' or 'three or four times each week', the frequency of engaging in exercise was associated with tooth wear, possibly suggesting a complex interaction between factors. These factors could include dehydration during exercise, the methods used to maintain hydration during exercise, or rehydration after exercise, dietary choices, supplements and the quality and quantity of saliva. Identifying an association between the frequency of exercise and tooth wear in an epidemiological study in teenagers is worthy of further investigation. As previously suggested in the discussion of dietary choices, future research should be in the development of a composite summary score composed of all elements that could contribute to tooth wear.

5.3.1.4.2 Dry mouth

Leading on from the possible indications above of why exercise could be associated with tooth wear, are the important associations of tooth wear with a perception of a dry mouth. In both the cross sectional and the longitudinal study at age 14 participants were asked about the perception of a dry mouth - *'Do you often suffer from a dry mouth?'*. Saliva, both unstimulated and stimulated were collected in the cross sectional study and analysis indicated that flow rates were not associated with tooth wear. In addition, the flow rates were within the accepted range of normal. This was perhaps to be expected in healthy school going children. However, when asked whether they had a dry mouth, participants in the cross sectional study who responded 'yes' had more buccal surfaces affected than participants who did not, (p<0.03). Similarly in the longitudinal study at age 14, participants who reported having a dry mouth also had more buccal surfaces affected than participants who did not, (p<0.006). In addition the mean increment between tp1 and tp2 was higher in participants who reported a dry mouth (p<0.04). A recently published large multi-centre study with adults conducted by general dental practitioners in the US indicated that along with patient age, gender and the number of teeth the perception of dry mouth was associated with the practitioners' judgement of tooth wear (Ramsay et al., 2015). Dsamou et al., (2012) also associated oral dryness with an increased prevalence of dental erosion in children 12 to 14-years of age.

5.3.1.4.3 Vitamins

In the longitudinal study, participants who reported taking vitamins had less tooth wear. Taking vitamins appeared to confer a benefit; this may be associated with other explanatory variables not expressed in the study. Other studies the use of vitamins in particular, vitamin C has been associated with an increase in tooth erosion (Li et al., 2012; Taji and Seow, 2010).

5.3.1.5 Concluding remarks on the questionnaire responses

The complicated multifactorial aetiology of tooth wear can be appreciated, from the list of explanatory variables and associations identified. The results suggest that the explanatory variables influence differently on the buccal/labial, palatal/lingual, occlusal or incisal surfaces and on the dental hard tissue whether it is enamel or dentine. When one reflects that tooth wear is a collective term used to describe the aetiologies of abrasion, attrition and erosion, the relevance to dental public health can be appreciated. None of the aetiologies exist in isolation and aetiologies are synergistic. Important associations identified such as the perception of a dry mouth in teenagers, the association with fruit consumption, the manner in which acidic drinks are consumed and exercise are significant to public health. A number of the associations identified with tooth wear in this research were identified in previous research conducted with participants of a similar age for dental erosion (Al-Dlaigan et al., 2002b; El Aidi et al., 2011; Hamasha et al., 2013; Hasselkvist et al., 2010). A real strength of the current research is that similar associations for tooth wear were identified in different populations and over the life course. The results are indicative that a focus on the development of a composite summary score and/or diaries would serve the advancement of knowledge. An holistic approach to oral health, cognisant of and informed by outcomes from the wider health agenda should be advocated.

5.3.2 The sample – demographic factors

5.3.2.1 Exposure to water fluoridation

Exploration of the impact of water fluoridation on tooth wear was one of the study objectives. The plan was to measure differences in the prevalence of tooth wear between lifetime residents of fluoridated and non-fluoridated areas. Despite extensive groundwork in preparation for the study, it was very difficult to recruit the target sample size who satisfied the criteria for the pure non-fluoridated group. Post primary schools with a transition year that were non-fluoridated were more difficult to recruit than anticipated. Children who attended small primary schools designated as non-fluoridated often went on to large urban fluoridated post primary schools. A further challenge encountered was that, where post primary schools were located in non-fluoridated areas they were often small and did not offer the students the option of a transition year. Future efforts to answer the research question, whether tooth wear levels between 16-year-old students living in fluoridated areas and those living in non-fluoridated areas, may necessitate a different approach. The sample size could be increased by including the measurement of tooth wear as part of a larger multi-site study. Another option would be to consider analysing the data with respect to the proportion of the lifetime exposed to water fluoridation rather than a dichotomy of a full life exposed to a fluoridated public piped water supply, or not exposed to a fluoridated public piped water supply. The latter would permit the data from subjects who moved between fluoridated and non-fluoridated areas to be analysed. Mullen et al., (2012) however, observed that this method did not provide much more information when compared to the simpler estimated fluoridation status method for caries experience up to the age of 16-years. The fluoridation status of the participant was included as an explanatory variable in the multivariable regression and was not identified as a significant variable.

In the longitudinal study, an individual's fluoridation status was also included as an explanatory variable in the statistical models. The result indicated that participants who were always exposed to a fluoridated water supply had more tooth wear on the occlusal surfaces of first permanent molars than those not exposed to a fluoridated water supply at age 14. A possible suggestion for this outcome that will require further analysis is the proportion of first permanent molars that were excluded in the non-fluoridated group. The study protocol indicated that ‘along with other conditions when a dental restoration(s) occupy greater than a quarter of a specific surface area, that surface will not be scored and will be marked R’. The participants with a lifetime exposure to fluoridated water were the participants who had more first permanent molar teeth that could be scored for tooth wear. Further research may indicate that similar to the root caries index, the proportion of surfaces that can be scored will need to be stated in populations with water fluoridation (Katz, 1984).

Important point 6: Further research is required to determine whether the number of first permanent molars at risk needs to be reported in addition to the number of surfaces affected when making comparisons between populations with a fluoridated water supply and those without.

Bardsley et al., (2004) compared fluoridated and non-fluoridated groups and indicated there were significantly more cases of tooth wear in their fluoridated group when all surfaces examined were considered. However, when dentinal exposure on labial and palatal surfaces was analysed separately, there was significantly less wear on smooth surfaces in the group of children with a fluoridated water supply. It would not be unreasonable to hypothesise that both outcomes could be attributed to fluoride. The first due to a systemic effect and the possible presence of fluorosis and a hypomineralised enamel, the second a topical effect with a constant low level of fluoride present in the oral cavity. In the last Irish national oral health survey, no difference existed when the proportion with tooth wear on the anterior labial surfaces was compared by age and fluoridation status (Whelton et al., 2006), nor did a difference exist in the Irish adult survey (Burke et al., 2010), both of these studies had a large sample size. In Australia (Teo et al., 1997) in a small sample, further reduced with subdivisions identified that the proportion with exposure to water fluoridation from birth to 12-years and who had a low caries experience had less tooth wear in the maxillary molar sextants. It is perhaps of note that Teo et al.,

(1997) identified the difference in the maxillary molar sextants of low caries individuals.

The final sample size for the non-fluoridated group precluded a direct comparison between a fluoridated and non-fluoridated group. Future investigations will be informed by the knowledge acquired in undertaking the cross-sectional and longitudinal study in this research.

5.3.2.2 Socioeconomic status – medical card ownership

Inequalities are a feature of oral health, with a higher burden with poor oral health being frequently identified in those of low socioeconomic status. This inequality has identified for tooth wear also (Al-Dlaigan et al., 2001b; Bardsley et al., 2004; El Aidi et al., 2011; Van Rijkom et al., 2002) but the opposite direction has also been reported (El Karim et al., 2007). The current studies failed to identify a difference even though a difference may have existed. In the longitudinal study when the participants were aged 5 a higher proportion of the children where the parents had a medical card had tooth wear (Harding et al., 2003). Medical card ownership was not identified as a risk indicator in the longitudinal study at tp1 or tp2. This may have been attributed to the higher proportion in the sample at tp2, who did not indicate whether or not they had a medical card. In Ireland entitlement to a medical card is means tested; therefore in the cross sectional study possession of a medical card was used as a dichotomous surrogate measure of low socioeconomic status. The proportion of the sample in possession of a medical card in the cross sectional study was 17%. This proportion was lower than in the general population. When the study was conducted the proportion nationally in possession of a medical card was 29% (Department of Health, 2013). The difference between the two values may be a true difference or a consequence of the manner in which the question on medical card ownership was phrased. The data collected was based on the question 'Does your family have a medical card?' It is now understood by the researchers that at 16-years of age the question may have been ambiguous. Sixteen-year-olds who are assessed on their family's income, can apply for their own medical card. At the time of the study the proportion of 16-year-olds nationally who were reported to hold a medical card was approximately 19% (Department of Health, 2013). In future research careful consideration and piloting of questions around medical card ownership will take place, to eliminate ambiguity and the impact that the recent years of austerity may have had on ownership of a medical card as a measure of low SES.

5.3.2.3 The longitudinal study - previous tooth wear scores as explanatory variables

The value of a life course approach to oral health and an integrated common risk factor approach (CRFA), (Watt and Sheiham, 2012) is clearly evident in the longitudinal study. The longitudinal study demonstrated that tooth wear present at age 14, was associated with tooth wear present at both age 12-years and age 5-years. Tooth wear present at age 12 was associated with tooth wear present at age 5 (Harding et al., 2010). At age 14 the participants who had erosive tooth wear into the dentine or pulp at 5 years of age were 1.46 times ($p<0.01$) more likely to have tooth wear on smooth surfaces. The erosive tooth wear at 5 years of age was also associated with the number of affected smooth surfaces ($p<0.0007$), tooth wear that had extended into dentine at age 14, ($p<0.02$) and the number of surfaces with tooth wear into dentine, ($p<0.0001$). The outcome '*count of the number of incisal surfaces with dentine exposed at age 12*' was associated with more surfaces affected at age 14, ($p<0.005$). At age 12 the participants who had erosive tooth wear into the dentine or pulp at 5 years of age were 5.06 times ($p<0.02$) more likely to have tooth wear on the occlusal surface of first permanent molar teeth (Harding et al., 2010), (Table 5-3).

Clinical data recorded at age 5 and age 12 that is associated with tooth wear at age 14 is significant from a dental public health perspective. It emphasises the value in examining each child from the earliest stage, rather than waiting until they present with problems.

Table 5-3: Summary of the associations when previous tooth wear as the explanatory variable

Variable	Age	Description	p-value
Erosive tooth wear into the dentine or pulp at age 5	12	**More** likely to have tooth wear on the occlusal surface of first permanent molar teeth at age 14	p<0.02
	14	**More** likely to have tooth wear on the smooth surfaces at age 14	p<0.004
	14	**More** likely to have tooth wear with dentine	p<0.02

Variable	Age	Description	p-value
		visible at age 14	
	14	**More** surfaces affected by tooth wear at age 14	p<0.0007
	14	**More** surfaces affected by tooth wear with dentine visible at age 14	p<0.0001
	14	A **higher** maximum increment between tp1 and tp2	p<0.004
Tooth wear on the incisal surface of incisors with dentine visible at age 12	14	**More** surfaces affected by tooth wear with dentine visible at age 14	p<0.02

As mentioned analysis of the cohort including data now available for the participants at age 16-years will commence shortly and may permit calculation of a relative risk value.

> ***Important fact 7:*** Tooth wear recorded at age 14-years is associated with tooth wear recorded at age 12-years and at age 5-years. Clinical assessments should include a measure for tooth wear at each examination

5.3.3 Possible sources of bias in the study types selected

In all research risk of bias must be recognised at the outset in the study design and in assessing the study on completion. The principle sources of bias to be considered are; selection, attrition, detection and reporting bias. In the cross sectional study efforts were made prior to commencing the study to minimise selection bias. The sample selected was a random, stratified sample of 16-year-olds in transition year. Selection bias was also considered at the completion of the study. In the cross sectional study the non fluoridated group would have been recruited from more rural locations. Thus in the study the proportion that were non fluoridated and from rural areas was less than anticipated. The proportion of participants in the study who had a medical card may have been less than the general population at the time of the study.

Both points could be sources of both selection bias and could affect the generalisability of the research. In the cross sectional study, the sole examiner (MH) was trained and calibrated prior to the study and carried out repeat examinations during the study to ensure reliability and to minimise drift. All clinical examinations were entered directly to a laptop and time stamped to ensure accuracy. At the time of the clinical examinations MH had no knowledge of how the participants had completed the various questions on, demographics (aside from the observable factors such as gender and age group), oral hygiene practices, dietary practices, health behaviours and lifestyles. To encourage honest responses MH developed and piloted an e-questionnaire with direct entry of the responses by the students, the students were asked to be as open and honest when answering each question (Harding et al., 2006). Entering the information directly to the laptop also reassured participants that others would not have easy access to their responses. These two measures ensured that detection bias was minimised. All findings and outcomes are reported minimising reporting bias.

The longitudinal study by the very nature of recruitment to the study is prone to selection and attrition bias. Attrition of the sample is evident, when participants were 5-years-old there were 168 that could be examined at age 12, and of the 123 examined, the number examined at age 14 had diminished to 83. The characteristics of the participants that remained in the study did not differ from the original sample. When the 5-year-olds were recruited, we were unsure whether a longitudinal study could be considered, and asked whether they would be prepared to be contacted in the future. This could alleviate selection bias, as a long term commitment was not immediately demanded. The same means of minimising detection bias were used in the in the longitudinal study as in the cross sectional study. The exception was that at tp0 the parent completed the questionnaire on paper and at tp2 the child completed the questionnaire on paper. To minimise the risk that the parent/guardian or participant would respond as they thought the examiner would like, they were assured that there was no right or wrong answer. They were encouraged to be as honest and open and to report what the habits/practices usually were, as that would be of great value to the research.

5.4 Salivary parameters

The ability of saliva to lubricate, dilute, transport, act as a buffer and an ion reservoir are important functions in the management of tooth wear.

5.4.1 Flow rate

In the study, the flow rate of both stimulated and unstimulated saliva was determined for all participants who provided a sample of saliva. Participants were then grouped according to the tooth wear outcome measure – 'none', 'mild' or 'moderate'. Both the unstimulated and stimulated mean salivary flow rates were within the accepted ranges of normal. This was different to Zwier et al., (2013), who collected saliva from individuals of a similar age and noted, individuals with erosive tooth wear had a lower unstimulated flow rate. A gender difference was however present for the mean stimulated salivary flow rate of subjects with mild and moderate tooth wear; males had a higher stimulated salivary flow rate. Crossner (1983) previously identified that males had a higher flow rates than females, and that as far as the flow rate was concerned the salivary glands, it seemed, were fully developed at the age of 15 years. There is an opportunity for further research with this aspect of salivary research. It could hypothesised that even though the mean stimulated flow rate was higher in males than females, and that buffering capacity increases with flow rate, it is the unstimulated saliva that is of the utmost importance, as it is present in the mouth for approximately 14 hours per day (Edgar et al., 2013). Buffering capacity was not determined in the current research. Bringing together these points and the fact that the participants who indicated that they had a 'dry mouth' had more tooth wear, adds to the complexity of saliva as a fluid. There is still a body of research to be undertaken with respect to saliva and tooth wear.

5.4.2 Salivary protein and carbonyl concentration

Tooth wear is referred to as a condition rather than a disease, however, the rationale in conducting the exploratory element with salivary proteins and carbonyl concentration was to investigate whether oxidative stress of salivary proteins was associated with tooth wear. The oxidative stress of salivary proteins could lead to protein damage and impaired function. Proteins in saliva function to ensure the availability of calcium and phosphate in solution, lubrication and buffering ability

(Edgar et al., 2013; Lamanda et al., 2007), and therefore the presence of carbonylated proteins could mean altered function and an increased susceptibility to tooth wear or an altered feeling of comfort within the oral cavity.

To determine oxidative stress, the protein carbonyl concentration in both unstimulated and stimulated saliva was measured. The measured mean protein carbonyl concentration in unstimulated saliva was significantly higher for the group with moderate tooth wear than those with no tooth wear. For stimulated saliva, mean total protein concentration was lower and the mean ratio of protein carbonyl to mean total protein concentration was higher for participants with moderate tooth wear. The quantitative differences observed in the protein concentrations could indicate a primary aetiological factor for tooth wear. It could also be an early indicator for underlying health conditions not yet identified. Researchers have identified associations between oxidative stress and chronic diseases including periodontal disease (Battino et al., 1999; Dalle-Donne et al., 2003a; Dalle-Donne et al., 2003b; Dalle-Donne et al., 2006). Research has also indicated that qualitative or quantitative alterations in salivary protein concentration can affect the acquired pellicle, lubrication and buffering capacity. The alterations in the total protein concentration identified in this research could suggest a reduced lubricant effect and contribution to buffering capacity (albeit small) from salivary proteins. Both of these effects could have an impact on the collective aetiologies of abrasion, attrition and erosion (Amaechi et al., 1999; Carpenter, 2013; Carpenter et al., 2014; Regano et al., 2008; Edgar et al., 2013). Zwier et al., (2013) however failed to demonstrate a difference in protein concentration in 16-year-olds with and without dental erosion (n=88, with erosion and (n=49, with no erosion).

5.4.3 Protein Identification – protein in participants with moderate tooth wear

A sub group (n=13) was selected for protein identification, to explore whether the protein profile in the saliva of participants with moderate tooth wear differed to the profile from participants with no tooth wear. The samples selected were based on (i) the carbonyl concentration in unstimulated saliva and (ii) the presence of either moderate or no tooth wear. In the subgroup selected that had moderate tooth wear, the prepared 2-DE images had protein spots present in the 62-63 kDa molecular mass range. In MALDI, TOF MS/MS analysis the protein spots in the 62-63 kDa molecular mass range were 'L3' and were likely to be, Chain A, crystal structure of human Fcari bound to IgA1-Fc. The Fc receptor fragment of IgA may or may not have a direct role in the presence of moderate tooth wear. Whether or not the Fc receptor fragment of IgA has a direct role or not, remains to be investigated further. The research has demonstrated a method where differences in salivary proteins can be explored. Wines et al., (2006) discuss IgA receptors in health and disease and their ability to exclude a multitude of dietary, microbial and environmental antigens. They also consider that they are important in looking for primary markers for such conditions as Sjogren's syndrome, although this would be more applicable to older populations. Wines and Hogarth (2006) conclude that their presence may serve as risk indicators; this is an area for future research.

Identifying biomarkers or risk indicators has particular relevance when the link between oral health and general health is considered, because tooth wear, has been associated with general health conditions such as gastro-oesophageal disease, alcoholism and eating disorders (Bartlett et al., 1998; Dalle-Donne et al., 2006; Järvinen et al., 1988). In addition, the impact of salivary disease and xerostomia on the oral cavity is well recognised. Davies et al., (2014) demonstrated how the properties of the mucin barrier is influenced by dietary components, using the polyphenols in green tea as an example. Davies et al., (2014) suggest that these interactions may alter the function of MUC5B, which is a major contributor to the lubricating and viscoelastic properties of whole saliva. It would not be unreasonable to conclude that perhaps dietary components in the current research project could have introduced changes to the lubricating and viscoelastic properties of whole saliva, thus contributing to increased tooth wear and or a perception of dry mouth.

It is possible that other proteins other than the constant region of IgA were present at 62-63 kDa but were not identified. As the Fc receptor fragment of IgA demonstrated the best ionisation characteristics for detection.

Important fact 8: The investigation of salivary protein profiles and proteomics is essential to understanding the relationship between saliva and tooth wear

5.4.4 *Protein Identification – proteins present in participants with no tooth wear or moderate tooth wear*

In the samples sent for analysis, proteins were also identified at 15-17 kDa, in participants irrespective of their tooth wear status. The spots at 15-17 kDa were identified as a glycoprotein and prolactin inducible protein (PIP), and cystatins of two different types. Cystatins have an important role in maintaining a healthy oral environment – having antibacterial and antiviral roles, a role in binding calcium and maintaining mineral balance, and were possibly identified because of their abundance. Lamanda et al., (2007) identified that salivary proteins played an important role in salivary buffering capacity in the range of pH 3 to 5, and demonstrated that changes occurred between samples in 1-DE band intensities. Ghafouri et al., (2003) also identified salivary proteins in the same mass range as in this research using 2-DE and peptide mass finger printing. Ghafouri et al., (2003) went on to theorise that in their research the levels of prolactin inducible protein (PIP) in saliva might reflect activities in the neuroendocrine and neuroimmune systems and raised the possibility that salivary PIP may be a new biological stress marker. The fact that attrition (bruxism) is associated with stress (Manfredini and Lobbezoo, 2009), may also contribute to future interest in research of salivary proteins and tooth wear. The laboratory *Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa* were unable to identify the protein spots in the 104-106kDa range because of difficulties with the sample.

The proportion of sequencing identified in this research for the protein spots at 62-63kDa and 15-17 kDa through MALDI TOF MS/MS was considered reliable. Cost and equipment were limiting factors in pursuing the proteomics element at this time point. The results however, suggest that research in this area should be pursued, and the potential to identify markers of disease or conditions prior to their manifestation is possible. Research could also advance in establishing individual protein profiles around food preferences. Previous research has shown that in infants the salivary protein profile is linked to bitter taste acceptance (Dsamou et al., 2012; Morzel et al., 2013). Research linking the salivary protein profile for, sweet, sour, salt and umami but in particular sweet and sour could build knowledge around food preferences and the impact on oral health.

5.4.5 *Whole mouth unstimulated and stimulated mean salivary calcium and phosphate results*

Dental enamel does not exist in a static state and there is a constant exchange of the tooth minerals calcium and phosphate between the tooth surface, the enamel pellicle, plaque fluid and saliva. The mean concentration of calcium determined for unstimulated saliva was 0.99 (0.43) mmol l^{-1} and for phosphate 4.90 (1.46) mmol l^{-1}. The respective concentrations for stimulated saliva were 1.06 (0.35) mmol l^{-1} and 3.93 (1.41) mmol l^{-1}. All values were determined using an isocratic IC method (Dionex 1,000 (ThermoFisher Scientific)). The concentrations identified for calcium and phosphate compared favourably with the values compiled by Dawes in (Edgar et al., 2013) although the methods of determination differed (Table 5-4).

Table 5-4: Mean calcium and phosphate concentrations in unstimulated and stimulated saliva current research and values tabulated in (Edgar et al., 2013)

Mean calcium current research mmol l^{-1} (SD)	Mean calcium Dawes mmol l^{-1} (SD)	Mean phosphate current research mmol l^{-1} (SD)	Mean phosphate Dawes mmol l^{-1} (SD)
Unstimulated Saliva			
0.99 (0.43)	1.32 (0.24)	4.90 (1.46)	5.69 (1.91)
Stimulated Saliva			
1.06 (0.35)	1.47 (0.35)	3.93 (1.41)	2.70 (0.55)

The mean concentrations determined also followed the trend that the concentration of phosphate ion is higher in unstimulated than in stimulated saliva and the calcium ion concentration is lower in unstimulated than in stimulated saliva. Calcium and phosphate concentrations demonstrated an inverse relationship, similar to previous research (Kavanagh and Svehla, 1998). The ionic phosphate form determined in the current research was HPO_4^{2-} with an anionic column and carbonate/bicarbonate eluent at a pH of approximately eight. No difference in HPO_4^{2-} concentration existed between the groups with or without tooth wear. Dawes in (Khan and Young, 2011) indicated that phosphate exists in four different ionic forms – H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, and the proportions are pH dependent.

The calcium ion concentration was significantly different between the groups with or without tooth wear. The mean calcium ion concentration in unstimulated saliva was significantly higher for the group with tooth wear ($p<0.001$) (Table 5-5), reproduced from data in Table 4-17. An increase in calcium ion concentration was previously identified in a longitudinal study of 18 adults with occlusal wear (Carlsson et al., 1985). The explanation provided was, that the higher concentration in those with moderate scores was a consequence of an increased mucin content. It was hypothesised that the higher mucin content prevented the deposition of calcium to repair small enamel defects (Carlsson et al., 1985). Conversely, Jarvinen et al., (1991) indicated that salivary calcium and phosphorus levels were lower in dental erosion cases than in the controls. Here the suggestion was that it was related to factors such as a low unstimulated salivary flow rate, or a higher critical pH. Research with young children found no difference in the salivary concentration of calcium or phosphate between children with or without erosive tooth wear (Wiegand et al., 2006a). The concentration of phosphate was similar in both the group with moderate tooth wear and the group with no tooth wear. The next stage in the current research would be to ascertain whether the increased calcium concentration is associated with the increased protein carbonyl concentration. Research examining possible associations between calcium concentration and the enamel pellicle have commenced (Carpenter et al., 2014).

Table 5-5: The mean total fluoride, phosphate and calcium concentrations (mmol l^{-1}) in unstimulated whole saliva of selected participants with and without tooth wear

	n	Fluoride F^- mmol l^{-1} (SD)	Phosphate HPO_4^{2-} mmol l^{-1} (SD)	Calcium Ca^{2+} mmol l^{-1} (SD)
No Tooth wear	26	0.12 (0.06)	4.57 (1.5)	0.87 (0.39)
Tooth wear	19	0.53 (0.1)	5.4 (1.73)	1.26 (0.4)*

statistically significant result * $p<0.001$

At this stage of the discussion it is helpful to bring the findings with respect to saliva and tooth wear together (Table 5-6). The data suggest that the quality of saliva both organic and inorganic elements are important with respect to the presence of tooth wear.

Table 5-6: Tooth wear and association with salivary elements

Outcome	Positive association	p-value
Number of buccal surfaces with tooth wear Cross sectional study	Perception of a dry mouth	$p<0.03$
Moderate tooth wear subset of cross sectional study	Higher mean protein carbonyl concentration in unstimulated saliva	$p<0.0001$
Moderate tooth wear subset of cross sectional study	Lower mean protein concentration in stimulated saliva	$p<0.0001$
Moderate tooth wear subset of cross sectional study	Higher mean calcium concentration in unstimulated saliva	$p<0.002$
Number of buccal surfaces with tooth wear Longitudinal study (age 14)	Perception of a dry mouth	$p<0.006$
Higher mean increment between tp1 and tp2 (age 14)	Perception of a dry mouth	$p<0.04$

5.4.6 Whole mouth unstimulated and stimulated salivary fluoride results

All subjects were asked to avoid tooth brushing with fluoride toothpaste from 9pm the previous evening. To determine the total fluoride content for both stimulated and unstimulated saliva, the Dionex 1,000 (ThermoFisher Scientific) ion chromatograph and bicarbonate/carbonate eluent were used. Fluoride consistently eluted at a time of 2.3 minutes, when preparing the IC and during the analysis. In cariology, the fluoride ion specific electrode with TISAB or Taves method of diffusion (Frant and Ross, 1966; 1968; Taves, 1968a) are predominantly the choice to estimate the concentration of ionic fluoride (Martínez-Mier et al., 2011). The values identified for total fluoride concentration were 0.12 (0.06) mmol l^{-1} in unstimulated saliva and 0.07 (0.04) mmol l^{-1} in stimulated saliva. These calculated concentrations varied by a factor of 100 and 10 respectively from the results set out by Dawes in (Edgar et al., 2013) for the mean free fluoride ion concentration (Table 5-7).

Table 5-7: The mean total fluoride values from this research project and the mean free fluoride values Dawes in unstimulated and stimulated saliva

Unstimulated saliva		Stimulated saliva	
Mean total fluoride current research mmol l^{-1} (SD)	**fluoride** Dawes µmol l^{-1} (SD)	**Mean total fluoride** current research mmol l^{-1} (SD)	**Fluoride** Dawes µmol l^{-1} (SD)
0.12 (0.06)	1.37 (0.76)	0.07 (0.04)	1.16 (0.64)

No difference existed in the mean total fluoride concentration between the group with moderate tooth wear and the group with no tooth wear. Differences did exist with concentration reported by other researchers. Martínez-Mier et al., (2011) validated the ion specific electrode for fluoride determination using concentrations of fluoride in µmol ml^{-1}; evaluation of the findings suggest that validation was conducted at concentrations of 0.19 (0.08) µmol ml^{-1}, this concentration is higher by a factor of 100 to the frequently recorded values for whole mouth saliva (Featherstone, 1999). Naumova et al., (2012) used the ion specific electrode and established baseline salivary fluoride concentrations at 0.39 (± 0.35) ppm and 0.44 (0.42) ppm, which equates to 0.02 (0.02) mmol l^{-1} in the two study groups. Although there was no difference in the concentration at baseline for fluoride between the two groups, they were both higher by a factor of ten than the concentration provided by Edgar et al., (2013), or the normal value for whole mouth saliva (Featherstone, 1999). Abudiak et al., (2012) presented results of a similar magnitude to the current research project. When Abudiak et al., (2012) validated the ion chromatograph and the ion specific electrode to determine plaque fluoride concentration, they used a 1,000 ppm fluoride mouth rinse and went on to state that the ion specific electrode was less reliable at low fluoride concentrations (0.01–0.02 ppm). With the fluoride ion specific electrode, the electrode responds only to free ions. The fluoride ion specific electrode measures only free fluoride .The total fluoride concentration, Ct, may include some bound or complexed ions (Cb) as well as free ions (CF) whose concentration is determined as: C_F = Ct – Cb. In 1968 when Taves (1968) conducted his seminal research, he concluded that there were two forms of fluoride present in serum and that the ionic form was approximately $^{1}/_{10}{}^{th}$ of the total (Taves, 1966;

1968 a; b). The fluoride ion specific electrode only measures free fluoride. The above variations reported in salivary fluoride concentrations would suggest that the development of standardised methods to determine fluoride concentration must be continued.

5.5 Discussion of the Aim of this research

The aim of this research was to investigate the characteristics, development and determinants of tooth wear among Irish schoolchildren. In the cross sectional study, sixteen-year-old participants were selected for a number of reasons – the length of time that the permanent dentition is present in the oral cavity (Berkovitz et al., 2009) and a degree of freedom around food choices and activities, as they move towards adulthood (Kelly et al., 2012; World Health Organisation, 2015) . The cross sectional study indicated a higher prevalence of tooth wear than that identified for 15-year-old children in the last Irish national survey (Whelton et al., 2006), but a value similar to that for the 16–24 year-old age group in the Irish adult survey (Whelton et al., 2007).

The longitudinal study followed a cohort of 5-year-old children over a nine-year period. Despite a loss of subjects to follow up, the characteristics of those remaining in the cohort were similar to the participants who did not continue. The results provide valuable information on the life course, and the transitioning from the primary dentition to the permanent dentition. The fact that tooth wear at age 12 and 14 were associated with the explanatory variable *the presence of tooth wear on the maxillary incisors at age 5* has important implications for dental public health and health policy and the results achieved provide valuable data for future research initiatives.

Data are now available for the cohort at a third time point (tp3) at age 16-years. It will be important to determine whether previous tooth wear at age 5 remains an explanatory variable for tooth wear when the participants are 16-years-old. It will also be important to establish if tooth wear at age 12- and 14-years is associated with tooth wear at age 16-years. Weight is given to the results in this research through the identification of similar dietary associations in both the cross sectional and longitudinal studies. The perception of a dry mouth was an explanatory variable in both studies and there were habits and practices at age 12 that were expressed as explanatory variables at age 14.

The value of a complete clinical examination at age 5-years and the value of advocating healthy dietary choices and oral hygiene habits informed by evidence from the earliest opportunity ought to be appreciated. This is particularly important when one considers the majority of this generation have a long life ahead of them. The ultimate goal for oral health professionals should be ensure a healthy functioning dentition. What that means specifically to the individual will differ.

5.6 Null Hypothesis

The results with respect to the null hypotheses identified for this research are presented below.

The results of the cross sectional study allow rejection of the following hypotheses:

H_0 There is no difference in the salivary properties of 16-year-old students according to their levels of tooth wear.

H_0 There is no difference in tooth wear levels between teenagers who frequently consume acidic foodstuffs and those who consume them less often

The results of the cross sectional study do not allow rejection of the following hypotheses:

H_0 There is no difference in the tooth wear levels between 16-year-old students living in fluoridated areas and 16-year-old students living in non-fluoridated areas

H_0 There is no difference in the tooth wear levels between male and female 16-year-old students at the subject level

H_0 There is no difference in the tooth wear levels between 16-year-old students of different socio-economic groups

H_0 There is no difference in tooth wear levels between 16-year-old students who brush twice each day and 16-year-old students who brush less frequently

The following hypotheses cannot be accepted based on the results of the longitudinal study:

H_0 There is no association between the levels of tooth wear at age 14 and the oral hygiene practices of participants at age 12 or 14 years.

H_0 There is no association between the levels of tooth wear at age 14 and the dietary habits or lifestyle choices of participants at age 12 or 14.

H_0 There is no association between the levels of tooth wear at age 14 and the general health or medical conditions of participants at age 12 or 14.

H_0 There is no association between the levels of tooth wear at age 14 and the clinical outcomes of participants at age 5 or 12

H_0 There is no association between the maximum increment score for tooth wear between age 12 and 14 and demographic factors, oral hygiene practices, dietary habits or lifestyle choices, general health or medical conditions and the clinical outcomes of participants at age 5 or 12.

H_0 There is no association between the mean increment for tooth wear between age 12 and 14 and demographic factors, oral hygiene practices, dietary habits or lifestyle choices, general health or medical conditions and the clinical outcomes of participants at age 5 or 12.

The results of the longitudinal study do not allow rejection of the following hypotheses

H_0 There is no association between the levels of tooth wear at age 14 and the demographic factors of participants at age 5, 12 or 14 years at the subject level

Chapter 6 Concluding comment

6.1 Introduction

In conclusion the research project presented had a single aim, to investigate the characteristics, development and determinants of tooth wear among Irish school children. The research involved two different study designs and examined the associations between demographic factors, oral hygiene factors, dietary factors, general health and lifestyles with tooth wear. The inclusion of salivary factors and previous tooth wear in the primary dentition added significantly to the knowledge of the determinants of tooth wear. As with a lot of research that creates new knowledge, it also poses further questions to be addressed. As with the work presented here, future research will require an interdisciplinary approach with dentists, statisticians, chemists, biochemists and nutritionists. New research, based on this work has already commenced in chemistry and statistics, as will be discussed further in the chapter.

6.2 Indices

The issues in measuring tooth wear in children and teenagers in epidemiological studies have been discussed in the literature review and in the results. Specific challenges when trying to measure progression have also been explored (Huysmans et al., 2011). In addition to the difficulties posed by differences in the indices differences also exist between the primary and permanent dentition (Lussi and Ganss, 2014). Many of the studies in child and teenage populations present data for dental erosion or erosive tooth wear, rather than for the more inclusive condition of tooth wear. Reporting only on dental erosion means there is potential for dental erosion to be overestimated. If abrasion and attrition are not included then it is possible that risk factors for tooth wear including the perception of a 'dry mouth' in the child and teenage population will be ignored. Anthropologists have uncovered a wealth of information on diet from the dentition, but still refer to tooth wear (Forshaw, 2014). In the longitudinal study, two indices (Bardsley et al., 2004; Bartlett et al., 2011b) were used to record tooth wear in the permanent dentition at age 12 and 14-years. Between age 12- and 14-years the more detailed full mouth index (Bartlett et al., 2011b) provided additional information, the prevalence increased between the two time points. At age 16-years (tp3) the nuances of that increase will be teased out. Further analysis particularly of measurement between time points is required, particularly if the emphasis of treatment is prevention. The data that are now available for the cohort at age 16-years will permit further research with the ETI index (Bartlett et al., 2011b). Research focussed on the determination of incremental wear and the management of reversals over time is of high priority. It is a relatively new area of research in tooth wear in teenage populations. Reversals have been acknowledged in previous research but how they were managed in the analysis or why they were ignored was not documented (Dugmore and Rock, 2003; El Aidi et al., 2008). It is an area of research that can borrow from the challenges similarly experienced in both dental caries and periodontal disease (Ismail et al., 2011; Leroy et al., 2010).

6.3 Questionnaire responses

From the research it can be seen that added value could be gained with the development of a summary score for all of the factors both damaging to and protective of the dental hard tissues. An electronic diet diary may go some way towards assisting this need. It could provide more complete information on diet, nutrition, lifestyle and activities. With computer 'Apps' available for almost every health condition, such measurement could be facilitated. It is not that all factors can be eliminated but rather the aim is to identify and to manage the modifiable factors.

6.4 Statistical methods

The extensive data set contained within the longitudinal study is being interrogated by a statistician as part of a PhD research project. The aim of the statistical research is to develop a mathematical model, that will take account of longitudinal data, appreciating that there will be attrition of the sample while at the same time the number of explanatory variables will increase from one time point to the next. The statistical research question was formulated, based on the experiences of the current research.

6.5 Proteomics

The novel methods employed in this research used whole mouth saliva and were capable of identifying differences in the salivary protein profile between individuals with either moderate or no tooth wear. This type of applied research in oral health is at a relatively early stage. From this research it can be tentatively suggested that there are altered protein profiles between those with and those without tooth wear. The samples of saliva were matched to the subjects tooth wear score after the dental examination. At the time that the fieldwork was undertaken the researchers had no ability to match saliva properties and the tooth wear score. Clinical research to establish whether the same proteins are consistently identified in individuals with moderate tooth wear will be the next stage in this work. It would be of considerable value to future research to collectively consider the mean total calcium concentration, protein profile and the perception of a dry mouth and establish whether or how they could be interlinked.

6.6 Ion chromatography

The variations in results in the literature reviewed suggests that further research in the area of IC and measurement is required. Refinement and validation of a method for IC analysis of salivary ions is desirable. The advantage of ion chromatography being that a number of inorganic and organic ions can be determined simultaneously. A Masters student in analytical chemistry has examined methods to maximise the number of ions and weak organic acids that can be identified from saliva in a single run. A focus on refinement of the methods will increase the number of ions and weak organic acids that can be identified in saliva, and allow further exploration of their presence and function.

6.7 Closing comment

The many strands of this research were included to further the knowledge of tooth wear, particularly in the young permanent dentition and focussed on teenagers. The results presented and the breadth of null hypotheses rejected indicates the value of the research undertaken. The new research questions that have arisen from the results are aligned with the promotion of oral health and prevention. The analysis of the data at a third time point (tp3) for the longitudinal study with the participants aged 16-years will commence shortly. This further work will permit research with respect to measurement and analysis of the risk factors and indicators. The methods used to identify oxidative stress and salivary proteins have the potential to advance the knowledge of the salivary protein profile with respect to tooth wear. Whether the higher calcium concentration and protein carbonyl concentration in unstimulated saliva are linked remains to be answered. In addition to investigating these physical elements, there also exists the opportunity to research the association in both the cross sectional study that those who perceived they had a dry mouth had tooth wear on more surfaces. The opportunity for further clinical, epidemiological and laboratory collaboration on tooth wear is enormous. Although it was more than 100 years ago GV Black (1908), expressed it quite eloquently.

Our information regarding 'erosion' is still far from complete and much time may elapse before its investigation will give satisfactory results

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Appendices

All appendices (Appendix 1 – 10) associated with the thesis are contained within Volume II.