Title: Sunshine, Vitamin D and Oral Health

Submitted by Jane Elizabeth Collingwood to the University of Exeter

as a thesis for the degree of

Doctor of Philosophy in Medical Studies

In February 2021

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……J Collingwood……

Acknowledgments

I would like to thank my supervisors, Dr Ben Wheeler, Dr Nick Osborne and Dr Zoe Brookes, for their huge amount of help, support and guidance throughout all stages of this project. In particular I would like to thank Dr Wheeler for his endless patience as a teacher and mentor, even during a global pandemic.

My sincere thanks also goes to Professor David Moles for giving me the opportunity to undertake this project, and his support and guidance in helping to get this whole process started.

I am also grateful to my family for their continued encouragement and unwavering support throughout the entirety of my studies.

Abstract

Poor oral health, in particular dental caries and periodontal disease, is a common problem in the UK population. Previous research has suggested an association between vitamin D deficiency and increased risk of poor oral health. The main source of vitamin D for people resident in the UK is via exposure to solar irradiance. This study aims to determine if an association exists between sunshine exposure and better oral health, also specifically asking if increased exposure to UVB light between the wavelengths 290-315 nm approximately, from natural or artificial sources, affects the incidence of caries and periodontal disease. The findings of the study aim to inform novel public health measures to improve oral health.

Following a review of the existing literature and a scoping review of previous relevant studies, a protocol was developed and a systematic review undertaken. The findings supported the presence of a positive association, but highlighted the lack of good quality observational trials and cohort studies. The scoping review highlighted a number of ecological studies, which further supported the existence of an association, but were outdated and limited in their methodology. These studies inspired the empirical studies undertaken in this thesis which used secondary data from modern epidemiological studies to investigate the hypothesis further.

The findings of these studies suggested that increased exposure to sunshine is associated with a small, but consistent, reduction in the presence of dental caries in children in the UK. The evidence did not support an association

between sunshine hours and a reduction in the prevalence of periodontal disease.

Further research could focus on repeating these analyses using other datasets to see if relationships are replicated. Furthermore, the relationship between sunshine and other pertinent oral health outcomes such as oral cancer should be considered. Longer term studies could be undertaken using more accurate personal exposure measures and personalised health data, a viable possibility with the increasing use of wearables for data collection.

This research undertaken uses a novel approach to investigate the relationship between sunlight, UVB and oral health, and to inform future public guidance, policy and health interventions.

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The Role of the Student

Specific contributions of the author and student, Jane Collingwood (JC), in each chapter.

Chapter 1

A number of similar questions and hypothesis were conceived by JC and presented to supervisors NO, DM and BW for feedback . JC completed an initial literature review and scoping of potential databases for each question and presented data to supervisors on the strengths and weaknesses of each of the proposed thesis topics. This was collated into a formal PhD proposal by JC.

Once the PhD proposal was approved JC completed appropriate training and completed a formal literature review to provide background and context for the research. JC wrote up the key findings of the review in Chapter 1.

Chapter 2

JC completed a scoping review and wrote the systematic review protocol, the latter completed after attending a relevant training course. Feedback from NO, DM and BW was incorporated in to the final protocol which was uploaded by JC to PROSPERO systematic review database. JC recruited the second and third reviewers, and undertook all searches, analysis and presentation of the systematic review findings.

Chapter 3

JC downloaded data from the relevant databases, collated and formatted the data in excel software and uploaded them to statistical software for analysis. The author also completed training and used GIS software to complete the weighted interpolation used to calculate the exposure measures with specific support from BW.

Chapter 4 and Chapter 5

JC completed short courses on statistics and the use of statistical software following which she created the descriptive analysis and regression models investigating the relationships between exposure measures and oral health outcomes. Analyses were amended and finalised following discussions with supervisors.

Chapter 6

JC identified the use of Hill's criteria as a way to evaluate the existing and novel evidence discussed in the thesis and the nature of the relationships found. JC summarised the findings and limitations of the study and developed the ideas for future research .

Overall

JC drafted all text and created all figures and tables in the thesis, with the exception of Figure 1.1 which is adapted from another resource. Supervisors provided constructive criticism to inform re-drafting by JC through the development of the thesis. JC created the visual layout of the thesis and formatted the thesis according to U of E submission guidelines.

Presentations Arising from this Work.

1) Poster Presentation:

Vitamin D, Solar irradiation and Gingivitis. IADR 2014 Pan European Region Meeting (Dubrovnik, Croatia)

J Collingwood[1 2], BW Wheeler[2], D Moles[1], NJ Osborne[2 3].
1. Peninsula Dental School, Plymouth University, UK. 2. University of Exeter Medical School, UK. 3. UNSW, Australia.

2) Poster presentation:

Increased Exposure to UVB Radiation Via Sunshine Is Associated with Lower Prevalence and Severity of Dental Caries. Vitamin D Workshop 2018 (Barcelona, Spain)

J Collingwood[1 2], BW Wheeler[2], D Moles[1], NJ Osborne[2 3].
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Abbreviations

µg: microgram

1, 25 (OH) 2D: 1, 25-dihydroxyvitamin D, the active form of vitamin D

25 (OH) D: 25-hydroxyvitamin D

7-DHC: 7-dehydrocholesterol

ADHS: Adult Dental Health Survey

BASCD: British Association of Community Dentistry

BMI: Body Mass Index.

CDHS: Child Dental Health Survey

CYP27B1: Cytochrome P450 family 27 subfamily B member 1.

DBP: Vitamin D Binding Protein

DMFT: Decayed Missing Filled Permanent teeth

dmft: Decayed Missing Filled Primary teeth

FDI: World Dental Federation

FGF: Fibroblast Growth Factor

GIS: Geographic Information Systems

HPLC: High Performance Liquid Chromatography

IFN-y: Interferon Gamma

IL: Interleukin

IOM: Institute of Medicine

IU: International Units

J/m2: Joules per metre squared

L: Litre

LC-MS: Liquid Chromatography Mass Spectrometry

MED: Minimal Erythemal Dose

MEDMI: The Medical and Environmental Data Mash-up Infrastructure Project

MIH: Molar Incisal Hypomineralisation

mL: Millilitre

Ng: Nanogram

Nmol: Nanomole

OHS : Dental Epidemiology Programme for England, Oral Health Survey of Five-year-old Children

ONS: Office for National Statistics

PTH: Parathyroid Hormone

S-ECC: Severe Early Childhood Caries

TNF: Tumour Necrosis Factor

UK: United Kingdom

UVA: Ultraviolet A light

UVB: Ultraviolet B light

UVC: Ultraviolet C light

W/m2: Watt per square Metre

WHO: World Health Organisation

μW/cm 2: Microwatts per square Centimetre

Chapter 1 Vitamin D Physiology and Health: A review of the literature.

1.1 Vitamin D

1.1.1 The Discovery of Vitamin D

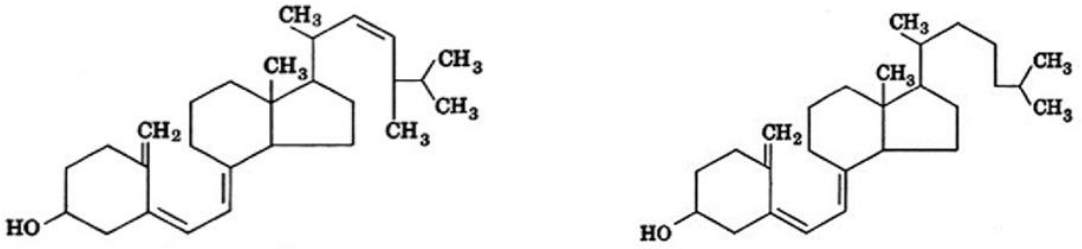
Vitamin D was first identified and named by Elmer McCollum, a scientist working at Johns Hopkins University, in the 1920's. Prior to McCollum's experiments Edward Mellanby, working in the UK, had described how rickets could be cured in dogs by feeding them a diet containing cod liver oil. He had attributed this effect to the vitamin A he knew to be present in the oil (Mellanby, 1919). McCollum however, found that even after destroying the vitamin A by bubbling oxygen through the oil, the dogs would still be cured if they ingested the vitamin A inactive oil. He attributed this effect to another unknown component, which he named vitamin D (McCollum *et al.*, 1922). Concurrently, it was noted that rickets could also be cured by exposure to sunlight (Hess, 1922), suggesting that vitamin D could also be produced by the body as well as ingested. The ability to cure rickets by both ingesting certain foods and by exposure to sunlight attracted the interest of Professor Steenbock, whose previous work had found that farm animals kept outside had better calcium metabolism than those kept inside. He conducted experiments exposing rats and their food to UV light and discovered that irradiating the rats could prevent rickets, but that irradiating their food also had the same effect (1924). He patented this process and when introduced to industry it had a major influence in reducing rickets in society. An old measure of vitamin D, Steenbock units, was named after him. In 1930 Askew was able to isolate vitamin D2 from a mixture of irradiated ergosterol (Askew *et al.*, 1930) and furthermore, in 1937 vitamin D3 was isolated in the skin by Windaus and Bock (Deluca, 2014), thus confirming that this micronutrient was present when people, or the food they ate, was exposed to sunlight.

1.1.2 What is Vitamin D?

It is now accepted that vitamin D is not a true vitamin, as the diet is not the only way vitamin D can be obtained by the body. It is in fact part of a group

of secosteroid hormones, steroids with a broken carbon ring, which are found in different forms in both plant and animal species. There are several forms of vitamin D and its derivative metabolites, which are all structurally similar, however the two main forms that are nutritionally useful to humans are vitamin D2, also known as ergocalciferol and derived from ergosterol in plant and yeast sources, and D3 or cholecalciferol, derived from cholesterol in animals and humans (Horst, 1997).

Vitamin D2 has the chemical formula $C_{28}H_{44}O$, contrasted with vitamin D3, which is $C_{27}H_{44}O$, and therefore the two have a different molecular mass. The structures are shown in Figure 1.1. As with other steroid hormones, such as cortisol and aldosterone, they are characterised by a carbon skeleton with four fused rings, also known as the cyclopentanoperhydrophenanthrene ring structure (Norman, 2008)



Vitamin D2 Vitamin D3

Figure 1.1 Molecular Structure of Vitamin D2 and D3. Adapted from Vitamin D and Health (SACN, 2016)

1.2 Sources of Vitamin D

1.2.1 Dietary Sources of Vitamin D

Dietary sources of vitamin D are essential for the maintenance of sufficient vitamin D levels, when exposure to sunlight is limited. Vitamin D can be present naturally in foods such as fish and dairy, where it has been produced in the same way in animals as in humans, via exposure to sunlight or obtained from the food chain. Examples of food sources potentially rich in vitamin D3 include cod liver oil, egg yolks, mackerel and tinned salmon (250 µg, 10.4 µg,

25.2 µg and 21.5 µg per 100 g respectively). Vitamin D2 is made from ergosterol, found in plants, yeasts and fungi, when it is irradiated by ultraviolet light, hence wild mushrooms can be a dietary source of vitamin D2 (United States department of Agriculture, 2015 (slightly revised May 2016)).

Vitamin D, in either of these forms, is used to fortify many types of food such as milk, margarine, breakfast cereal and orange juice. In the UK, many margarines are still supplemented voluntarily with vitamin D even after the mandatory basis to do so was removed in 2013. EU law Directive 2006/141/EC states all infant formula and follow on milk also be fortified with 40-100IU per 100kcal and 40-120 IU/100kcal respectively (SACN, 2016)

Although ingestion of both forms leads to an increase in vitamin D status, in humans there is evidence to suggest that vitamin D2 is not as effective in this aspect as vitamin D3. A systematic review and meta-analysis of seven studies compared D2 and D3 supplementation in raising serum 25-hydroxyvitamin D status and found vitamin D3 to be more effective (Tripkovic *et al.*, 2012). It should be noted that the studies included in the review mainly gave supplements at doses higher than 600 IU, which was the Institute of Medicine (IOM) recommended daily dietary allowance for adults (age 70 and under) at the time of publication (Institute of Medicine, 2011). It is thought that the difference in effectiveness between vitamin D2 and D3 in elevating vitamin D status is because vitamin D2 and its metabolites have a lower binding affinity to the vitamin D binding protein in the blood, than vitamin D3 (Houghton and Vieth, 2006). It is the vitamin D binding protein (DBP) that transports vitamin D and its metabolites throughout the body to its activation and end use sites. When absorbed from the gut, vitamin D enters the circulation on chylomicrons first and then is slowly transferred to DBP. This is in contrast to the vitamin D made in the skin, which is mostly bound to the DBP on formation. This is significant as vitamin D bound to chylomicrons may be taken up by other tissues in the body before becoming bound to the DBP and become unavailable for use (Jones, 2008).

1.2.2 Production of Vitamin D via Exposure to UVB

As previously discussed, humans are not reliant on obtaining vitamin D from their diet alone as it can also be produced endogenously in our skin when we are exposed to UV light, for example via sunshine. Indeed, cutaneous production is considered the most important source for most people in the UK as this is the major factor in maintaining sufficient levels in the UK (Webb *et al.*, 2010).

The pathway for formation of vitamin D via UVB exposure starts when the skin is exposed to UVB radiation which has wavelengths between the ranges of approximately 290-315 nm. This converts 7-dehydrocholesterol (7-DHC) via photolytic cleavage to previtamin D, whereupon it is quickly isomerised in a heat dependent thermal reaction, also within the skin, to vitamin D3. The 7-DHC is found throughout the epidermal strata and the dermis but it is in highest concentrations in the stratum basale and stratum spinosum, hence, previtamin D is formed throughout the whole epidermis and a small part of the dermis (Holick, 1981). However, even if exposed to UVB light for a prolonged time only 12-15% of the 7-DHC is converted to previtamin D3 (Webb, DeCosta and Holick, 1989) and, following that, up to 50% of the available previtamin D3 will then isomerise to vitamin D3 (cholecalciferol). This happens within 2.5 hours, with maximum levels in the skin being achieved 12 to 24 hours after sunlight exposure and continuing to be formed for up to 2 to 3 days after (Holick *et al.*, 1980).

1.2.3 Pharmacokinetics of Vitamin D

Once the vitamin D is produced, it enters the bloodstream by diffusion into the dermal capillaries and binds to vitamin D binding protein (DBP), which transports it to the liver for further transformation. These rate limitations in the production of vitamin D mean that excessive sun exposure does not lead to a further increase in vitamin D production and is therefore not beneficial in that regard. In fact, prolonged exposure leads to the degradation of previtamin D3 to inactive forms of non-toxic sterols in the skin, and thus it is not possible to achieve vitamin D toxicity from sun exposure (Holick *et al.*, 1980).

1.2.3.1 Metabolism of Vitamin D.

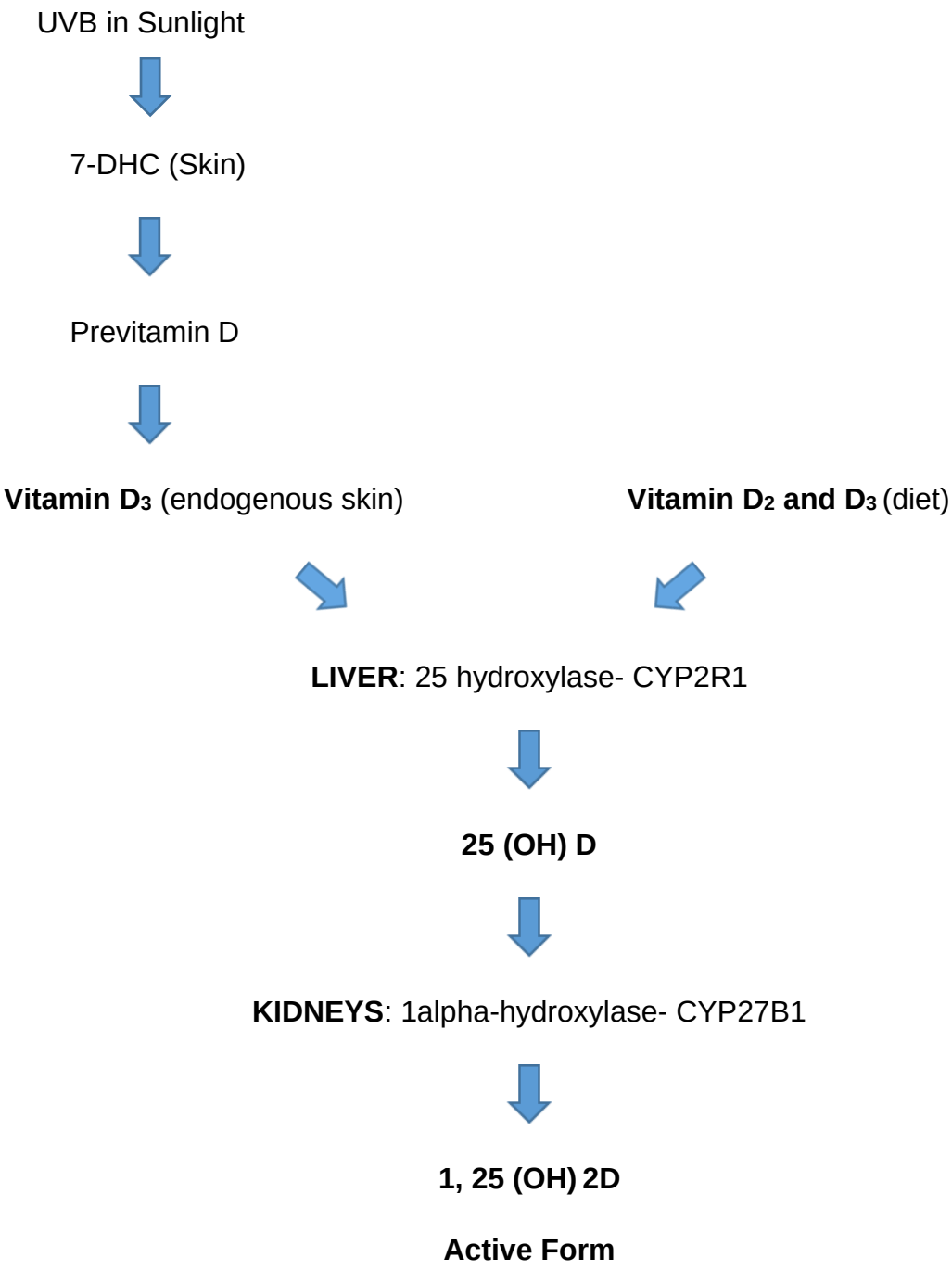


Figure 1.2 Vitamin D Metabolic Pathway (adapted from Vitamin D and Health (SACN, 2016))

The two forms of vitamin D, vitamin D2 and D3, are prohormones and are therefore inactive until they have undergone two hydroxylation steps within the body (Figure 1.2). Enzymes known as cytochrome P450 proteins (CYPs) mediate these hydroxylation steps. The first step in the process is in the liver where vitamin D is converted to 25-hydroxyvitamin D (25(OH)D) via the action of 25-hydroxylases from this cytochrome P450 group (Prosser and Jones, 2004). This enzyme, thought to be CYP2R1 although several cytochrome P450 oxidases exhibit vitamin D25-hydroxylase activities, can hydroxylate both vitamin D2 and D3 (Henry, 2011). The 25(OH)D produced once again binds to the DBP and is carried to the kidney (Speeckaert *et al.*, 2006). Once there, the 25(OH)D undergoes further hydroxylation where a second hydroxylase, 1-alpha-hydroxylase, converts it to its active form of 1, 25-dihydroxy-vitamin D or calcitriol. This process is regulated by a feedback mechanism in response to circulating levels of parathyroid hormone (PTH).

Vitamin D half-life in plasma is 4-6 hours but for the whole body is 2 months (Jones, 2008). Once it has passed through the liver and been converted to 25(OH)D the circulating half-life is about 12-15 days (Jones *et al.*, 2014), however once activated by the kidney the resulting 1,25(OH)2D has a much shorter half-life of approximately 7 hours (Lips, 2007).

Throughout each of the described steps vitamin D is transported by vitamin D binding protein (DBP) found in plasma, to which it is bound, whilst travelling throughout the body. DBP is an albumin protein and is produced in the liver. It has a rapid turnover rate and a half-life of approximately 2.5 days (Speeckaert *et al.*, 2006).

It is now known that there is also extra-renal production of 1,25(OH)2D occurring in several tissues including keratinocytes, lung, colon and macrophages. This extra-renal production is enabled by CYP27B1 and is thought to be regulated in a different way, possibly related to the cell function and status. For example, there may be increased production in response to an increase in inflammatory cytokines such as gamma interferon (Prosser and Jones, 2004).

1.2.4 Vitamin D Receptor and the Mechanism of 1, 25-Dihydroxy-Vitamin D

Once formed 1, 25-dihydroxy-vitamin D (1,25(OH)2D or calcitriol) is carried to the cells by the DBP and there binds with the vitamin D receptor in the region of its nuclear ligand binding domain. Once bound, this vitamin D/ vitamin D receptor complex forms a heterodimer and binds to the target element of a responsive gene. This then causes concurrent binding of other DNA binding proteins followed by the transcription, translation and formation of the proteins required. Classically these are proteins to aid calcium absorption in the intestine, via active transport, or release of calcium from stores in the bones. It is now known, that the vitamin D receptor is found in many cells throughout the body, including immune cells, and vitamin D regulates the expression of thousands of genes. Production of 1,25(OH)2D is controlled through several negative feedback mechanisms from itself, PTH and fibroblast growth factor (Henry, 2011). Increased levels of 1,25(OH)2D decrease its production and high calcium levels supress PTH production which in turn leads to less 1,25(OH)2D being produced (Lips, 2006).

1.3 Factors Affecting Production of Vitamin D and Vitamin D Status

Apart from sun exposure and amount of vitamin D ingested there are other factors that are known to influence an individual's vitamin D status. It is recognised that many of these factors have been identified from observational studies and so the evidence to decide whether they are associated with or directly causing vitamin D insufficiency can be limited. This was discussed by the Institute of Medicine in their report on vitamin D in 2011 (Institute of Medicine, 2011). Several factors have a more direct relationship to vitamin D production as they affect the availability of UVB, dietary vitamin D or the synthesis of vitamin D and some of these are discussed further below and summarised in Table 1.1.

Table 1.1 Factors affecting Vitamin D Production

Environmental	**Behavioural**	**Systemic**
Sun exposure	Dietary intake	Obesity
Living at lower Latitude	Physical activity	Increased Age
Seasonal change	Use of Sunscreen	Genetics
Cloud cover	Clothing /Covering of skin	Skin Type and Pigmentation
Ozone	Outdoor activity	
Surface reflection	Sunbed use	
Altitude		
Air/ Pollution		
Zenith Angle		
Glass		
Full Spectrum Lighting		

1.3.1 Environmental Factors

Sun exposure, or solar irradiation, includes wavelengths across the full electromagnetic spectrum, including ultraviolet radiation (UVR). UV light is separated into UVA (315 to 400 nm), UVB (280 to 315 nm) and UVC (100 to 280 nm). Virtually no UVC reaches the ground as it is absorbed when passing through the Earth's atmosphere. Of the remaining UV which passes through 95% is UVA and only 5% is UVB, and it is the UVB which contains most of the action spectrum for the synthesis of vitamin D in the skin of humans (MacLaughlin, Anderson and Holick, 1982; Norval, Björn and de Gruijl, 2010)

As UVR is required to synthesise endogenous vitamin D it follows that the main environmental factor affecting vitamin D status is sun exposure, and any factors which modify this sun exposure will modify the size of this effect. An example of this would be the zenith angle, which is the angle at which the sun's rays hit the earth with respect to the vertical (that is, when the sun is overhead). The zenith angle is increased in certain circumstances, for example in the early morning, late afternoon, in the winter and at higher latitudes. An increase in the angle means the sun's rays have to travel further through the atmosphere, in

particular the stratosphere, which absorbs much of the sun's radiation. All of the UVC radiation is absorbed as well as 99% or more of the UVB, which means very little UVB is available to make vitamin D. If this is further reduced, vitamin D production becomes unviable (Wacker and Holick, 2013a). For example, at latitudes below 37^{o} North UVB radiation from the sun is sufficient to produce vitamin D endogenously all year round; however, above this latitude vitamin D cannot be produced in winter months (Webb, Kline and Holick, 1988). This means in the UK vitamin D production from sunlight exposure is only effective between April and October and the population is at risk of deficiency during the winter period. If the sun's rays pass through air pollution or glass, this absorbs more of the energy and further reduces or completely inhibits production of vitamin D (Wacker and Holick, 2013a). Conversely living at higher altitudes is associated with increased vitamin D production (Holick *et al.*, 2007). Despite these geographical limitations, Webb estimated that exposure to the UK midday sun for 17 mins daily, wearing shorts and tee shirt, would be enough to achieve sufficient vitamin D levels, ≥20 ng/ mL (50 nmol/L), in Caucasian individuals (Webb *et al.*, 2011).

It should be noted that, as exposure to UVB between the wavelengths of 290-315 nm produces pre vitamin D, artificial lighting containing this wavelength can also be used to induce cutaneous vitamin D production. Therefore, exposure to full spectrum lighting, mercury vapour lights and even sunbeds will also increase vitamin D levels if they contain these wavelengths.

1.3.2 Behavioural Factors

An individual's behaviours and habits can also affect their vitamin D status. For example, people who take supplements or have diets naturally high in vitamin D will have higher vitamin D levels, as would be expected.

Other behaviours that influence status include use of sunscreen, physical activity and types of clothing worn. The use of sunscreen is associated with reduced vitamin D production. However, a recent review suggested that although sunscreen use has the potential to reduce vitamin D production when used very strictly, in reality normal use does not in itself cause insufficiency (Norval and Wulf, 2009).

1.3.3 Systemic Factors

Several systemic conditions and illnesses are known to reduce vitamin D status, which given the many body systems involved in its production and therefore providing opportunities for disruption, is not surprising. Some examples of these will be discussed below.

1.3.3.1 Skin Pigmentation, Integrity and Health

The first stage in endogenous vitamin D production starts in the skin, thus anything that reduces the ability of the skin to absorb UVB radiation will affect this process. Indeed, some skin types, those with increased skin pigmentation and melanin content, whilst giving increased protection against the damaging effects of UV light also make it more difficult to produce vitamin D from sun exposure (Webb, 2006). This can be a problem for dark skinned individuals, particularly when living at higher latitudes, and some populations can be at increased risk of vitamin D deficiency and even rickets (Holick, 2006)., A study in Sweden found children with darker skin types required higher doses of supplements to achieve and maintain vitamin D sufficiency, >50 nmol/L, than fair skinned children (Ohlund *et al.*, 2017). Another study of 51 black women and 39 white women aged 20-40 years in Boston USA showed black women had consistently lower vitamin D status at all points throughout the year compared to white women, even though both groups demonstrated seasonal variation in status (Harris and Dawson-Hughes, 1998). In the Boston study the group of black women never achieved a mean 25(OH) D serum concentration above 41 nmol/L, whilst the group of white women achieved a mean of 85 nmol/L in the summer months.

Aging is also associated with decreased cutaneous vitamin D production, as the skin's ability to produce vitamin D reduces with increasing age due to reduced presence of 7-DHC in the skin (MacLaughlin and Holick, 1985). Aging may also influence vitamin D status through lifestyle changes and clothing choices, such as less time outside and increased coverage with clothing (including cultural aspects such as veils and hijabs).

1.3.3.2 Genetic Influences

Some individuals have genetic alleles associated with reduced circulating 25-hydroxyvitamin D concentrations (Wang *et al.*, 2010). These are mainly genes associated with cholesterol synthesis, hydroxylation and vitamin D transport. Cholesterol is the basis for 7-dehydrocholesterol (7-DHC) which is in turn needed to make vitamin D in the skin.

1.3.3.3 Obesity

Obesity, defined as having a body mass index (BMI) above 30, is given as an example here of a systemic condition affecting vitamin D status as it is now a common condition in many developed countries, including in the UK where it affects 26.9% of the adult population. (OECD, 2017). People with obesity are at increased risk of vitamin D deficiency. It is suspected that although obesity causes low serum 25-hydroxyvitamin D concentrations the possibility of a bidirectional relationship existing, and low circulating 25-hydroxyvitamin D in some way inhibiting weight loss, is unlikely (Vimaleswaran *et al.*, 2013). A possible explanation for obesity causing low vitamin D status is storage of vitamin D, a fat soluble vitamin, in adipose tissue contributing to low circulating 25-hydroxyvitamin D concentrations. Other suggested causes of obesity related deficiency include increased breakdown and increased excretion due to obesity related inflammation (Earthman *et al.*, 2012).

1.4 Comparative Contributions of Different Sources of Vitamin D

Exposing the whole body to UVR to the extent that a slight pinkness occurs in the skin is known as the minimal erythemal dose (MED). An MED is personal to each individual as it varies according to skin type, time of day, location and other factors previously discussed. However, 1 MED of sunlight is equivalent to an oral vitamin D dose of 10,000 IU and exposure of a quarter of the body (skin surface) to a quarter MED provides the equivalent of an oral dose of 1000 IU of vitamin D (Engelsen, 2010). Even though sunlight exposure is the most efficient and common way of receiving vitamin D it can be hard, given all the possible modifying factors, to recommend a “dose” of sun

exposure. Casual sun exposure in clear atmospheric conditions where vitamin D production is possible is estimated to be equivalent to 400 IU/day but if higher doses of vitamin D are needed, 1000-4000 IU/day, the amount of time required in the sun is more likely to be of a duration where sunburn and skin damage is increased. For these higher levels, large amounts of skin would have to be exposed for durations of more than an hour (Webb and Engelsen, 2008).

Knowing how much effect vitamin D from different sources will have can be further complicated as the increase in serum vitamin D achieved can vary depending on the pre-exposure serum levels. An individual with a lower starting serum level will see a bigger increase of serum 25(OH) D than those with higher pre-exposure status (Engelsen, 2010; Vieth, 1999).

Currently it appears although cutaneous and dietary vitamin D intake are still leaving many individuals deficient, there is insufficient evidence to give specific advice on how much sun exposure an individual will need.

1.5 Adverse Effects of UVR Exposure

Although exposure to sunlight has the beneficial effect of increasing vitamin D production, which is good for overall health, paradoxically sun exposure also has associated negative health risks. These include increased risk of several forms of skin cancer including malignant melanoma, basal cell carcinoma and squamous cell carcinomas, the formation of cataracts and skin damage (MacKie, 2006). The risks seem to be higher generally in those with fair skin, prone to burning and freckling and with intermittent and chronic sun exposure (Gallagher and Lee, 2006). As well as some enhancement of some immune functions (e.g. production of antimicrobial peptides (Hart, Gorman and Finlay-Jones, 2011)), it is known that immunosuppression, both local and systemic, occurs after UV exposure and it is possible this plays a role in the development of skin cancers (Norval, 2006). These negative effects are due to exposure to the UV radiation, both UVA and UVB, found in the sunlight spectrum. The UVC in sunlight is almost entirely absorbed by the atmosphere and is thought therefore to only have negligible effects. These adverse effects should inform any guidance on sun exposure and vitamin D production.

1.6 Measurement of Vitamin D Status and Deficiency

1.6.1 Measuring Vitamin D Status

The measurement of vitamin D status can be challenging as, like many hormones, the levels of the active form in the blood are carefully regulated by feedback mechanisms. This means that some of the measurable metabolites vary very little and are not representative of overall reserves of vitamin D in the body. It is generally accepted however that the serum plasma concentration of 25-hydroxyvitamin D (25(OH)D), the major circulating active metabolite of vitamin D, can be used as a proxy measure for vitamin D status, is the industry standard and correlates with vitamin D stores in the body. This has been endorsed by the Institute of Medicine and is used in its reports advising on required intake of vitamin D (Institute of Medicine, 2011). 25-hydroxyvitamin D can be expressed in nmol/L or ng/ml with 25 nmol/L being equivalent to 1 ng/ml. The benefit of using this metabolite includes the numerous assays available which can measure it. Also, the step between conversion from vitamin D to 25(OH)D by the liver is not regulated by feedback mechanisms and so is reflective of the amount of potential vitamin D available (Norman, 2008). There are several methods to measure vitamin D status. These include liquid chromatography mass spectrometry (LC-MS/MS), high performance liquid chromatography (HPLC), and several ligand-binding assays. The use of different measuring techniques and preparations of blood samples can lead to variation in results and there has been some effort to try and standardise the laboratory measurement of 25(OH)D and other vitamin D derivatives by the Vitamin D external quality scheme (DEQAS) (Burdette *et al.*, 2017).

1.6.2 Guidelines for Optimal Vitamin D Status

There are a number of guidelines for achieving optimum vitamin D status, which are reflective of the variation in the availability and interpretation of evidence, as well as the differing opinions regarding the optimum levels for skeletal and non-skeletal health outcomes and needs of different ages and ethnicities. A recent summary review of guidelines from around the world recommended that for a general healthy population the chosen vitamin D supplementation guideline should be specific for age group, body weight, ethnicity (skin type), and latitude of residence (Pludowski *et al.*, 2018).

Previously, a 2006 review of previous RCTs and meta-analysis advised an optimum blood serum concentration between 30 ng/mL (75 nmol/L) and 40 ng/mL (100 nmol/L). However, with the recommended daily intakes of 200 IU (5 µg) for children and 600 IU (15 µg) for older people, this could not be achieved in the majority of people. The review looked at skeletal and non-skeletal health outcomes and recommended all adults take a supplement of at least 1000 IU (25 µg) daily to bring 50 % of the population up to serum levels of 30 ng/mL (75 nmol/L) (Bischoff-Ferrari *et al.*, 2006)

In 2011 the North American Institute of Medicine also reviewed all the available evidence. They concluded that evidence of sufficient quality was only available to make recommendations that aimed to achieve optimum calcium absorption, bone mineral density and avoidance of rickets or osteomalacia. Their recommendation was that a serum 25(OH)D concentration of 20 ng/ml (50 nmol/L) would achieve these outcomes in 97.5% of healthy individuals and this translated to an RDA (recommended dietary allowance) of 600 IU/day (15 µg), for ages 1-70 years and 800 IU/day (20 µg), for above 70 years (IOM, 2011a). For extra-skeletal outcomes, including cancer, cardiovascular disease, diabetes, and autoimmune disorders, the evidence was inconsistent and inconclusive as to causality. It was therefore deemed there was insufficient evidence to inform nutritional requirements in these cases (Ross *et al.*, 2011). The Endocrine Society USA recommends higher doses of supplements for adults of 1500-2000 IU/day (37.5-50 µg/day) and advises that the general healthy population should optimally have 75 nmol/L serum levels to achieve good skeletal and genetic outcomes (Holick *et al.*, 2011). Furthermore, in the UK, Public Health England released new guidelines in July 2016, also reviewing the evidence on vitamin D and health (SACN, 2016). They recommended an intake of 400 IU/day (10 µg) for everyone above the age of 4 years old to achieve health outcomes and they recommend the use of supplements over the winter to reduce the risk of deficiency.

Generally, a blood serum measurement of 25(OH)D less than 20 ng/ml (50 nmol/L) is considered to be at least insufficient as at this level muscle weakness and bone disorders can occur (Holick, 2009). Although there is variation in the recommended intakes ranges and optimum serum levels there is agreement on the skeletal effects of deficiency. Deficiency causes an

increase in parathyroid hormone, or secondary hyperparathyroidism, and this leads to release of calcium from bones to compensate for the lack of absorption from the intestine. The outcome of this is a reduction of calcium in the bone matrix causing osteomalacia in adults and rickets in children (Lips, 2011; Holick, 2006). Interestingly, in groups who are exposed regularly to high levels of sunshine, their mean 25(OH)D serum levels are often found to be above 40 ng/ml (100 nmol/L), with some individuals having levels above 80 ng/ml (200 nmol/L), with no ill effects (Vieth, 1999).

1.6.3 Hypervitaminosis and Vitamin D Toxicity

Vitamin D toxicity is rare and is usually associated with incorrect manufacture or use of supplements. As already discussed, although it is possible to achieve high levels of serum vitamin D from sun exposure, toxicity is not possible from endogenous production due to feedback mechanisms and rate limiting steps. Therefore, when toxicity occurs it is due to oral vitamin D intake. Using serum 25(OH) D as the accepted measure of vitamin D status the threshold suggested for producing toxic symptoms is variable. One study suggests 200 ng/ml (500 nmol/L) (Heaney, 2008) whilst others suggest as low as 80 ng/ml (200nmol/l). This lower level, in some individuals exposed to frequent sunshine, for example farmers or lifeguards, could occur naturally and could therefore be considered to be within physiological levels (Cannell *et al.*, 2008). This is much higher than levels considered sufficient for all health outcomes, average levels or even levels achieved with daily use of supplements of 1000-2000 IU/day. Toxicity is associated only with excessive intake usually well above 20,000 IU/day (Heaney, 2008). Excessive vitamin D causes toxicity via hypercalcemia and hypercalciuria, the symptoms of which include weakness, lethargy, headaches, nausea, polyuria, unwanted calcification of tissues and blood vessels, and eventually confusion and coma.

1.6.4 Prevalence of Vitamin D Deficiency in the UK

Reporting of vitamin D deficiency is relatively widespread, with deficiencies in studies frequently associated with risk factors such as female gender, indoor lifestyles, low dietary intake, obesity and sunscreen use. For example, high prevalence of vitamin D deficiency have been reported in North

American and Canadian populations, where black and Hispanic individuals and those living at higher latitude were shown to be more at risk (Calvo, 2003); in Dutch populations where 45% had serum levels less than 50nmol/L (Brouwer-Brolsma *et al.*, 2016) and in Korea where 69% of adults were thought to be deficient (Lee *et al.*, 2015). Even countries and continents that have abundant sunshine have populations and groups reported to have sub optimal levels of vitamin D (van Schoor, 2011; Munns *et al.*, 2012). Due to differences in the serum 25(OH) D level thresholds defined as deficient, insufficient or sufficient it is hard to make comparisons between studies.

Several studies exist describing deficiency in the UK population, particularly amongst older adults, and some are summarised in Table 1.2.

One study in the UK, looking at 45-year-olds from the 1958 British birth cohort survey, measured serum vitamin D status in 7,591 white individuals (Hyppönen and Power, 2007). Data were collected on demographics including sex, age, weight, diet, outdoor activity, supplement use, sunscreen use, season of sample collection and region of residence. Vitamin D levels were strongly associated with month of collection, which was the strongest predictor for status, explaining 21.5% of the variation. Highest levels were found in September and lowest in February (northern hemisphere). Levels were strongly associated with time outdoors in the summer but, as expected, not in the winter when production of vitamin D is not possible throughout most of the UK. The participants were grouped into three different levels of severity of deficiency. Less than 10 ng/ml (25 nmol/L), above which was considered sufficient to prevent rickets, less than 40nmol/L which was considered the threshold for requiring supplementation and less than 75 nmol/L with 75 nmol/L being considered the lowest threshold for optimum general health outcomes. Using these severity thresholds nearly 50% of this population had 25(OH) D serum concentrations of less than 40 nmol/L in the winter and spring and 90% had less than 75 nmol/L. There was also a significant north-south gradient with average levels consistently higher in the south than in the north, the lowest levels being in Scotland. This supported the findings of a previous study (Hirani and Primatesta, 2005), that used data for 1297 individuals taken from the Health Survey for England 2000 dataset, to investigate vitamin D status of older people (over 65 years old) living in private households and institutions. Their findings

were that vitamin D deficiency was consistently lower in individuals living in institutions, women living in private residences and was associated with increasing age and general ill health. The mean serum vitamin D levels were significantly lower for both men (38.1 nmol/l) and women (36.7 nmol/l) in institutions, than among men (56.2 nmol/l) and women (48.4 nmol/l) in private households ($P<0.05$). Using a cut off of 25 nmol/L as a threshold for deficiency almost a third of those in institutions were deficient compared to approx.12% of those in private residences. Furthermore, in private households, women (15.0%) were significantly ($P< 0.001$) more deficient than men (9.6%). A further study of older adults (mean age 72) found 25% had serum levels of 25(OH)D less than 25nmol and reduced levels were again associated with non- white ethnicity, lack of supplementation and blood sampling in the winter/spring seasons (Jolliffe *et al.*, 2016). Mean serum 25(OH)D concentration was 42.7 nmol/L (SD 22.0). Non-white ethnicity was associated with an 8.6 nmol/L lower serum 25 (OH)D concentration (95% CI -14.9 to -2.3, $p = 0.008$), whilst a lack of vitamin D supplement consumption was associated with a 17.1 nmol/L lower serum 25(OH)D concentration (95% CI -23.3 to -10.9, $p < 0.001$). Conversely, vitamin D levels in a study of 9-year-olds, taken from the UK Avon Longitudinal Study of Parents and Children (ALSPAC) (a birth cohort study that recruited pregnant women, their partners and their babies in 1991-1992 and collected longitudinal health data,) found only 0.5 % of the children were vitamin D deficient. However, having fairer skin was associated with higher levels of 25(OH) D. This study used a deficiency threshold of 25 nmol/L (Bonilla *et al.*, 2014). Deficiency in the UK has also been shown to be high amongst ethnic minorities, although interestingly in these groups, particularly South Asians, deficiency was not associated with increased rates of osteoporosis, suggesting the clinical indications of deficiency may differ from white populations (Patel *et al.*, 2013).

Table 1.2 Summary of Studies of Vitamin D Deficiency in the UK

Study	Population	Deficiency thresholds	% Deficient
(Jolliffe *et al.*, 2016)	Adults, Mean age 72 Sheltered housing	<25nmol/L	25%
(Bonilla *et al.*, 2014)	Children 9yrs ALSPAC study	<25nmol/L	0.5%
(Patel *et al.*, 2013)	Adults South Asian ethnic heritage Afro Caribbean ethnic heritage	<30nmol/L	 76.2% men 75.7% women 45.7% men 49% women
(Hyppönen and Power, 2007)	Adults 45-year-olds 1958 British Birth Cohort	<25nmol/L <40nmol/L <75nmol/L	15.5% 46.6% 87.1%
(Hirani and Primatesta, 2005)	Adults >65 yrs. age. Institutions Private Home	<25nmol/L	 30.3% men 32.5% women 9.6% men 15.0% women

1.7 Vitamin D, Bone Metabolism and Calcium Homeostasis

Normal calcium levels are essential for multiple processes in the body needed for health and function, and vitamin D is essential to maintaining calcium homeostasis. Vitamin D, in its active form of 1,25(OH)2D, opens up calcium channels in the entire length of the intestine, although the greatest effect is in the duodenum and jejunum, which stimulates the absorption of calcium and phosphate to be available for the passive process of bone mineralisation. In vitamin D deficiency, less calcium is absorbed leading to a decrease in extracellular calcium levels. This triggers an increase in the release of parathyroid hormone PTH, which stimulates both hydroxylation of 25(OH)D to 1,25(OH)2D and bone resorption. The 1,25(OH)2D again increases calcium absorption and to this effect levels of 1,25(OH)D and calcium levels are maintained, but at the expense of increased bone resorption (Lips, 2011). The process is inhibited by increased levels of calcium and 1,25(OH)2D in a negative feedback loop. Finally, 1,25(OH)2D and PTH also act in synergy to stimulate reabsorption of calcium in the renal distal tubule of the kidneys, decreasing its excretion.

1.8 Vitamin D and Immune Regulation

Although vitamin D has mainly been associated with calcium and phosphate metabolism and bone health, it is now recognised that it plays a notable role in immunoregulation through the modulation of the activation, proliferation and differentiation of immune and inflammatory cells.

1.8.1 Innate Non-Specific Immunity and Vitamin D

Innate immune defence refers to the natural existing defences the body has to combat infection. This includes the barrier defences, such as the keratinised tissues provided by the gingivae and the antimicrobial substances found in saliva, such as lysozyme, an enzyme that break downs some bacterial cell walls. Innate immunity also refers to the cells of the innate immune system. These cells consist of chemical secreting cells and cells which can undertake phagocytosis. Cells of the innate immune system derive from the common myeloid progenitor line of cells and include neutrophils, mast cells, basophils,

eosinophils, dendritic cells, monocytes and macrophages. Mast cells and basophils can secrete histamine, whilst neutrophils and eosinophils are phagocytic against invading pathogens. The innate immune system is characterized by a lack of specificity and lack of memory (Scully, Georgakopoulou and Hassona, 2017).

There is evidence that vitamin D can influence the innate immune response. An *in vitro* study found that 1,25(OH)2D3, the active form of vitamin D, increased the expression of LL-37, a cathelicidin with antibacterial activity against periodontal pathogen A. actinomyecetemcomitans, in cultured gingival epithelial cells (McMahon *et al.*, 2011; Wang *et al.*, 2013). A clinical study found that in patients with periodontal disease, Human Beta Defensin (HBD-2) levels, an antibiotic innate peptide, were higher in the gingival tissues of patients who were vitamin D sufficient rather than deficient. The difference was statistically significant (2.58 ng/30 s; range: 2.39–2.86 compared to 2.34 ng/30 s; range: 1.92–2.54, p=0.028). A similar pattern was found in patients with gingivitis; with highest HBD-2 being found in those who had gingivitis and were vitamin D sufficient 1.67 (0.90–1.87) compared to those with gingivitis who were vitamin D deficient 0.76 (0.58–1.53) ($p < 0.001$) (Bayirli, Öztürk and Avci, 2020). The cells of the innate immune system are often found in the tissues, skin and epithelium. UV exposure is known to lead to immunosuppression to antigens encountered within a few days of irradiation by moderating the response of antigen presenting cells in the skin. These dendritic cells express vitamin D receptors and it is possible that extra renal cutaneous production of the active metabolite of vitamin D explains this response (Norval, 2006).

Inflammation is a form of innate immunity which is partly facilitated by cytokines and complement, proteins that help coordinate the inflammation process. These immune proteins include amongst others cytokines, interleukins (IL), interferons, (IFN) and tumour necrosis factor (TNF). Exposure of cells to calcitriol is known to decrease the production of some of these pro inflammatory cytokines including IL-12, IFN-y, IL-6, IL-8, TNF alpha, IL-17 and IL-9 (Colotta, Jansson and Bonelli, 2017). Conversely, the production of anti-inflammatory cytokines including IL-4, IL-5 and IL-10 is increased (Colotta, Jansson and Bonelli, 2017; Zhang *et al.*, 2012).

1.8.2 Adaptive Immunity and Vitamin D

Adaptive, or acquired, immunity is highly specific and discriminatory. It takes longer to develop a response than the innate system but it also has memory. Therefore, a second exposure to the pathogen will evoke a faster and stronger immune response. The adaptive response is characterised by leukocytes such as T cells and B Cells. The B cells are responsible for humoral immunity and the production and release of antibodies that bind to a specific antigen on an invading pathogen. The antibodies help neutralise the antigen, encourage phagocytosis and activation of the complement cascade. The cell mediated immune response targets intracellular pathogens via the use of T Lymphocytes, T-helper cells or cytotoxic T cells. T lymphocytes recognise the antigen proteins that are presented by antigen presenting cells such as dendritic cells and macrophages. The cytotoxic T cells destroy infected cells directly while the T helper cells produce cytokines that enhance the B cell response (Scully, Georgakopoulou and Hassona, 2017). Vitamin D receptors are known to be present on activated T and B cells and activated vitamin D has been shown to inhibit differentiation of B lymphocytes to plasma cells (Stein, Livada and Tipton, 2014). It is also established that 1,25(OH)2D3 suppressed T helper cell proliferation and production of pro inflammatory cytokines (Lemire *et al.*, 1985).

1.9 Vitamin D and Other Non-Skeletal, Non-Oral Health Outcomes

Vitamin D deficiency is associated with many non-calcaemic diseases and health outcomes. These include reduced male and female fertility (Ozkan *et al.*, 2010; Blomberg Jensen *et al.*, 2011), mental health disorders, dementia, cardiovascular disease, cancers, respiratory disease, allergies, multiple sclerosis and autoimmune diseases including, diabetes type 1, rheumatoid arthritis and systemic lupus erythematous (Wacker and Holick, 2013b; Wacker and Holick, 2013a). However, despite the reports of vitamin D deficiency being associated with disease, there is often disconnect between the results from these observational studies and the interventional studies, which give vitamin D supplementation as a treatment modality.

Vitamin D deficiency is widespread in the population and unhealthy diets and some behaviours predispose to both vitamin D deficiency and other poor outcomes. One review that looked at vitamin D and ill health concluded that results from intervention studies did not show an effect of vitamin D supplementation on disease occurrence and vitamin D deficiency was a marker of ill health, not a cause (Autier *et al.*, 2014). The picture remains unclear as evidence also exists showing the widespread actions of vitamin D and the extent of vitamin D receptors and extra renal production of vitamin D throughout the body. Vitamin D appears to be vital to health and yet the exact relationships appear hard to quantify.

1.10 Vitamin D and Oral Health

The literature on the links between oral health and vitamin D has increased substantially in recent years. To gain an initial indication of the extent and consistency of evidence on oral health and vitamin D, a general survey of the literature was carried out, with the results summarised in Table 1.3. This shows a range of study types finding an association between oral disease (caries and periodontitis) and vitamin D status, usually measured by serum levels or by proxy using dietary intake. The nature of these relationship being either associative or causal will be discussed in later sections, as will the direction of this relationship and the oral and systemic health outcomes.

Table 1.3 A Summary of Examples of the Evidence Investigating the Relationship Between Oral Health and Vitamin D

Study	Year	Country	Population	Results
Caries				
(Schroth *et al.*, 2016)	2016	Canada	6-11 yrs.	Association between caries and lower serum vitamin D
(Herzog *et al.*, 2016)	2016	USA	5-12 yrs. Non institutionalised children	No significant association between caries experience and 25(OH)D status
(Antonenko *et al.*, 2015)	2015	Buenos Aires	Mean age 23.7±0.4	Group with highest caries scores had significantly lower calcium and serum 25(OH)D levels
(Hofilena, 2015)	2015	USA	Children	A relationship between the prevalence of Early Childhood Caries (ECC) and low serum levels of vitamin D in children 0 to 6 years of age
(Schroth *et al.*, 2014)	2014	Canada	Children 1 yrs. age	Inverse relationship between untreated decay in the 1st year of life, enamel hypoplasia and maternal prenatal vitamin D
(Schroth *et al.*, 2013)	2013	Canada	Mean age 40.8 months ±14.1	Association between Severe Early Childhood Caries (SECC) and lower serum vitamin D levels
(Schroth *et al.*, 2012)	2012	Canada	Children	Children with SECC have lower vitamin D levels and increased PTH levels than age matched controls
Gingivitis				
(Millen *et al.*, 2012)	2013		920 postmenopausal women	Gingival bleeding was inversely associated with vitamin D status

(Dietrich *et al.*, 2005)	2005	USA	6700 never smokers 13 -90 yrs. NHANES Male and female	Association between decreased gingival inflammation and higher serum concentrations of 25-hydroxyvitamin D
Periodontal Disease				
(Abreu *et al.*, 2016)	2016	Puerto Rico	Case control 19 matched pairs cases 35-64 yrs.	Periodontitis is associated with lower serum vitamin D levels in Puerto Rican adults
(Lee *et al.*, 2015)	2016	Korea	Adults >19yrs	A statistically significant association was not found after adjusting for covariates in the total sample However, an association between poorer periodontal status and lower serum vitamin D levels was found in current smokers.
(Alshouibi *et al.*, 2013)	2013	USA	VA Dental Longitudinal Study (DLS), Men Mean age 62 yrs.	Total vitamin D intake ≥ 800 IU was associated with lower odds of severe periodontal disease and moderate-to-severe alveolar bone loss relative to intake < 400 IU/day.
(Antonoglou *et al.*, 2013)	2013	Finland	Male and Female Mean age 38.6 ± 12.3 Type 1 diabetes	An association was found between better periodontal health and a higher serum level of 1,25(OH)D The serum level of 1,25(OH)D increased after anti-infective periodontal therapy

(Millen *et al.*, 2012)	2013	USA	Postmenopausal women	Clinical categories of chronic periodontal disease were inversely associated with vitamin D status.
(Zhang *et al.*, 2013)	2013	China	Mean 27yrs age	Generalised Aggressive Periodontitis is associated with elevated plasma vitamin DBP levels.
(Boggess *et al.*, 2011)	2011	USA	Pregnant women	Maternal periodontal disease during pregnancy is associated with vitamin D insufficiency (serum 25 [OH] D< 75 nmol/l)
(Bastos Jdo *et al.*, 2013)	2009	Brazil	Adults with CKD	Chronic periodontitis is associated with vitamin D deficiency in patients with chronic kidney disease not yet on dialysis.
(Miley *et al*, 2009)	2009	USA	Men 50-80 years Compared to subjects who did not take vitamin D and calcium supplementation,	Shallower probing depths, fewer bleeding sites, lower gingival index values, fewer furcation involvements, less attachment loss, and less alveolar crest height loss, not significant, found in those that took supplements
(Naito *et al.*, 2007)	2007	Japan	Men. 22-59 yrs. age	Association between vitamin D receptor gene haplotypes and chronic periodontitis among Japanese men
(Dietrich *et al.*, 2004)	2004	USA	11,202 NHANES Over 20yrs Male and Female	Association between periodontal disease and serum concentrations of 25-hydroxyvitamin D3 and in the US population Low serum 25(OH)D3 concentrations may be associated with periodontal disease independently of Bone Mineral Density
Other				
(Yuan-yuan *et al.*, 2017)	2017	China	380 Children	Increased risk of primary tooth decay was associated with vitamin D receptor gene Bsml polymorphism.

			4-7yrs	
(Cogulu *et al.*, 2016)	2016	Turkey	150 children 6-12 years Divided into high risk, moderate risk and caries free groups	Statistically significant difference in the frequency of vitamin D receptor polymorphism Taq1 genotypes in caries active and caries free children.
(Kühnisch *et al.*, 2015)	2015	Germany	10yr children primary and permanent teeth	Lower odds of molar incisor hypomineralisation and lower number of caries affected permanent teeth associated with higher serum 25(OH)D.

1.10.1 What is Oral Health?

The World Dental Federation (FDI) has defined oral health as "multifaceted and includes the ability to speak, smile, smell, taste, touch, chew, swallow, and convey a range of emotions through facial expressions with confidence and without pain, discomfort, and disease of the craniofacial complex" (Glick, 2016). The World Health Organisation says "Oral health means more than good teeth; it is integral to general health and essential for wellbeing. It implies being free of chronic oro-facial pain, oral and pharyngeal (throat) cancer, oral tissue lesions, birth defects such as cleft lip and palate, and other diseases and disorders that affect the oral, dental and craniofacial tissues, collectively known as the craniofacial complex. Oral Health is a determinate factor for quality of life" (Petersen, 2003) Both of these definitions emphasise the multiple causes and aspects of poor oral health and the importance of good oral health to overall general health and wellbeing. By far the most common diseases affecting oral health in the UK are dental caries and periodontal disease and so the focus of this project will be on these conditions. Caries remains a common childhood condition in the UK. Approximately 31% of 5 year olds have untreated tooth decay and 46% of 15 year olds have obvious decay experience (Office for National Statistics, 2015). A diagnosis of dental caries is the most common cause of admission to hospital for those aged under 19 each year and in 2013-14 this number was approximately 46,500 (*The Royal College of Surgeons of England.* 2015). According to prevalence data from the 2009 UK Adult Dental Health Survey, 37% of the adult population suffer from moderate levels of chronic periodontitis (with 4-6mm periodontal pocketing), while 8% of the population suffer from severe periodontitis (with pocketing exceeding 6mm) (Office for National Statistics., 2012). The WHO recognises a burden of oral disease and that there is increasing periodontal disease and untreated caries globally (Marcenes *et al.*, 2013).

1.10.2 Vitamin D and Caries

Teeth consist of a hard outer highly mineralised surface layer of enamel, which is formed from densely packed hydroxyapatite crystals lying over a less mineralised dentine layer and a central dental pulp. The pulp contains the blood and nerve supply to the dentine, but not enamel, which is avascular and

acellular. There is no enamel over the roots of the teeth and instead it is cementum, a much softer substance, not enamel, that covers the root surface (Berkovitz *et al.*, 2018b). Caries is the decay of these hard tissues of the teeth, namely the enamel, dentine and cementum.

1.10.2.1 Development of the Teeth, Enamel and Dentine

The very initial stages of tooth development are visible in the embryo from six weeks *in utero.* Cells contributing to the formation of the teeth and jaws derive from embryological structures, called the neural crest and the first pharyngeal arch. The cells from the neural crest migrate from the margins of the developing neural tube to form surrounding mesenchymal tissues, and the cells from the ectoderm of the first pharyngeal arch form the primitive oral epithelium. There they organise to form a thickened epithelial band extending into the surrounding mesenchyme. The band divides to form the vestibular lamina which will form the mouth, cheeks and lips, and the dental lamina which will form the teeth. From here individual tooth germs start to form in the dental lamina from approximately week eight in utero. This starts with the bud stage, where swellings develop to form simple spherical condensations of epithelial cells surrounded by mesenchyme, and continues to the cap stage at approximately eleven weeks in utero. In the cap stage morphogenesis starts to occur with the formation of internal and external epithelial layers and by the 14th week tooth development enters the bell stage where the final shape the tooth starts to become visible. At the end of the bell stage, approximately eighteen weeks, the hard tissue development of the tooth begins, with dentine development commencing before enamel formation.

1.10.2.2 Enamel Formation

Enamel formation is known as amelogenesis and is completed by cells called ameloblasts, derived from the internal enamel epithelium cells. Firstly, in the pre secretory phase, inner enamel epithelium cells differentiate into ameloblasts. The morphogenic phase of the pre secretory phase is at the end of the bell stage, which is when the shape of the crown starts to become visible. Then follows the differentiation phase when the inner epithelial cells go from low

columnar/cuboidal cells to polarised columnar ameloblasts. This phase also triggers the odontoblasts to start laying down dentine, which will be discussed separately. In the secretory stage the ameloblasts develop a process in their distal portion called the Tomes process. This process secretes enamel matrix, consisting of proteins called amelogenins and non amelogenins (enamelin, tuftelin and amelobalstin). The proximal part of the process secretes what will become the interrod enamel and the distal part secreting what will become the rod/prism enamel.

In the maturation phase the ameloblast morphology alters again. The Tomes process recedes and the proximal surface now alternates in form between a ruffled surface with tight junctions and a smooth form with leaky junctions. The ruffled ended ameloblasts release enzymes to degrade the matrix which is then removed by the smooth ended ameloblasts. The ruffle ended ameloblasts, simultaneously to their removal of the matrix, release calcium binding proteins and calcium ATPase to mineralise the developing enamel. The calcium release is derived from serum calcium and is vital to achieving the required 96% mineralisation of enamel with hydroxyapatite.

1.10.2.3 Dentine Formation

The differentiation of the pre ameloblasts to ameloblasts in the enamel organ trigger the differentiation of pre odontoblasts to odontoblasts, the cells responsible for the laying down of dentine. The pre odontoblast cells, located at the periphery of the dental papilla, become columnar in shape and develop cell features such as endoplasmic reticulum and Golgi apparatus which will later produce the secretory granules released by the odontoblast processes. The cells polarise and the nucleus moves to one end of the cell. At the other end of the cell, adjacent to the ameloblasts, the odontoblastic process starts to develop. The processes lay down a matrix, predentine, which will then become mineralised. The odontoblasts move inwards towards the future pulp leaving the processes elongating behind them and becoming enclosed in dentine tubules as mineralisation occurs. The mineralisation of dentine involves the removal of proteins from the predentine and the secretion of vesicles containing crystallites. These crystallites seed continuous calcification and the formation of

calcospherites. These areas of calcification grow and fuse together until the dentine is 70% non-organic material.

1.10.2.4 The Healthy Tooth and Oral Environment

Healthy enamel is subjected to a constant process of demineralisation and remineralisation as a normal cycle within the oral environment. During eating the saliva glands are stimulated to change the contents of the saliva produced from an unstimulated mucous secretion to a stimulated serous form. This stimulated saliva contains increased levels of bicarbonate, which buffers acids, and increased calcium and phosphate, which aid remineralisation. This helps “repair” any early increase in enamel porosity that has occurred and as long as this cycle remains in equilibrium caries will not develop.

1.10.2.5 Caries

Caries is the process of tooth decay and is caused when bacteria in the oral cavity metabolise dietary fermentable carbohydrates, producing acid by-products. When this happens during eating, the pH in the oral environment can drop below the critical pH of approximately 5.5, which is the level at which enamel starts to demineralise. As previously discussed, the fundamental organisational unit of the enamel is the hydroxyapatite rods, or prisms, as they are also called. The prisms are densely packed and so enamel is a highly mineralised structure of up to 96% inorganic material. The region between the prisms is the interprismatic substance, an area of less mineralised residual organic material, left from when the enamel formed and the matrix was removed, which is slightly more susceptible to initial acid dissolution. When the pH drops the crystals of hydroxyapatite will start to dissolve and the porosity of the enamel will increase. The hydroxyapatite may include impurities, for example in the form of carbonated hydroxyapatite, or ions such as fluoride, magnesium or strontium substituting ions in the crystal lattice. The presence of these impurities can convey different properties on the enamel. For example, high levels of incorporated fluoride (fluorapatite) can reduce the critical pH to levels around 4.5, making teeth more resistant to acid dissolution (Rošin-Grget *et al.*, 2013).

1.10.2.6 The Early Carious Lesion

Once teeth erupt into the mouth, or immediately after cleaning, an enamel pellicle begins to form on the teeth almost immediately. The pellicle consists of proteins derived from saliva and the gingival crevicular fluid (GCF) that have an affinity for carbonated hydroxyapatite, the main mineral form of hydroxyapatite in teeth and the enamel surface (Odanaka *et al.*, 2020). Following this, early colonising bacteria attach to the pellicle and start to form a biofilm or dental plaque (Siqueira, Custodio and McDonald, 2012). Over time the plaque biofilm thickens, and late bacterial colonisers begin to appear also. The interface between the enamel, biofilm and saliva is in a constant state of flux with an ongoing equilibrium between mineral deposition and mineral loss to the enamel surface and immediate subsurface. Saliva itself is maintained in a supersaturated state by the action of statherin, meaning it contains high levels of calcium and phosphate ions. These are the constituents of hydroxyapatite and at normal oral pH supersaturation of saliva with calcium phosphate salts is maintained, therefore favouring mineral deposition into porous areas of the enamel and consequently remineralisation. The plaque bacteria on the teeth surface use dietary fermentable carbohydrates for their metabolism, which leads to the production of acid waste products. The pellicle and biofilm act as a diffusion barrier slowing the process of enamel demineralisation, controlling the supply of saliva buffers and remineralisation agents and helping remineralise early cavities (Dawes *et al.*, 2015).

The caries process starts with a phase of nonbacterial enamel crystal destruction. This happens when the amount of demineralisation occurring is greater than the capacity for remineralisation, such as occurs during frequent intakes of sugar in the diet. The acid by-products initially begin to dissolve the enamel in the weakest regions, between the surface perikymata, leading to an increase in porosity. The surface layer stays intact at about 1% porosity, due to proximity to mineral in the pellicle and oral environment, but the subsurface region, forming the body of the lesion, increases to about 25% porosity. This is surrounded by deeper progressing fronts of increasing porosity of approximately 4% in the dark zone and 1% in the peripheral translucent zone. At this stage there is no bacterial invasion and changes are mostly confined to the enamel, however if the porosity and noxious products extend deep enough along the enamel prisms changes in dentine can occur, as irritation of the odontoblast

processes can lead to laying down of tertiary and sclerotic dentine around the pulp or within the dentinal tubules respectively.

Clinically this early lesion is called a white spot lesion as it appears on the tooth as a white chalky lesion, which can become a discoloured brown spot lesion over time. If the balance of demineralisation/remineralisation is restored at this point the lesion will arrest and restoration of the tooth will not be required.

1.10.2.7 The Progressing and Advanced Lesion

Once the lesion progresses further cavitation will occur, bacteria will enter the tooth and decay progresses to the dentine. Clinically at this point, when any inflammation of the pulp is reversible rather than irreversible, restoration of the tooth may still be possible. Destruction spreads along the mantle dentine (surface dentine) and the enamel dentine junction.

As already discussed, in the early stages of enamel demineralisation, remineralisation is possible under favourable conditions, which clinically translates as the arrest in progression of the caries and hardening of the early lesion (Silverstone, 1977). Once the enamel is cavitated however, bacteria can enter, and the lesion will not generally arrest without clinical intervention. The caries and bacteria will then progress through dentine, along the tubules and towards the pulp. The dentine defence mechanisms include the laying down of sclerotic dentine, within the affected tubules, and tertiary dentine at the pulp dentine interface under the tubules that have been affected by the caries insult. This action of this defensive mineralisation ahead of the progressing lesions slows the caries and protects the pulp. Eventually however bacterial noxious waste products cause further demineralisation and inflammation of the central vascular pulpal tissues, causing pain and eventually pulp necrosis (Berkovitz *et al.*, 2018b). The process of caries progression is multifactorial with many elements such as saliva flow rate, saliva buffering capacity and mineral content, and the bacterial species present all interacting to favour either the remineralisation or demineralisation equilibrium in one direction or another. It can be seen therefore that the fundamentals needed for healthy teeth is conditions favouring optimal mineralisation, either during the formation of the tooth or in the maintenance of the tooth structures. It can also be seen that

saliva and its constituents play a major role in maintaining a healthy oral environment. This extends beyond providing the buffers and minerals to reverse early demineralisation, but also containing proteins with antimicrobial substances which help reduce bacterial load (Kidd, 2016). Unless the environment is modified, for example by placing a restoration or removal of plaque retentive factors, the carious lesion will progress finally causing pain, pulp necrosis and abscess.

For many years, it has been suggested that vitamin D deficiency may be a cause of increased dental caries. May Mellanby, an English physiologist and nurse, worked extensively during the 1920s when she completed a number of experiments from which she concluded vitamin D not only gave a protective effect against dental caries, but was also required for the healthy development of teeth (Mellanby and Pattison, 1928; Mellanby, Pattison and Proud, 1924; Mellanby, 1919; Mellanby and Pattison, 1932). More recently, a systematic review by Hujoel concluded that vitamin D supplements in early life could reduce the risk of dental caries. Taking supplemental vitamin D was associated with a significant reduction in the development of dental caries compared with no supplement (RR 0.53, 95% CI 0.43 to 0.65) (Hujoel, 2013). A further recent study by Schroth also showed an association between serum vitamin D and childhood caries in a Canadian cohort and suggested that supplementation with vitamin D may be a possible novel public health measure to tackle childhood dental caries (Schroth *et al.*, 2016). In contrast to the majority of published studies however Herzog did not find an association between serum vitamin D and caries risk. In that study of US population data, children aged 5-12 years old who had been examined as part of the National Health and Nutrition Study (NHANES), were grouped as being vitamin D deficient (serum 25(OH)D<30nmol/L) or inadequate (25(OH)D 30-49nmol/L). The total number of children in the adjusted analysis model was 1,137 of which 68 (5.98%) had deficient vitamin D status and 285 (25.07%) had inadequate vitamin D status. No association was found between vitamin D deficiency or inadequacy even after adjusting for age, sex, race and ethnicity, ratio of family income to poverty threshold and sugar consumption (Herzog *et al.*, 2016).

1.10.3 Vitamin D, Gingivitis and Periodontal Disease

The other common oral diseases considered here are gingivitis and periodontal disease. Gingivitis, which is inflammation of the gingivae, is a sign of poor gingival health and plaque control and was shown definitively by Loe *et al*. to be caused by substances derived from microbial plaque accumulating at the gingival sulcus and margin (Loe, Theilade and Jensen, 1965). It may be identified by bleeding on probing from periodontal pockets (depth <3.0mm) (Tonetti, Greenwell and Kornman, 2018). In some individuals, gingivitis can progress to periodontal disease (periodontitis) with further soft tissue inflammation and bone loss (periodontal pockets >3mm), due to a build-up of plaque and bacteria, mainly Gram-negative species, initiating an inappropriate host immune response. Immune cells then release mediators that cause local inflammation and destruction of the supporting underlying alveolar bone, increasing mobility of the teeth and can eventually cause tooth loss (Kinane, 2001). Several studies exist, consistently demonstrating an association between reduced vitamin D serum levels and poorer periodontal health. For example, Hiremath et al. showed in a randomised controlled trial (RCT) that supplementation with vitamin D led to a significant reduction in gingival inflammation and that the reduction was dose dependent, $p<0.001$(Hiremath *et al.*, 2013). Another study found that there was an inverse association between increasing serum vitamin D status and a reduction in bleeding on probing in a US population (Dietrich *et al.*, 2005). The odds ratio for bleeding for the highest quintile of 25(OH)D (median: 99.6 nmol/L) compared with lowest quintile (median: 32.4 nmol/L) 25(OH)D was 0.74 (95% CI: 0.64, 0.86). An increase in serum concentration of 25(OH)D of 30 nmol/L showed a linear association with sites having 10% (95% CI: 5%, 14%) lower odds for bleeding. The same group also found an association between low serum 25(OH)D and increased attachment loss in adults over the age of 50, but not in younger adults (Dietrich *et al.*, 2004). This was also supported by Millen who found higher serum vitamin D to be inversely associated with lower odds of gingival bleeding in postmenopausal women, OR 0.67, 95% CI 5-53 (Millen *et al.*, 2013). Miley reported that in subjects receiving periodontal therapy those that took supplemental vitamin D and calcium had shallower pocket depths, reduced sites with bleeding on probing, lower gingival index values, fewer furcation

involvements, less attachment loss and less loss of alveolar crest height than those who did not (Miley *et al*., 2009).

It is also known that periodontal disease has a bidirectional relationship with other diseases of chronic inflammation and immunity, including diabetes (Shlossman *et al.*, 1990; Simpson *et al.*, 2015) and cardiovascular disease (Li *et al.*, 2014), both with inflammatory pathological pathways. This recognition that poor periodontal health can exacerbate systemic conditions is a drive to improve population periodontal health through regular dental care.

1.11 Literature Review Summary

Vitamin D is clearly vital to human health and yet the extent and direction of the relationship can often be unclear, as is the case with its relationship to oral health. There is consistent evidence from observational studies that higher vitamin D status is associated with improved oral health outcomes, yet there are few intervention studies to support a causal relationship, and there are many confounding factors. Whilst dietary sources of vitamin D can be significant in some individuals, for the vast majority of the population sunlight exposure is the main contributor for individuals in the UK. Given these observations, it was decided this project would investigate the relationship between sunlight, UV exposure and oral disease in adult and child populations in the UK.

1.12 Overall Aims and Objectives for this Thesis

AIM

To investigate the relationship between sunlight, UV exposure and oral disease in adult and child populations in the UK, with specific reference to dental caries and periodontal disease.

OBJECTIVES

To review available evidence of the effect of UV exposure on oral health outcomes by undertaking a systematic review, presented in Chapter 2.

To use the results of the systematic review to inform the design and methodology of the following ecological studies, described in Chapter 3.

To present and discuss the results of the ecological studies undertaken in Chapters 4 and 5.

To summarise the evidence presented throughout the thesis and conclude findings and recommendations in Chapter 6.

Chapter 2: A Systematic Review of Exposure to UVB Radiation and Oral Health

2.1 Background to the Review

In the early part of the 20th century, it was observed anecdotally that some populations had poorer oral health, with the focus of discussion being on dental caries having a varying geographical pattern of rates (East, 1939; Dunning, 1953). Discussion about possible causes for this variation included social factors, diet, mineral content of soil, humidity, temperature and sunshine hours (Blackerby, 1943; Hadjimarkos, 1956; Hadjimarkos and Storvick, 1950; Hadjimarkos and Storvick, 1951; Ludwig and Bibby, 1969). Currently in the UK a geographical variation in caries still exists, as shown in the Adult Dental Health Survey, with some areas of the country having higher caries prevalence than others (O'Sullivan *et al*., 2011). The University of Malmo regularly publishes global caries maps which continue to show a wide variation in dental caries throughout different countries of the world (Malmo University, 2014). The data shows reported mean decayed, missing, filled teeth (DMFT) for 12 years olds range from 0.2 in Bermuda to 6.9 in the Republic of North Macedonia (Kassebaum *et al.*, 2015). Current evidence suggests that most variations in oral health are explained by socioeconomic factors (Costa *et al.*, 2012), oral hygiene behaviours (Bellini, Arneberg and von der Fehr, 1981), diet, genetics and exposure to fluoride (Chapple *et al.*, 2017). Dental caries and periodontal disease are multifactorial in origin and a set of complex interactions and influences over the course of a lifetime affect oral health status (Ramos-Gomez, 2002; Genco and Borgnakke, 2013). It is known that environmental factors can play a role in the development of healthy teeth and the prevention of dental disease, a noticeable example of this being water fluoridation reducing dental caries (Buzalaf *et al.*, 2011).The early researchers were also aware of work completed by May Mellanby in the 1920s, who had reported in her experiments that vitamin D was required for healthy development of teeth and seemed to give a protective effect against dental caries (Mellanby., 1928; Mellanby and Pattison., 1932). They were also aware that formation of an antirachitic substance, a substance that protects against and is curative of the disease of rickets, occurred in the skin after exposure to sunlight. Exposure to sunlight, providing the same effects as from vitamin D intake, was thus proposed as one

possible reason for such geographical or seasonal variation in caries rates (McBeath, 1937; Erpf, 1938) .

Researchers in the 1950s (Sainsbury., 1956) performed a study in 300 five-year olds entering State school in Hertfordshire, England. The children's teeth were examined visually, using a lighted spatula but without the use of a dental probe or x-rays, for evidence of dental caries and hypoplastic tooth structure. This was indicated by decayed missing filled teeth (dmft) and normal or defective tooth structure. The children had been born in the years 1946-47 and total hours of sunshine and mean temperature for each of the months of birth during this time was obtained from the County observatory. The data were analysed to review correlations between month of birth, sunshine and temperature, and dental health status. These data are shown in the Table 2.1, modified from the original paper for presentation purposes. Findings suggested children born in winter months were more likely to have a higher mean dmft than those born in summer and those with defective enamel were also more likely to have an increased dmft, with the 20% of children who had enamel defects accounting for 39% of the carious teeth. When the data for the percentage of children with teeth showing no evidence of enamel defects, is plotted graphically against the monthly mean hours of sunshine, there is a striking visual correlation. Sainsbury reports a Pearson's r value of 0.5 for sunlight and 0.6 for temperature suggesting a moderate correlation. Whilst this age group would have mostly primary teeth present at the time of examination, those teeth would have still been forming at the month of birth and would not have been present in the mouth. The development of the crown, and therefore the enamel, of the primary teeth mostly takes place between 15 weeks in utero and 13 months post-partum with the incisor crowns completed by 4 months of age (Sunderland, Smith and Sunderland, 1987; Berkovitz *et al.*, 2018b). Therefore, those children born in summer months, or their mothers, would have potentially had higher levels of vitamin D at the time of their birth, but not necessarily in the 6 months prior to their birth during tooth formation. Later separate research showed that vitamin D production is not possible from approximately October to March at the latitude of the UK (Webb, Kline and Holick, 1988; Engelsen *et al.*, 2005), therefore children born in the winter months would not have had endogenous vitamin D and would have been

dependent on their mother's vitamin D reserves obtained through breast milk. It could be argued that those children born in 1946 had teeth erupted into their mouth longer at the time of examination and were therefore more likely to have caries. However, children born at the end of 1947 during the non-vitamin D producing months of September to December also had higher mean dmft even though their teeth were more recently erupted. At this time food rationing was still in place, until 1953, and sugar consumption was low, meaning the results of this study may not be translatable to today's populations (The National Food Survey Committee, 1956). Furthermore, factors affecting sunshine exposure such as clothing choices, housing and environmental factors and lifestyle would all have been very different.

Table 2.1 Relationship between Month of Birth and Caries and Enamel Defects. Adapted from Sainsbury (Sainsbury, 1956)

Caries	Period of Birth	Dec 1946-Feb 1947	March-May 1947	June-Aug 1947	Sept-Nov 1947
	Number of children	40	58	50	58
	Total dmft	184	194	170	225
	Mean dmft	4.6	3.3	3.4	3.9
	S.D of Mean	7.5	6.9	6.4	5.9
Enamel defects	Number of children	38	58	49	59
	Number with enamel defects (%)	13 (36)	15 (25)	9 (17.5)	8 (14.5)

More recently, studies have also associated lower levels of vitamin D with poorer gingival and periodontal health outcomes (Jonsson *et al.*, 2013; Miley *et al*, 2009; Millen *et al.*, 2013; Antonoglou *et al.*, 2013).

A summary of the literature relating to vitamin D and oral health has been discussed in chapter one and it can be seen the evidence is inconclusive. Whilst there are several publications relating vitamin D deficiency to caries or periodontal disease, others have found no significant relationships to be present.

2.1.2 Background to the Hypothesis

In order to explain why a relationship may exist between vitamin D and oral diseases of caries or periodontal disease, it is important to consider some developmental and physiological aspects of the dentition and oral environment. Consideration of these factors could identify stages in the development of oral disease that are potentially influenced by vitamin D, or the lack of it. These factors are described in the following sections.

2.1.2.1 The Development of the Teeth: Formation of the Dentition

The human dentition starts developing before birth. Initial enamel formation of the primary teeth starts from approximately 15 weeks *in utero* for the central primary incisors and complete enamel formation of the second primary molars, usually the final primary teeth to form, is at about 11 months old. The primary teeth then erupt into the mouth, on average, from 6 months of age until 3 years of age when all 20 primary teeth should be present. They are later replaced by the permanent adult dentition, which starts to form around the time of birth. The permanent dentition erupts from approximately 6 years of age and so a mixed dentition of primary and permanent teeth is present until approximately 12 years of age when the second permanent molars erupt. If third molars also develop, a full complement of 32 teeth will be present (Berkovitz *et al.*, 2018b). Given this lengthy development process, disturbances in mineral metabolism during formation of the teeth are not seen until several years later when the teeth erupt. Defects in enamel formation can take the form of hypoplastic enamel (thin or insufficient in amount), hypocalcified (deficient in mineral content but correct amount / thickness) or hypomaturation (organic matrix insufficiently removed). Therefore, enamel, unlike bone, is formed by apposition not turnover or remodelling, and once formed its overall structure cannot be altered. Underneath the enamel is the dentine layer containing

dentinal tubules, within which odontoblast processes are contained. The odontoblasts are cells of neural crest origin responsible for dentinogenesis and are part of the outer surface of the pulp.

2.1.2.2 Carious Disease Processes of the Teeth

When damage, decay or attrition occurs to the enamel or dentine, as described in chapter 1, the odontoblasts can lay down further areas of mineralisation, called tertiary dentine, which forms a reparative thickened layer to “repair” the teeth from within and prevent pain and inflammation of the pulp. The thickening of the dentine blocks the tubules and slows the progress of bacteria and their noxious by-products through the dentine, towards the pulp. This mineralisation process utilises serum derived calcium and phosphate (Berkovitz *et al.*, 2018a).

2.1.2.3 Development of the Periodontium

The periodontium are the tissues that support the teeth and are collectively made up of the alveolar bone, the periodontal ligament (PDL) and the gingivae. The alveolar bone is the compact bone that forms the tooth sockets, the periodontal ligament is the connective fibres that attach the teeth to the bone and the gingivae is the mucosal soft tissue which covers the periodontal complex. The periodontal ligament is of mesenchymal origin and is derived from the dental follicle, which reduces to a thin layer of connective tissue that will eventually become the PDL when the tooth erupts. It is composed of several connective fibres, 90% collagen (70% type 1, 20% type 3 and the remainder made up of types 5, 6, 9, 10, 12) and 10% other fibres such as oxytalin, elastin and elaunin. The surrounding ground substance consists mainly of water and also glycosaminoglycans, including hyalurin, proteoglycans and glycoproteins. Therefore, the main cell present is the fibroblast, which is responsible for the synthesis and degradation of the collagen fibres, which have a high turnover in periodontal tissues, and the ground substance proteins. Immune cells such as monocytes and macrophages are also found around the blood vessels and nerves in the PDL space and play a role in host defences against oral pathogens (Berkovitz et al, 2018b).

2.1.2.4 Disease Processes of the Gingivae:

It was shown definitively by Loe (Loe, Theilade and Jensen, 1965) that gingivitis, inflammation of the gingivae, is caused by substances derived from microbial plaque accumulating at the gingival sulcus and margin and presents clinically as red bleeding swollen gums. In some individuals this progresses to periodontal disease which is loss of the periodontal ligament and supporting alveolar bone. Initial gingivitis begins to manifest after approximately four days of plaque accumulation and is associated with ulceration of the gingival sulcus allowing infiltration of microbial substances into the tissues. Even in healthy tissues a constant flow of neutrophils is present providing a first line of defence against the oral flora, however the plaque and ulceration invokes increased migration of neutrophils into the gingival tissues in a classic acute inflammation reaction. There is also local damage of collagen in the gingival tissues caused by collagenases and enzymes released from neutrophils (Attstrom and Schroeder, 1979). The early gingivitis lesion develops approximately one week after initial inflammation (Page., 1976) and is characterized by infiltration into the inflammatory infiltrate of mostly T lymphocytes as well as macrophages and some plasma cells. Neutrophils continue to increase, vasculitis is present, the fibroblasts become altered and clinically gingivitis is now visible. Six to twelve days after the onset of clinical gingivitis there is a large increase in transmigrating leukocytes and the histological features in the gingivae are similar to delayed hypersensitivity reaction as the host is sensitized to the bacterial antigens via a specific T cell mechanism (Wilde, Cooper and Page, 1977). Once the established lesion is formed, which can take several weeks, the cellular infiltrate changes to mostly B lymphocytes and plasma cells as well as large numbers of neutrophils in the gingival pocket. The established lesions may remain stable or in some individuals progress to destructive periodontal disease lesions.

If the lesion progresses to periodontal disease the epithelium migrates apically along the root surface and leads to irreversible attachment loss, periodontal pockets and crestal bone loss. This is caused by the host response to the mainly gram-negative anaerobic bacteria found in the pockets and the histopathology is similar to gingivitis (Darveau, Tanner and Page, 1997).

2.1.2.5 Development of Alveolar Bone

In utero, the alveolar bone develops around the developing tooth germ and is separated from it by the enamel organ. First woven bone is laid down which is later replaced by lamellar bone. As the teeth erupt the bone resorbs and remodels around it and remodelling of alveolar bone can continue throughout life. The periodontal ligament extends from the bone into the cementum on the root surface. The bone and cementum are both mineralised tissues however cementum is avascular and does not continuously remodel as a normal physiological process. Therefore, the periodontium complex develops along with the roots of the teeth, which start forming once the crown is complete and coincides with eruption of the tooth (Berkovitz *et al*, 2018b).

2.1.2.6 Disease of the Alveolar Bone in Periodontal Disease

In periodontitis an exaggerated host immune response to Gram negative plaque bacteria occurs which leads to destruction of the alveolar bone and periodontal tissues. Bone resorption and remodelling is a normal physiological process, and it is osteoclasts, specialised migratory cells derived from haematopoietic stem cells, which are responsible for bone resorption. Osteoclast activity is therefore an essential requirement for alveolar bone loss in periodontal disease and it is influenced by both microbial and host-derived factors which can increase or modify osteoclast activity. Biologically active substances derived from bacterial plaque induce a local inflammatory response in the gingival soft tissues and periodontium. The cellular inflammatory infiltrate of T cells, B cells, macrophages, and neutrophils within gingival connective tissue is increased in disease states, along with associated secreted inflammatory mediators. These include amongst others, 1,25(OH)2D, parathyroid hormone (PTH), PTH-related protein, PGE2, thyroxine, and IL-11. The proinflammatory cytokines (IL-1 and IL-6, TNFs) and the immunoregulatory cytokines (IL-2 and IL-4, interferon gamma) have also been implicated in the disease process. (Hienz, Paliwal and Ivanovski, 2015)

2.1.3 Proposed Hypothesis of Action of Vitamin D in Periodontal Disease and Caries Disease Processes

To describe the proposed hypotheses for how vitamin D (and therefore solar UVB radiation exposure) can influence periodontal disease and caries, they will be split into pre-eruptive effects, local post eruptive effects and systemic post eruptive effects as these are key stages in the development of the dentition and the oral environment.

2.1.3.1 Hypotheses of Pre-Eruptive Effects: Caries

Hypotheses about the pre-eruptive effects of vitamin D on the development of caries are summarised as:

i. Directly related. Vitamin D is involved in calcium/phosphate metabolism and these elements are important for tooth formation. Poorer formation of the teeth may predispose to an increased risk of dental caries once the teeth erupt into the mouth.

During the formation of the teeth vitamin D is required during several of the stages of enamel formation and maturation. For example, Amelogenin and Enamelin, proteins associated with the formation of enamel, are downregulated in vitamin D deficiency. Down regulation of these proteins is thought to contribute to disorganisation of the enamel prisms, decrease of intraprismatic enamel and resulting enamel hypoplasia (Papagerakis, MacDougall and Berdal, 2002). Furthermore, Calbindin D9k (CB9k), an intracellular soluble calcium-binding protein associated with active calcium transport, is localised in maturation ameloblasts, where active calcium transport has been observed and is part of the enamel maturation process. Its expression in ameloblasts has also been shown to be regulated by vitamin D (Onishi *et al.*, 2008)

2.1.3.2 Hypotheses of Pre-Eruptive Effects: Periodontal Disease

It is less likely that vitamin D has pre-eruptive effects on the development of periodontal disease, which is reflected in the fact that periodontal disease overwhelmingly affects adult populations and increases with age (Genco and Borgnakke, 2013). Periodontal disease is characterised by the host response, which in some individuals is so severe that destruction of host tissues occurs.

Exposure to sunlight is known to be followed by a period of immunosuppression (Schwarz, 2008) and some studies have suggested a decrease in development of food allergy and eczema in later life if sun exposure is greater in childhood (Osborne *et al.*, 2012; Mullins and Camargo, 2012).

Systemically, the risk for individuals in the UK of developing autoimmune conditions, has been associated with season of birth, with increased risk being associated with periods of reduced sunshine, UVB radiation and UVB exposure in the second trimester of pregnancy (Disanto *et al.*, 2012). Locally, the immune response to periodontal disease does show some features common to other autoimmune reactions and is associated with diseases where immune regulation is disordered, such as diabetes (Kaur, Mohindra and Singla, 2017; Hasturk and Kantarci, 2015). There is also evidence that the immune suppression caused after UV exposure may modify the host response, or trigger a change in the immune system, initiating an autoimmune response (Ponsonby, Lucas and van der Mei, 2005). It may be therefore that in some individuals the immune system develops in such a way that autoimmune diseases and excess host response are more likely if vitamin D levels are low at certain times during development of the immune system.

Possible hypotheses about the pre-eruptive effects of vitamin D on the development of periodontal disease are therefore summarised as:

i. Indirectly related. UV exposure can modify the development of the immune system, possibly through increasing vitamin D production. This will impact on the individual immune response to plaque bacteria later in life once the teeth have erupted and possibly an individual's susceptibility to developing periodontal disease.

2.1.3.3 Hypotheses of Post Eruptive Effects

The proposed post eruptive effects on caries and periodontal disease can be divided further into local and systemic and are summarised here.

2.1.3.3.1 Hypothesis of Local Effects: caries

i. Vitamin D sufficiency may increase the amount of calcium and phosphate ions in the saliva, as it does in serum. Supersaturation of saliva with

these ions produces the remineralising properties of saliva and reduces caries progression. Vitamin D deficiency may decrease the availability of these ions in the saliva or plaque and so decrease the protective effect of saliva.

ii. Vitamin D will optimise the presence of calcium and phosphate in the blood and so could lead to more efficient formation of tertiary dentine. This is a reparative mineralising response to damage to the enamel and dentine which delays the progression of caries to the pulp (Goldberg and Smith, 2004). Improved deposition of tertiary dentine is the effect which was proposed by Mellanby in the 1920s (Mellanby and Pattison, 1928). Vitamin D is also purported to moderate inflammation. The inflammatory response of the pulp to carious lesions can cause pulp death if excessive, but moderate inflammation is important to stimulate the deposition of tertiary dentine (Cooper *et al.*, 2010).

iii. Vitamin D sufficiency may support saliva flow rates leading to a more effective anti-caries action from the saliva. Vitamin D deficiency has been associated with reduced parotid gland function and reduced volume of saliva produced (Glijer, Peterfy and Tenenhouse, 1985). Glijer et al showed that the primary source of parotid saliva calcium is the extracellular fluid, and the concentration of calcium changes in parallel with the changes in serum calcium. Optimum flow and composition of saliva is important for caries protection via the buffering and remineralisation processes.

2.1.3.3.2 Hypothesis of Local Effects: plaque

i. Vitamin D and its metabolites may be present in the saliva and gingival crevicular fluid. It is proposed that these metabolites have a direct effect on the pathogenicity of plaque bacteria, changing it in quantity or quality. It is known that UV exposure modifies the gut microbiome and similar could be possible for the oral flora (Bosman *et al.*, 2019).

ii. Vitamin D sufficiency leads to increased production of antibacterial peptides (AMP) such as cathelicidins and defensins, and Vitamin D supplementation is associated with increased AMP secretion in saliva (He *et al.*, 2016). This may modify the bacteria present in the oral environment and reduce the cariogenicity of the dental biofilm (Wang *et al.*, 2013).

2.1.3.3.3 Hypothesis of Systemic Effects: periodontal disease

i. As an immune moderator, sufficiency in vitamin D status is associated with a decrease in inflammation. As discussed previously, the increase in inflammatory mediators stimulates osteoclast activity leading to alveolar bone loss in some individuals (Hienz, Paliwal and Ivanovski, 2015). Gingival fibroblasts exposed to periodontal pathogens produce several pro-inflammatory cytokines including IL-6 and IL-8 (Steffen, Holt and Ebersole, 2000) whilst IL-1, TNF, IL-6 are found in gingival crevicular fluid of periodontitis patients (Reinhardt *et al.*, 1993) and IL-4, IL-5, Interferon gamma (IFN-gamma) and TGF- Beta are present in inflamed gingivae (Matsuki, Yamamoto and Hara, 1993).Vitamin D inhibits the production of cytokines (Baeke *et al.*, 2010) related to T1 helper lymphocytes such as IFN-gamma and interleukin -2 (IL-2) and may also reduce the production of these inflammatory proteins. The active metabolite of vitamin D reduces the expression of IL-8 in periodontal ligament cells when they are exposed to the bacteria *Porphyromomas gingivalis* (Tang, Pan and Zhao, 2013).1,25(OH2) D3 has also been shown to inhibit IL-1 beta, IL-6, and IL-8, TNF- alpha and neutrophil recruitment in many diseases of acute and chronic inflammation (Mei-Lang Xue, 2002; Takano, Mitsuhashi and Ueno, 2011). A small systematic review of three papers found that vitamin D sufficiency was associated with increased healing after periodontal surgery and this was attributed to moderation of post-surgery inflammation (Fakheran, Khodadadi-Bohlouli and Khademi, 2019)

ii. Vitamin D is integral to calcium metabolism and homeostasis. Vitamin D deficiency leads to increased calcium reabsorption from the bones and

can lead to decreased bone mineralisation which may be of relevance in periodontitis, a disease characterised by alveolar bone loss.

iii. Furthermore, certain Vitamin D receptor (VDR) polymorphisms are associated with vitamin D deficiency (Wang *et al.*, 2010) and some studies have found associations between periodontal disease and some genetic polymorphisms of the VDR receptor gene. Polymorphisms of the gene TaqI were associated with periodontal disease in a Chinese and Japanese population (Tachi *et al.*, 2003) and a Brazilian population (de Brito Junior *et al.*, 2004), although conversely Gunes did not find this in a Turkish population (Gunes *et al.*, 2008). This could be due to limitations of the different studies (e.g., design or sample size) or because the VDR polymorphisms have a different effect on periodontal disease in different ethnic groups.

2.1.4 The Development of Outcome Measures for use in a Systematic Review

These hypotheses discussed above were used to generate the possible outcome measures for a systematic review as shown in Table 2.2

Table 2.2 A List of Potential Outcome Measures Derived from Proposed Hypothesis of Action of Vitamin D on Oral Health

EFFECT	MEASURED BY	EXAMPLE OUTCOME
PRE-ERUPTIVE		
Poor mineralisation of teeth	Increased caries present	dmft / DMFT (Decayed, Missing, Filled, teeth) primary teeth = dmft, permanent teeth = DMFT
	Increased caries rate	New lesions over specified time
	Increased past caries experience	dmft/ DMFT primary teeth = dmft, permanent teeth = DMFT
	Increased dental abscesses	GA (General Anaesthetic) extractions Antibiotic use
	Increased childhood extractions	GA extractions/ admissions
	Increased enamel defects	Enamel defects score
Poor formation of salivary glands	Decreased saliva flow rate	Saliva flow rate
	Decreased salivary gland growth	Anatomical measurement

POST ERUPTIVE		
LOCAL		
Increased calcium and phosphate in saliva	Decreased caries	Saliva pH and mineral content dmft/ DMFT No. new carious lesions Clinically arrested lesions
Increased tertiary dentine	Increase in arrested lesions	Microscopic examination of exfoliated teeth
Antibacterial effect, reduced plaque	Decreased plaque deposits	Plaque score indices Gingivitis score indices Saliva content and protein analysis
Optimal saliva production	Decreased / Increased saliva flow rate	Saliva flow rate Analysis salivary content
SYSTEMIC EFFECTS		
Modified immune response	Less plaque deposits	Plaque score indices Gingivitis score indices
Modified inflammation	Less loss of attachment Less gingival inflammation	BOP (bleeding on probing) Pocket depth CAL (Clinical attachment loss)

2.2 Summary of Observational Epidemiological Studies, Geographical Distributions of Caries and Sunshine Hours.

The previous chapter and sections have summarised the hypotheses and possible mechanisms explaining how vitamin D deficiency may be a cause of poorer oral health, and also explained how vitamin D is obtained by exposure to sunlight. The literature on environmental ecological studies that show an association of poorer oral health, specifically difference in caries prevalence with varying sunlight exposures, will now be summarised. The purpose of this initial scoping review is to help demonstrate the background and context of the systematic review and was used to develop the systematic review protocol. The papers discussed do not fit the criteria developed for the review but do help to generate hypotheses and contribute to the overall narrative of the relationship between vitamin D, UVB and caries. No papers were found in the scoping review which addressed the relationship between UVB, sunlight and gingivitis or periodontal disease and so this is not discussed here.

A scoping search was completed using Medline to identify the studies that investigated sunlight or UVB exposure and geographical variation in oral health. Studies that had proxies for sunshine, such as number of cloudy days or mean temperature were also included. Four papers, plus one series of papers, discussed here as one study, were identified spanning from 1939 to 1982 (Table 2.3). Three of the papers used sunlight hours as the exposure of interest and two used proxies of mean temperature or number of cloudy days. The study population for five of the studies was composed of children between six and sixteen years, two of these only included male participants and three only included Caucasian populations. One study did include a small amount of data on child dental health from South Africa, but the majority of the study was a summary of dental data that had been collected from armed forces personnel in America and Australia and then correlated with the mean annual hours of sunshine. Although there were several outcome measures across the studies, all included some variation on the measure of dmft/ DMFT (decayed, missing, filled primary/ permanent teeth respectively).

East published his paper “Mean Annual Hours of Sunshine and the Incidence of Dental Caries” in 1939 (East, 1939). His study followed research suggesting a

seasonal variation in dental caries due to changes in the amount of sunlight throughout the year (Erpf, 1938). He first calculated the number of cavities (including permanent and primary teeth if present) in 12-14-year-old white boys living in rural areas of the United States. The mean annual amounts of sunshine hours for each area of interest were determined using data from the US Weather Bureau map and the areas were then categorised by mean hours of sunshine into four ascending groups. The number of subjects analysed from each geographical region varies, with the area with the most sunshine hours having 1,775 subjects and the second highest group having 48,718. The mean caries per 100 boys was compared for each group and from their results, the authors of the study concluded that the mean caries incidence varied inversely with the hours of sunshine in the area of residence.

Blackerby, in 1943, published a paper discussing the variations in intrastate caries rates that he had noticed in his work as a dental officer (Blackerby, 1943). He studied the caries experience of white schoolchildren in 39 counties in the state of Tennessee over a period of 3 years. The children included had primary, mixed and permanent dentitions and his findings were grouped by age but not sex. The examinations were all undertaken by the author who was therefore not blinded to the geographical locations of the schools. The State of Tennessee was divided into 16 East, 15 Middle and 8 West Counties. By grouping the schools into these three groups, the areas became large and it is possible that there was a wide variation of environmental influences within them. Blackerby then tested to see if any correlations between environmental factors and oral health outcomes existed and concluded that the data showed a correlation between increased sunshine and decreased caries. Cloudiness, which he used as an inverse measure of sunshine exposure, is however, a very crude measurement UVB radiation as many other factors such as time of day or year, hours of sunshine and altitude may affect overall UV exposure on the ground (Fioletov, Kerr and Fergusson, 2010). The poor exposure measure and lack of individual data are limitations of this ecological study.

Following this, Hadjimarkos, published a report (Demetrios M Hadjimarkos, 1950) and a series of papers from 1949-1956 (Hadjimarkos and Storvick, 1950; Hadjimarkos and Storvick, 1951; Hadjimarkos, 1956) looking at geographical variations in caries rates among different populations in the State

of Oregon. Caries experience was recorded as decayed, missing and filled teeth and the environmental factors considered included climatological data and altitude. Other factors such as fluoride, diet and access to dental care were also reported. The author discussed the results from all the papers and compared the oral health of the children in different regions and altitudes of the Willamette Valley region. He concluded that when data from all the studies were analysed the only significant factor noted was an inverse relationship between the prevalence of caries and amount of sunshine in combination with altitude.
Following this, in 1953, Dunning collated epidemiology studies which seemed to show a geographical or latitudinal variation in oral health (Dunning, 1953). His paper summarises large geographical studies of dental disease among relatively homogenous groups and plots the disease patterns onto maps.
The first study looks at the prevalence of dental defects among men rejected from conscription to military service due to poor oral health in the First World War and the results are based on examination of over 967,486 men. The second study by Ferguson gives the average DMFT per recruit by region, among 4602 white naval recruits seen in one induction centre in 1934. The third study by Nizel and Bibby in 1943 gives average DMFT for 22,117 soldiers, at an army camp in World War II. Dunning used internal comparisons to rank these studies for prevalence of oral disease by state. It was not possible to determine outcomes for every state and so for some states, where data were available for a larger area, the states in that area were pooled. The same ranking was then given to each state in that area. It is noted that this approach reduced the accuracy of outcome measures and is a limitation of the ranking system. When the mean rank of the states were plotted onto a map and contour lines drawn grouping the ranks by 10 as much as possible (lines drawn around states grouped 1-10, then 11-20 etc.) a pattern emerged. The pattern suggested that in the USA, using these studies, dental disease is more prominent at lower latitudes and nearer the seacoast. Some data were presented in the paper to demonstrate this. The correlation coefficient for States on the Atlantic coast between dental disease ranking and latitude was 0.844. The correlation coefficient for inshore states along the one hundredth meridian of west longitude is 0.923. The correlation coefficient reported between distance from seacoast along the forty third parallel and dental disease rank was -0.847.
Dunning repeated this process with studies from South Africa by Ockerse,

whose research recorded the percentages of children with dental caries in districts of South Africa. In the case of South Africa there was also a higher caries prevalence closer to the seacoast, reducing in the districts as they moved in land. More recent research has shown that people who live near the coast in the UK have higher levels of vitamin D (Cherrie *et al.*, 2015), although this cannot be generalised to other countries at different points in time as lifestyles may now be very different.

Finally, Dunning analysed the work of Andrews, who studied 2000 members of the Australian RAF in 1948. Again, he found that average DMFT increased in the Australian states as the latitude (degrees south) increased. The correlation coefficient was 0.771, but he was unable to show any correlation to the coast. This may be because the majority of the population lived near the coast and not inland. The latitude of a state however related to its mean daily sunshine, mean temperature and mean relative humidity. Dunning recognised in the papers that many factors affect the intensity of sunshine and the amount of UV radiation reaching the earth's surface and suggested a more accurate measurement of UV radiation, rather than sunshine hours alone, is required. He concluded that latitudinal patterns of caries exist in the United States, South Africa, Australia and New Zealand.

The limitation of this study is that associations like this can be spurious, especially when no individual data are considered (i.e., the ecological fallacy). The geographical areas considered were very large in many cases and it would be difficult to get a representative figure for either caries or UV radiation. The strength of this study is that it was a review of several studies and so the population numbers were large. The latitudinal pattern was consistently seen in all the countries studied, and in two hemispheres, thus it provided a plausible explanation in the form of UV radiation variation and vitamin D production. Populations now however, have very different diets and socioeconomic profiles compared to when these studies were undertaken, so caution should be taken when assessing the relevance to populations today.

In 1982, Valentine et al published a paper that investigated whether the influence of geographical variation could be detected in the prevalence of dental caries in children living in twelve areas of Burma (now Myanmar) (Valentine *et al.*, 1982). This study was more robust in its methods and tried to address potential confounders, as well as recording the characteristics of the

participants more accurately. Following on from a pilot study, 2,639 12-year-olds were selected for the main study. A cluster sampling method was used whereby a school in each of the twelve areas was assigned a number. A school number was then chosen randomly and all 12-year-old children in that school were examined, although it is noted the randomisation process was not described. In the process of examination and data collection, caries was recorded only if a cavitated lesion was present, and only permanent teeth were reported in the results. This recording method excludes non-cavitated enamel lesions or white spot lesions but does have the benefit of making the diagnosis of caries more reliable. The outcome was presented as DMFT and DMFT per 100 teeth. As Myanmar is 900 miles north/south and 250 miles east/west, the geographical variation of the schools was large and the towns were also classified as inland or coastal as well as by latitude. The most extreme point of Myanmar in the north is at latitude 28°32' N and the southernmost point is at a latitude of 09°59' N above the equator. It is mostly tropical and therefore length of daylight does not vary much between seasons. However solar irradiation still shows monthly variation and geographical variation with higher levels generally in the central dry zone and at decreasing latitude (Janjai, Masiri and Laksanaboonsong, 2013).

The results indicated that inland towns consistently had lower levels of caries than the coastal areas, with 62.3% (95% CI) of inland children being caries free, compared with only 48.7% (95% CI) of coastal children. The mean DMFT of the inland children was 3.82 (se 0.18), whilst the mean of the coastal children was 6.42 (se 0.24). There was also a significant correlation between latitude, hours of sunshine and DMFT counts. Caries decreased as latitude increased, and also as mean daily sunshine hours increased, which is in agreement with the earlier studies. The latitude in this study varied from 26’ north to 12’ north and so it is noted vitamin D production should have been possible all year round at these latitudes. The amount of sunshine actually observed on the ground however, would have been affected by different weather events. Although the results show these associations, the authors of the study felt that climactic differences were not in themselves the cause of caries variation, through for example the action of vitamin D, but rather that the hotter climates caused changes in the soil content of trace elements, which led to a protective soil composition. The authors also considered that this explained why caries

increased when closer to the coast, as soil content along a river also changes as it flows towards the sea. They did not specify whether other factors such as differences in diet, socioeconomic factors or healthcare access differed between children residing in coastal or inland areas.

In summary, several studies exist each demonstrating an association between increased sunlight hours or sunshine and lower rates of caries. The evidence suggests that this pattern is replicated in several countries and at different points in time. Conversely, it is also noted that these studies suffer limitations. Epidemiological studies such as these are considered relatively weak evidence (Coggon and Barker., 2003; Beaglehole, Bonita and Kjellström, 1993). Although individual data may have been collected for the oral health measures, such as DMFT or missing teeth, the participants are grouped to give mean levels of disease and so the exposure measures are not reported at individual level. Likewise, the exposure measurement is also relatively crude. By amalgamating individuals into large groups determined by geographical boundaries, and assuming the exposure is the same for all individuals in that region, an accurate assessment of exposure is not possible, only an overall average picture. These errors in exposure estimation can lead to bias in the measures of association with the outcome (Webb and Bain, 2011). Furthermore, the older papers had no blinding of the clinical examiners whilst only the most recent Valentine paper attempted to account for confounders. These older papers were also mostly descriptive in their results and lacked the robust statistical analysis that would be expected now. Finally, although an association may be shown with sunlight or sunshine hours this cannot be taken as evidence of causality. Whilst there is a correlation between sunshine exposure and vitamin D synthesis, it cannot be definitively concluded that an inverse relationship between sunshine hours and caries is explained by increased vitamin D synthesis. It is therefore suggested that to investigate further, future ecological studies should utilise more accurate UVB data, either at individual or small area level, to see if the associations between exposure and outcome persist.

Table 2.3 Summary of Ecological Studies Showing Associations between Sunshine Exposure and Oral Health

Study	Year	Country	Study population	Exposure	Outcome Measure	Results
East, 1939)	1939	USA	12-14yr boys, n= 94,337	Mean Annual Hours of Sunshine as 4 groups <2200/yr. 2200-2599/yr. 2600-2999/yr. >3000/yr.	Mean DMFT per 100 children	Caries varied inversely with sunshine hours. Increased sunshine hours gave protective effect.
Blackerby, 1943	1943	USA	6-15yr old white n= 26,576	Average no. of clear, partly cloudy and cloudy days As 3 groups.	Mean DMFT per 100 children	Inverse relationship between caries and sunshine. Lower no. of cloudy days seems to give protective effect.

Hadjimarkos papers in a series (Hadjimarkos, 1956; Hadjimarkos and Storvick, 1950; Hadjimarkos and Storvick, 1951; Demetrios M Hadjimarkos, 1950)	1949-1951	USA	White 14-16 yrs.' old	Average number of clear, partly cloudy and cloudy days.	Mean DMFT per child	Inverse relationship between caries and sunshine. Lower no. cloudy days seem to give protective effect
Dunning, 1953)	1953	USA South Africa	Male WWI army recruits 1918 N=967,486 N=4602 white navy recruits N=22,117 men Army camp WWII Children N=not stated	Mean Annual Hours of Sunshine Grouped as <2200/year 2200-2599/yr. 2600-2999/yr. >3000/yr. Mean Daily sunshine	Ranked states Mean no. dental defects/1000/state Ranked by mean DMFT/ recruit/ state Ranked states mean DMFT/ recruit/ state.	Inverse relationship between increasing sunshine and caries. Inverse relationship between increasing sunshine and caries. Inverse relationship between increasing sunshine and caries.

		Australia	Air Force N=2000	Mean Daily Sunshine Ranks states	Percentage of children with dental caries Mean DMFT / recruit/ state. Ranks states	
Valentine *et al.*, 1982	1982	Burma	12yr children Burmese N=2,639	Mean daily hours of sunshine	Mean DMFT Per cent caries free	Significant relationship between caries and sunshine. Caries reduces as sunshine increases

2.3 Methodology of the Systematic Review

Having developed an overview of the literature regarding sunlight, UVB exposure and caries the information was used to inform and develop the systematic review on the same topic. The purpose of this systematic review was to examine the existing evidence investigating the association between UVB exposure and oral health, focussing on studies that measured outcomes of gingivitis, periodontal disease and caries. This review aimed to determine whether increased UVB exposure improves oral health outcomes and whether this information could be used to inform novel interventions to improve oral health. A protocol was developed (appendix 1) and published on PROSPERO, the international database of prospectively registered systematic reviews, prior to commencing the review (Collingwood, 2016), and the methodology of the systematic review is discussed further here.

2.3.1 Protocol- Review Question

The overarching review question was defined as “Do human populations exposed to higher levels of UVB radiation have better oral health than those exposed to lower levels of UVB radiation” and was explored through two sub-questions:

Question 1: What are the effects of sunlight exposure on caries, gingivitis, periodontal disease and plaque deposits?

Question 2: “What are the effects of artificial UVB exposure on caries, gingivitis, periodontal disease and plaque deposits?

The light sources were separated into natural sunlight and artificial exposure as it was hypothesised that the spectrum of wavelengths in each light source could be different and have varying levels of UVA, UVB and UVC. Although plaque is not a disease of the oral cavity, it was included in the review as an outcome measure as it is a primary risk factor for gingivitis, periodontal disease and caries and essential for each disease process.

2.3.2 Details of the Conditions Studied

2.3.2.1 Dental Caries

The carious process has already been described in detail in chapter 1, to summarise here salivary glycoproteins and bacteria form a plaque biofilm on the surface of the teeth. The bacteria, mainly *Streptococcus mutans*, then use dietary fermentable carbohydrates, especially extrinsic sugars, in their metabolic processes producing acidic waste products. These acids lower the pH of the oral environment and cause dissolution of the hydroxyapatite into solution and its constituent ions (Kidd, 2016). In the early stages of enamel caries, before there is cavitation in the enamel, teeth can remineralise under favourable conditions, such as when the saliva is supersaturated with mineral or fluoride is present (Arends and Christoffersen, 1990). Once the lesion is cavitated and in deeper enamel and dentine, the caries cannot be reversed (Silverstone, 1977). Clinically early lesions can appear as white or brown discolouration of the enamel, later lesions present as black brown cavities in the tooth surface which may be soft or leathery in texture.

2.3.2.2 Gingivitis

Gingivitis is inflammation of the gingivae, which can progress to periodontal disease and bone loss in some individuals and is a sign of poor periodontal health and poor plaque control. Clinically it presents as superficial inflammation of the gingivae causing red, swollen, bleeding gums. The underlying bone is not affected. It is noted that studies relating to acute necrotising gingivitis will not be included in this review, as this is a separate disease of different underlying aetiology and not typical of chronic gingivitis.

2.3.2.3 Periodontal Disease

Periodontal disease (periodontitis) is chronic inflammation of the gingivae and periodontium. A build-up of plaque and bacteria can initiate a host response, which causes inflammation and destruction of the underlying alveolar bone and can cause tooth loss (Kinane, 2001). Clinically it presents as bleeding, inflamed gingivae, alveolar bone loss and the development of pocketing around the teeth. Eventually due to the loss of bony alveolar support

the gingivae can recede exposing the roots of the teeth and the teeth can become mobile, eventually exfoliating in extreme cases.

2.3.2.4 Plaque Deposits

Plaque is the build-up of bacteria and food debris to form a biofilm on all the oral hard tissue surfaces, clinically appearing as soft yellow/cream coloured debris. This biofilm is present in both oral health and oral disease, however the quality and quantity of the biofilm may differ for each state. The bacteria ferment dietary carbohydrate and produce acidic waste products, which lead to caries; others form noxious by-products and cause gingival inflammation. Therefore, the presence of oral plaque is a prerequisite for dental caries, gingivitis and periodontal disease (Marsh, 1994) and although plaque is not a disease of the oral cavity it will be included in this review as it is the significant risk factor for these diseases. Any intervention that reduces plaque deposits will reduce the risk of developing the oral diseases of interest.

2.3.3 Literature Search Strategy

A preliminary search was completed as part of an initial literature review of evidence of vitamin D, UVB exposure and gingivitis, periodontal disease and caries. This literature review used keyword search strategies in MEDLINE to identify relevant papers and previous reviews on this topic. The reference lists of papers and reviews were also searched for relevant papers to get an overview of the number and type of studies available. The findings of this initial searching informed the protocol full search strategy which was used on the following electronic databases.

- MEDLINE via EBSCO
- EMBASE via OVID
- Dentistry and Oral Sciences Source via EBSCO
- Cochrane Central Register of Controlled Trials- Wiley
- ERIC via EBSCO
- British Education Index via EBSCO

- Child development and Adolescent studies via EBSCO
- Proquest dissertation database via Proquest
- Web of Science via Thomson Reuters

Two databases, ZETOC Conference Proceedings and Scopus were removed from the published search strategy. No date restriction was placed on searches, which were conducted from earliest recorded records on the database to the date when the search was undertaken (20th October 2016). This was because initial scoping searches had suggested the number of papers available would be small and spanned across the 19th and 20th centuries. Once the initial search was completed the search strategy for MEDLINE was adapted for use in the other databases. The exposure search terms used in the protocol were as follows:

UV radiation OR UVB OR Ultraviolet OR Irradiation OR Broad spectrum light

OR sunshine OR sunbed OR sunlight OR Solar irradia*

OR Latitud* OR seasonal variation OR geographic variation

OR zodiac

Whilst the outcome terms were:

gingiv* OR periodont* OR carie* OR plaque

OR enamel hypoplasia OR enamel dysplasia OR enamel defects

OR tooth loss

OR DMFT OR DMFS OR PUFA OR CPITN

The review was undertaken between 20th October 2016 and January 2017. After the first database search results were returned the term PUFA was removed from the search strategy and the search repeated without this term. PUFA in dental epidemiology refers to the PUFA index, which is designed to evaluate the prevalence and severity of oral conditions resulting from untreated dental caries. It records the presence of severely decayed teeth with visible pulpal involvement (P), ulceration caused by dislocated tooth fragments (U), fistula (F) and abscess (A) (Monse *et al.*, 2010). It is also an acronym for

Polyunsaturated Fatty Acids and more commonly appears in the literature in this context, therefore its addition had very poor specificity without gain in sensitivity. In addition, several MESH terms were included in the search strategy and were derived from the individual search terms. The final search strategy is shown in Appendix 2 as it appeared for MEDLINE and was adapted as necessary to different databases. The search results were imported into Endnote and screened according to the review's inclusion criteria.

2.3.4 Inclusion/Exclusion Criteria

Only papers in English were included and this is recognised as a limitation of the review, however no translating services were available. Papers needed to relate directly to UVB exposure, either from natural or artificial sources, and needed to be a primary study. Whilst reviews of studies were not included in any narrative or analysis, reference lists of reviews found using the search strategy were backwards searched for relevant studies.

For question one studies had to include the following:

- A comparison of at least two populations, or of the same population, at two or more time points.
- Different sunlight exposures, including no exposure *versus* exposure or two different exposures (natural sources of varying UVB for example could be exposure caused by latitudinal variation, sunlight hours variation, seasonal variation, monthly variation).
- Prospective study design, assessing two points in time.
- Having the start of the study less than one year since changes in the UVB exposure status occurred
- A measure of the sunlight exposure or a proxy of exposure.
    - Suitable proxies included sunshine hours.
- Reports the oral health of the study population.

For question two studies had to include:

- A comparison of at least two populations, or of the same population, at two or more time points.

- Differing artificial UVB exposures in two populations/same population, including no exposure versus exposure or two different exposures.
- Measures of UVB exposure
- Outcome measures of oral health
- A prospective study design, assessing at least two points in time.
- Start of study less than one year since change in UVB exposure status.

For question 1 all types of longitudinal observational studies of humans were included in the analysis including ecological studies. Case reports, case series and cross-sectional studies were excluded, as were studies that looked at only temperature, season, month or latitude but no actual sunshine or UVB measure. This was because these were judged to be of insufficient study design for a systematic review or were considered poor UV exposure measures.

For question 2 the same criteria were applied as for question 1 except observational studies were excluded (i.e., the focus was intervention studies). The studies suitable for inclusion for question 2 had artificial sources of UV exposure, therefore, the researchers had control over the exposure, unlike natural exposures considered in question 1.

2.3.5 Outcome Measures

The primary outcome measures were developed from the scoping literature review and the proposed hypotheses of action of vitamin D as described in Table 2.1. In the scoping search some of the studies were several decades old and the measures of oral health reflected this in their obscurity. Therefore, to try and maximise the number of studies that could be included, the systematic review included any recognised or reproducible measure of caries, gingivitis, plaque or periodontal disease including the following:

Caries:

(Lifetime) DMFT (Decayed, Missing, Filled Teeth)

DMFS (Decayed Missing Filled Surfaces)

New carious lesions during a trial period, caries incidence

Lifetime Tooth loss

Gingivitis:

Any gingival bleeding index, for example the Loe and Silness Gingival Index (Silness and Loe, 1964; Loe, Theilade and Jensen, 1965)

Periodontal Disease:

Bleeding on probing

Pocket depths

Alveolar bone loss

Loss of attachment

Lifetime tooth loss

Community Periodontal Index (CPITN)

Plaque deposits:

Plaque scores including the Silness and Loe Plaque Index and the O'Leary Plaque Index

Any other published plaque score.

The above outcome measures could be obtained clinically and/ or where appropriate, for example in the case of caries or bone loss, radiographically.

Secondary Outcome Measure: As there are recognised risks to UVB exposure a secondary outcome measure of any harmful health outcomes, to the population, individual person or teeth, which were reported in any of the papers were included as secondary outcome measures.

2.3.6 Data Extraction

Three reviewers in total were involved in the screening process. JC the main reviewer reviewed all papers from all search strategies whilst the second and third reviewers, LB and CM (members of academic staff at Peninsula Dental School), reviewed a sample of the papers. LB and CM each screened half of the references obtained from the first database searched (MEDLINE by EBSCO) and requested full text for papers, as they felt necessary. For all studies that appeared to meet the inclusion criteria, or where the abstract did not contain sufficient information to determine if the inclusion criteria were met, a full report was requested. Duplicates that were created when papers were returned from more than one database were removed from the full list of returned papers, as were duplicates that occurred when the full text was requested by more than one reviewer. From the resulting list of full texts, a decision was made by JC whether the paper should be included in the final review according to the protocol criteria. CM and LB also each read all the papers recorded in the final review to confirm they agreed they met the criteria and should be included in the final review. Any disagreements were to be settled by a majority vote; however, none arose. Once a final list of included references had been confirmed JC read the full texts and completed the data extraction forms. In addition to conducting systematic searches of electronic databases, JC crosschecked the reference lists of key articles identified through the searches. This led to an additional eight studies being added for assessment via reading of the full text, of which one was included in the final review, giving a total of six studies altogether. When dealing with missing data, due to the age of the studies and limited resources authors were not contacted to request missing data.

The Cochrane data collection form for intervention reviews: RCTs and non-RCTs version 3 April 2014 was used to record data for each study in this review (Higgins, 2011).

For each study the following was recorded where available

- Year of publication, country of origin and source of any study funding
- The primary study aims

- Details of the participants including demographic characteristics (socio-economic status (SES), ethnicity), primary/permanent dentition and criteria for inclusion and exclusion
- Details of the type of intervention environment where it was delivered, who provided the intervention, comparator and co-interventions.
- Details of the outcomes reported, including method of assessment, and time intervals
- Details of confounding factors considered (potential confounders of relevance to this review include sealant use, exposure to other UVB sources, different latitudes of place of study, exposure to fluoridated water, dietary content, SES, ethnicity and the use of other fluoride sources)
- Details on comparability of groups with regard to confounding factors.
- Details on methods used to control for confounding
- Details on results and effect estimates
- Details of quality assurance/ risk of bias

2.3.7 Quality Assessment/ Risk of Bias

The Hamilton Quality Assessment Tool for Quantitative studies (Hamilton, 2008, (Updated 13 April, 2010)) was chosen for rating selection bias risk, design, confounders, blinding and data collection methods, withdrawals and drop-outs in the studies as strong, weak or moderate. This form was chosen as it allowed for multiple study types rather than only randomised controlled trials and therefore reflected the variation of study types in the review. The tool includes a dictionary to aid interpretation of studies.

2.3.8 Data Presentation and Analysis

2.3.8.1 Presentation

The results of the searches are displayed in Figure 2.1 according to PRISMA guidelines (Liberati *et al.*, 2009). A flow diagram provides the number of citations retrieved from electronic searches and summarises the number of articles retained at each screening stage along with reasons for any exclusions (Moher *et al.*, 2010).

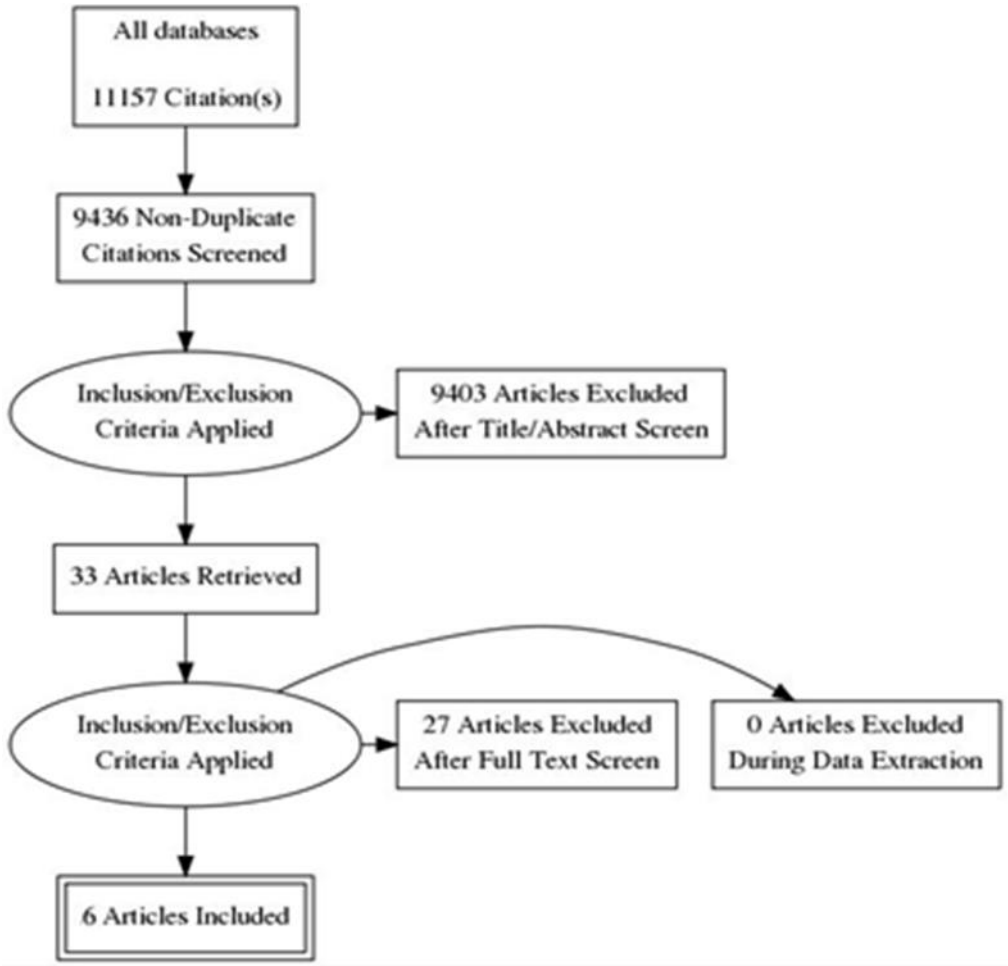


Figure 2.1 Flow Diagram of Study Selection

2.3.8.2 Analysis:

The analysis included all the papers identified irrespective of their quality assessment rating; however, papers were grouped for analysis according to those addressing question one and those addressing question two. Differences in how the UVB is produced, amount of UVB/sunlight exposure, outcome measures and technique of measuring the outcomes were all possible sources of clinical heterogeneity.

In this review a meta-analysis was not possible as the studies did not contain enough data for statistical analysis and there was heterogeneity between the studies in their outcome measures. However, a narrative synthesis was undertaken to compare the strength and direction of associations between UVB exposure (natural or artificial), and each of the oral diseases of interest. This narrative synthesis aimed to develop the theories of how the intervention of UVB exposure may work; develop a preliminary synthesis of findings of

included studies; explore relationships within and between studies and assess the robustness of the synthesis. Due to the age of the studies, they included minimal statistical analysis and so, where possible, the data were re-analysed to enhance interpretation.

2.4. Question 1: What are the Effects of Sunlight Exposure on Caries, Gingivitis, Periodontal Disease and Plaque Deposits?

2.4.1 Results

Of the papers included in the final review, only one investigated the effects of sunlight exposure on oral health using hours of sunlight per month as a proxy for sunlight exposure (Erpf, 1938). The paper did not present any outcomes on gingivitis, periodontal disease or plaque deposits therefore only caries outcomes can be discussed.

It is summarised in Table 2.4.

As stated, only one paper was found to meet the necessary criteria for inclusion, which was a prospective ecological study using a proxy for sunlight exposure and had a measurement of oral health made at more than one point in time. The subjects of the study are not specifically described in the text other than to say they have an adult dentition and the patients are recruited from the University of California dental department. Factors such as age, sex or SES are not presented or discussed so it was not possible to ascertain how these may have confounded the results. The proxy used to measure the exposure in this study was mean annual hours of sunshine for the geographical location of the study at different times of the year. This paper looked at seasonal variation in the caries susceptibility index (CSI), which is derived from the life caries index (LCI) over a period of two years, however; neither of these indices are used in modern epidemiology. Charles F Bodecker first described the life caries index in 1931 (Bodecker, 1939). There are 32 adult teeth normally, each with five surfaces, which gives 160 surfaces in total. For each carious surface a score of one is given whilst an extracted tooth scores three. The total number of carious surfaces a patient has is divided by 160, and then multiplied by 100 to give a percentage and this is the LCI. The difference between two LCIs is the CSI.

Table 2.4 A Summary of Papers Studying the Effects of Sunlight Exposure

Study	Year (of study)	Country and Location	Setting	Population	Control	Exposure	Fluoridated Water Area
Erpf (Erpf, 1938)	1934-1936	USA San Francisco latitude 37°º	Dental School Patients	Not stated Adults	Own control in ecological study	Seasonal variation of sunshine hours	Water not artificially fluoridated. Natural levels unknown

Table 2.5 Summary of Data (Erpf, 1938)

Month	Mean Hours of Sunshine	Season	Mean seasonal sunshine hours	Mean seasonal CSI %
September 1934	280	Fall 1934	218.33	4.36
October	225			
November	150			
December	190	Winter 1934	175	4.76
January	175			
February	160			
March	280	Spring 1935	276.67	2.72
April	260			
May	290			
June	380	Summer 1935	336.7	2.28
July	350			
August	280			
September 1935	180	Fall 1935	216.7	2.7
October	260			
November	210			
December	175	Winter 1935	158.33	2.87
January	140			
February	160			
March	190	Spring 1936	236.67	1.08
April	180			
May	340			

June	350	Summer 1936	338.33	0.58
July	375			
August	290			
September 1936				

2.4.2 Analysis- Effects of Exposure

The paper included mean CSI measurements for the population group taken over the duration of the study on eight different occasions, each occasion being a different season. The data were presented as a table for the mean CSI% and a line graph for hours of sunshine. The measurements used for the analysis, which was completed by the author for this review as additional analysis using the data from the published paper, are approximated from the graph in the paper and shown in Table 2.5. As individual markings are not present on the x and y axis the intersects are estimated. Using the estimated data, it was possible to calculate values for mean sunshine hours per season for eight consecutive seasons from fall 1934- start of fall 1936. This was plotted against Mean CSI on a scatter plot (Figure 2.2).

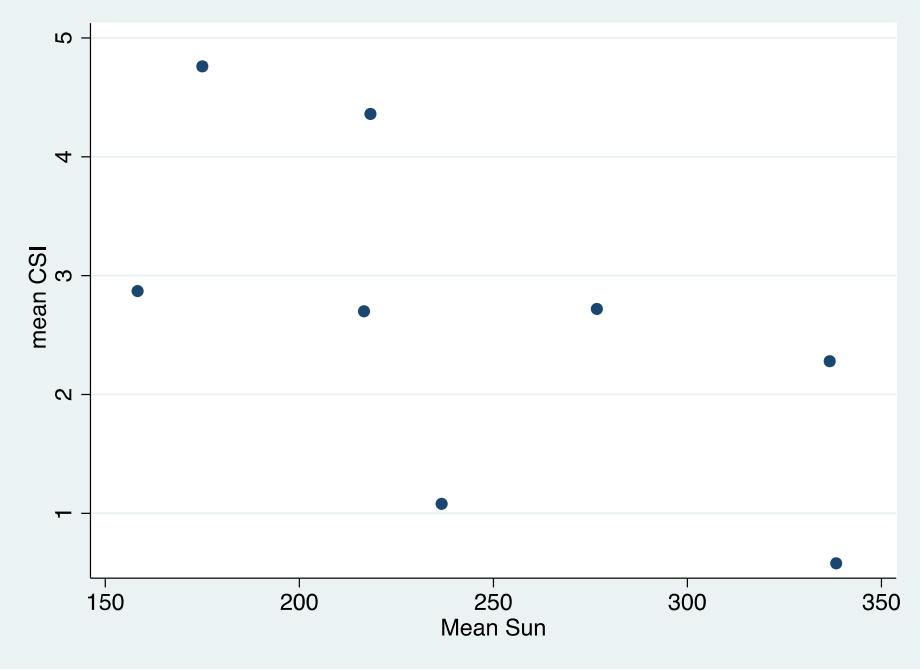


Figure 2.2 Mean CSI/Season Plotted Against Mean Sunshine Hours/Season (Estimated) Correlation (Coefficient of -0.74, p = 0.037).

As there are only eight values it was not possible to determine whether the data were normally distributed, therefore it was decided to use Spearman's coefficient to estimate correlation between the variables. The results demonstrated a correlation coefficient of -0.74, p = 0.037. Despite the small number of observations, this indicated statistical evidence of a moderate negative correlation between sunshine hours and the caries index.

From the limited data available in this paper, an increase in the mean sunshine hours in a season appeared to be associated with a decrease in the mean number of new carious lesions forming in the study population during that season. One possible reason for this association could be that there was an increase in endogenous vitamin D production and this had a protective anti-caries effect. However, there are many other possible factors that could explain this association. There may have been changes in diet throughout the year which showed seasonal variation, for example increased carbohydrate intake in cold weather or drinking more water in hot weather.

2.4.3 Discussion

This paper was published in 1937 and although it was very comprehensive for its time, it has many limitations. As stated previously the demographics of the study population were not considered making accounting for confounders impossible. Much of the data regarding the study population, selection criteria, recruitment and other factors were not reported and therefore the paper is rated as a weak study using the Hamilton Quality Assessment Tool for Quantitative studies (Table 2.6).

This study was undertaken in the US in 1936-7. The diet and demographics of UK populations today would be very different to this study population and so it would not be appropriate to assume these findings could be applied to modern populations. However, it is important to look at these older data sets as even though the clinical implications of findings may be different in modern populations the underlying causative factors will remain unchanged.

A limitation of ecological studies is that it is not possible to know individual exposure, only population level data. As it is not known how much time the study participants spent in the sun, it cannot be assumed their

individual exposure corresponds to the mean sunshine hours for the area. Finally, although the Spearman's coefficient showed a statistically significant negative correlation between increasing mean hours of sunshine and a decrease in mean new carious lesions, this was calculated from a small sample of only eight values. No conclusions can be drawn from such a small data set, although the analysis was still felt to be useful in the context of the prior literature review and proposed hypothesis that increased UVB exposure leads to better oral health

Table 2.6 Quality Assessment for Erpf Study (Erpf, 1938)

Domain	Quality Assessment	Reason
Selection Bias	Weak	Not described
Study design	Weak	Temporal ecological study
Confounders	Weak	Less than 60% of confounders accounted for
Blinding	Weak	No blinding described
Data Collection Method	Strong	Previously published methods used to record data
Withdrawals and Dropouts	Weak	Withdrawals nor reported. Cannot determine the percentage of participants completing the study.
Overall rating	Weak	2 or more weak ratings

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2.5. Question 2: What are the Effects of Artificial/Controlled UVB Exposure on Caries, Gingivitis, Periodontal Disease and Plaque Deposits?

Five different intervention studies were identified from the review process. Three of the most recent studies conducted in the 1970s and 80s showed considerable homogeneity in their study design. These three studies were all conducted in schools and for the UVB exposure they used the same type of UV full spectrum bulb, Vita-Lite. This is advantageous for this review as it leads to some similarities in exposure dose. Two of these studies took place in Alberta, Canada and one in Florida, USA, two countries with similar standards of health provision, and all the studies were in areas with fluoridated water supplies. All the studies' participants were children, although two of the studies included children aged 10-12 years and one study had younger subjects aged 6-7 years. The latter study only looked at newly erupted permanent teeth and excluded the primary teeth making it more aligned with the other two prior studies in older children which also only examined permanent teeth. The other two, of the five studies included, are earlier studies and use mercury vapour lamps as the UVR exposure. Although, they again show some homogeneity to each other in that their institutional settings, exposure type and age groups were all very similar. However, the two studies used different outcome measures for recording disease. The studies are summarised in Tables 2.7 and 2.8. Given the similarities between some of the studies they will be described in more detail below separated into two groups; those where the exposure is from a full spectrum light bulb in the form of environmental lighting to a classroom group, and those where the exposure is from a quartz lamp applying light topically to the skin of individuals.

Table 2.7 Summary of Study characteristics- Exposure: Mercury Lamps

Study	Year (of study)	Country and Location	Setting	Type of Study	Population	Control	Exposure	Fluoridated Area
Schoenthal (Schoenthal and Brodsky, 1933)	1929-1931	New York USA Latitude N40°	Dental Hospital	Intervention at individual level	Children 4-16yrs of age 48:52% M:F Primary and permanent teeth	Good diet no treatment Poor diet no treatment	UVB Exposure from Hanovia Chemical and Manufacturing company Mercury vapour lamp Good/Poor diet plus Light treatment 2 x per week, 6 weeks, starting at 2 min. duration increasing to 30 min. duration, approx. every 6 months	Not Recorded
Mcbeath (McBeath, 1937; McBeath, 1934)	1937	New York USA Latitude N40°	Institution	Intervention at individual level	Children 6-14 yrs. of age. Primary and permanent dentition	Institutional diet	Exposure twice weekly to back and chest under quartz mercury lamp, institutional diet.	Not Recorded

Table 2.8 Summary of Study Characteristics- Vita Lite Bulb, Environmental Lighting

Study	Year (of study)	Country and Location	Setting	Type of study	Population	Control	Exposure	Fluoridated Water Area
Mayron (Mayron *et al.*, 1975)	Sep 1973-June 1974	Sarasota, Florida Latitude N27°	School, 4 Window-less class-rooms	Inter-vention at populat-ion level	Children 1st grade permanent 1st molars	Conventional cool white lighting in 2 rooms	Full spectrum fluorescent lighting (Vita-Lite lightbulb) in 2 rooms	Yes Naturally fluoridated at "optimal levels"
Hargreaves (Hargreaves and Thompson, 1989)	June 1982-1984 22 months	Wetaskiwin, Alberta, Canada Latitude N53°	Element-ary School	Inter-vention at populat-ion level	Children Average age at start of study Exposure group 10.55 yrs.+/- 0.89 Control group 10.42+/- 0.58 Mixed dentition	Convention cool white lighting	Full spectrum fluorescent lighting (Vita-Lite Inc lightbulb)	Yes All sites 1.0ppm in water supplies
Hathaway (Hathaway, 1993)	June 1987-	Edmonton, Alberta Canada	Element-ary School	Inter-vention at	Children	Site 1: High pressure sodium	Site 3: Full spectrum fluorescent lighting, with	Yes All sites 1.0-1.1

	June 1989	Latitude N53°		populat-ion level	Average age 12.02 yrs. on June 30th 1989 Whilst in grade 5 and grade 6 No significant age differences between the sites Mixed/ Permanent dentition	vapour lighting Site 2: Full spectrum lighting, no UV enhancement-(Vita-Lite) Site 4: Cool white fluorescent lighting	UV enhancement-(Vita-Lite Duro-Test Canada, Inc) Site 5: Full spectrum fluorescent lighting, with UV enhancement-(Vita-Lite, Duro-Test Canada, Inc)	ppm in water supplies

2.5.1. Results: Mercury Lamp Studies

The first group to be discussed is that of the two older papers, where the UVB exposure was given in the form of mercury lamps, reported to have emitted wavelengths between 200 and 600 nm, therefore inclusive of the vitamin D active range estimated to be between 290-320 nm (Norval, Björn and de Gruijl, 2010; MacLaughlin, Anderson and Holick, 1982).

2.5.1.1 Schoenthal Study

The first of these is by Schoenthal (Schoenthal and Brodsky, 1933) published in 1933, which looked at the dentition of children of lower socioeconomic status, in a Dental Hospital in New York. Considering the date of publication, quite extensive baseline investigations were conducted within the initial exam. This included collecting data about the amount of baseline caries from clinical and radiographic examination, the parents' dental status, oral hygiene, cultures of oral bacteria, and blood tests of calcium and phosphate. Unfortunately, these data were not fully presented in the published paper and because of the limited development of statistical analysis at the time of publication, they were not used in regression models. In 1929, there were 319 subjects, and in 1930 a further 134 were added to the study, making this the largest study in this review. The subjects were allocated into groups and assigned to receive different diets, although the process of allocation was not described. The groups were classified as good diet, good diet plus vitamin D drops, good diet plus exposure to mercury vapour lamp (UV light), poor diet (control), poor diet plus vitamin D drops, poor diet plus fruit and poor diet plus exposure to mercury vapour lamp. Only the control groups and the UV exposure groups (good and poor diet) will be discussed here, as the impact of dietary vitamin D is beyond the scope of this review. A good diet, provided by supplementing with various food types, was defined as including three or four glasses of milk, one piece of fruit, one serving of green vegetables, one ounce of butter and fish or meat or eggs daily. A poor diet was deficient in these items and was considered to be a "normal" diet of the participants. The regimen for UV lamp exposure was twice a week for six weeks with exposure times increasing over that time period from two minutes to thirty minutes. This course was delivered three times over the course of the study period, approximately

every six months. The exact dose was not reported; however, it is reported in the paper that this treatment would be sufficient to cure rickets in children of this age group and so it has been assumed that this light exposure induced cutaneous vitamin D production. The study recorded and reported on the number of extractions the children had and the number of new carious lesions that occurred during the study period. This review, reported only on the number of new lesions not the number of extractions, as the number of extractions could be evidence of, and influenced by, disease present prior to the intervention being delivered. The new lesions are lesions that have formed during the study period after the first exposure to UV light were given and so these can inform whether the intervention has any effect on disease prevention. The study and results are summarised in Table 2.9.

Table 2.9 Number of New Cavities / Child (Schoenthal and Brodsky, 1933)

Group	No. of children	Average age	Period of observation	Average number of new cavities per child		
				Primary teeth	Permanent teeth	Total
Good diet	117	10.62	13 months	0.18	0.38	0.56
Good diet + UV lamp	12	9.6	13 months	0.25	0.33	0.58
Poor diet + UV lamp	11	9.7	14 months	0.45	0.73	1.2
Poor diet	46	12.32	13 months	0.09	1.87	1.96

As the raw data are not available and the groups were small, so it cannot be determined if the data are normally distributed. As no further data were given

in the paper, such as standard deviation around the mean, statistical analysis as to whether these results are significant is also not possible. There were also differences in the mean ages and time of follow up which make useful comparison between groups limited, and so it is difficult to draw conclusions from this study. As expected, those on the poor diet developed more new carious lesions than those on the good diet in their permanent teeth. The exposure to the lamp was however associated with fewer new lesions in permanent teeth amongst those with poor diet. In primary teeth the UV lamp seemed to offer no protection at all and those exposed to UV lamp developed on average more new lesions than those who did not receive the light exposure. Looking at the mean age of this group however, the remaining few primary teeth may have been close to exfoliation. If primary teeth with new lesions had been extracted or exfoliated, this would give an inaccurate picture of any effect the UV had on caries prevention. There appeared to be a protective trend in the permanent dentition, which was more prominent in those children on a poor diet, but as the number of children in the UV lamp exposed groups was very small, the result may have been inaccurate. In conclusion, no statistical evidence was presented in this paper to support a protective effect from UVB exposure, but the overall direction of findings appears to favour caries protective effect from UVB exposure.

2.5.1.2 McBeath Study

The second early paper, which is similar to the Schoenthal study, is a publication by Mcbeath and Zucker in 1937 (McBeath, 1937). It is discussed here along with a short paper, also by McBeath, published in 1934 that reports some further results on the final year of the full study (McBeath, 1934) and the papers are considered as one study within this review. This study again took place in New York, in six different sites (orphanages and schools), over four years, including children from 6 to 14 years of age. The children were also given different regimens including vitamin D rich diets, supplements and mercury vapour lamp exposures, but only the light exposure cases and controls will be considered in this review. The exposure regimen described was two exposures per week from a quartz mercury lamp 60 inches (152.4 cm) from the body,

starting with 30 seconds and increasing each week by a further 30 seconds up to a total of 12 minutes (24 weeks).

In the first shorter paper published in 1934 the outcome was recorded as the mean percentage of carious surfaces per mouth, and is presented here in Table 2.10. Although again, in this paper, not enough data was present to allow statistical analysis, there appears to be a general trend of less new lesions occurring in the UV exposure group.

Table 2.10 Change in Mean Percentage of Carious Surfaces per Mouth (McBeath, 1934)

Group	N (Sex)	Mean percentage of carious surfaces /mouth			
		Nov 1933	Feb 1934	April 1934	Overall Increase
UV exposed	29 (M)	5.55	6.58	7.00	1.45
Control	33 (M)	5.50	8.28	10.31	4.81

In the second paper published in 1937, and reporting on the full study over the preceding years, the trend persists (Table 2.11). These data also showed continued association between exposure to UV light and reduced incidence of new caries, but unfortunately as no other variables were considered, and other raw data or standard deviations were not supplied, statistical analysis is again not possible.

With regards to the secondary outcome measures considered in this review, it was reported that none of the children had any negative effects from the UV lamp treatment during this short study period.

Table 2.11 Change in Carious Surfaces/ 100 days/ Mouth (McBeath, 1937)

Study Site	Mean increase in carious surfaces/ 100 days/ mouth (dates between examination)	
	UV exposed	Control
X	**0.27** N=19 (3rd Dec 1931 - 27th May 1932)	**1.93** N=23 (3rd Dec 1931 - 27th May 1932)
Z	**0.97** N=27 (18th Nov 1932 - 2nd May 1933)	**2.92** N=26 (3rd Nov 1932 - 9th May 1933)
Z	**0.96** N=26 (11th Oct 1933 - 24th April 1934)	**3.09** N=29 (11th Oct 1933 - 24th April 1934)

2.5.2 Results: Environmental Lighting Studies

The second group of later twentieth century studies used environmental lighting in the form of classroom lighting bulbs (Vita-Lite) to deliver the UV exposure.

2.5.2.1 Mayron Study

The first discussed here, a small study conducted from September 1973-June 1974 (Mayron *et al.*, 1975), is the first of the more "modern" studies conducted in the latter half of the twentieth century. There was an initial pilot study conducted for a short period from January to June 1973, which did not look at dental outcomes, only at hyperactivity levels. Following this pilot study, the children from the pilot group moved into 2nd grade and were included in the second study along with new 1st grade students. This second main study included measures of oral health at the start and end of the trial period. The results relating to these 2nd grade students have been analysed here separately from the main study population, even though they were reported in the same

publication. This decision was taken by the author as some of these children swapped classrooms after the pilot study. Therefore, there were four possible subgroups: pilot study exposed and main study exposed, pilot study exposed and main study unexposed, pilot study unexposed and main study unexposed and pilot study unexposed and main study exposed. These groups were an older age group than the new 1st grade students and had been in the study for a longer period of time. They did not undergo dental examination at the start of the pilot study. It was therefore felt that there was too little clinical homogeneity between this cohort and the new 1st grade students to be considered the same study population, so the decision was taken to analyse the subgroup of second year students separately.

The mean age of the children was not reported, however 1st grade children in the United States are 6/7 years of age and the study looked at the 1st permanent molars, which erupt approximately at this age, as well as the presence of disease in these teeth. The only oral health outcomes measured were the number of children with carious 1st molars, the number of new cavities in the group and the number of children who developed new cavities. No measures of oral hygiene or gingival/periodontal health were reported and so cannot be commented on here. Furthermore, no confounding factors, e.g., diet, oral hygiene or socioeconomic status were reported, so this study still scored weakly on the quality assessment tool outcome shown in detail in Table 2.12

Table 2.12 Quality Assessment Rating (Mayron *et al.*, 1975)

Domain	QA Rating	Justification
Selection Bias	Moderate	Likely to be representative but participation rates not reported
Study design	Strong	Non randomised intervention study
Confounders	Weak	Less than 60% of confounders accounted for
Blinding	Weak	No blinding described
Data Collection Method	Moderate	Previously published methods used to record data- valid Recorded by dentists, calibration training not reported- reliability not confirmed
Withdrawals and Dropouts	Strong	Withdrawals reported in text. 30/128 (23%)
Overall rating	Weak	2 or more weak ratings

From the available data for the 1st grade students in the main study it was possible to complete the following descriptive data and analyses. There was a small sample size consisting of 18 children in the full spectrum light rooms and 24 in the cool white light rooms. The distribution of new carious lesions over the study period can be seen described in Figure 2.3. This demonstrates that a greater number of children developed new lesions in the cool white light group than the full spectrum classrooms, where the majority developed no new lesions.

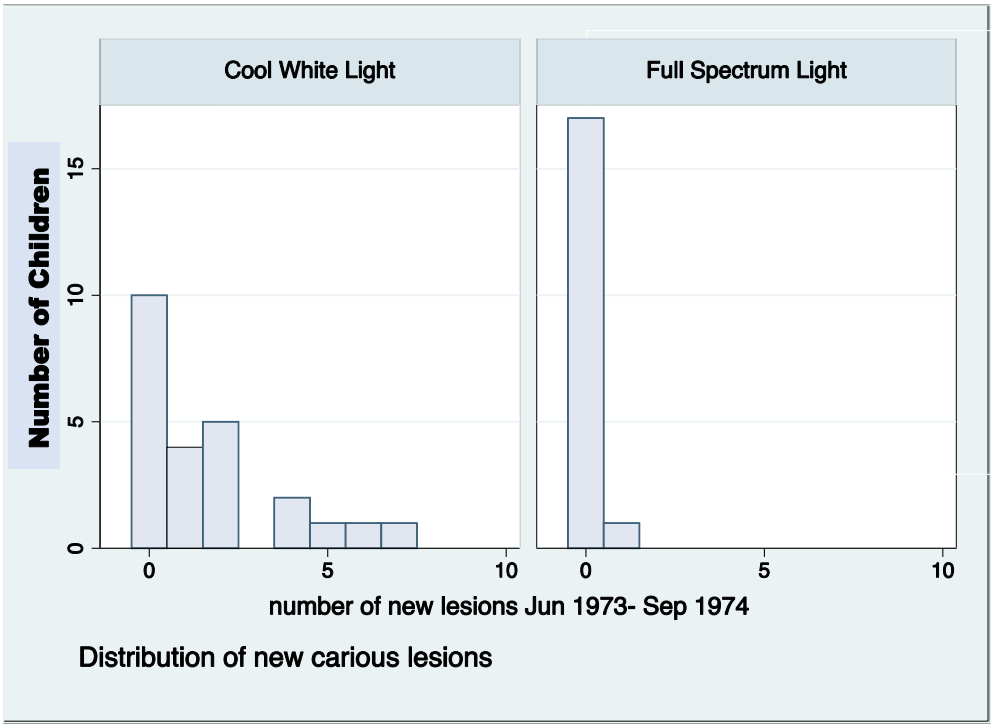

Figure 2.3 Distribution of New Carious Lesions over Main Study Period (Mayron *et al.*, 1975)

The data presented in this paper were then used to complete further analysis for this review, using a statistical software package (Stata, College Station, USA). Looking at the number of lesions in children at the beginning of the study, 18 out of 18 children in the full spectrum group had no carious lesions in their first molar teeth at the beginning of the study. Only 22 out of 24 had no lesions at the start of the study in the cool white group. In order to calculate an odds ratio (OR), the data were analysed according to whether any new caries developed over the study period. When comparing the number of children who

developed new lesions in the exposed group, to the number of children who developed new lesions in the unexposed group, the odds ratio was 0.095 with a 95% Confidence Interval (CI) (CI 0.014 - 0.660 and p=0.0012). The confidence interval is wide due to the very small sample size, but these statistics suggest that the true number of children developing new carious lesions in the exposed population is reduced between 98.62 % and 34.09%, being statistically significantly different from a null reduction (of zero).

Observing the pilot study separately, the distribution of new carious lesions is shown in Table 2.13. These data can be used to calculate OR for developing new lesions for those previously in Cool White classrooms (no previous UVB light exposure in the pilot). For this group of students, exposure to full spectrum lighting gave no protective effect (OR 0.94 95% CI 0.33- 2.66 p=0.92). With the second group of students (those with previous exposure to full spectrum lighting in the pilot study), exposure to full spectrum lighting was associated with an OR of 1 (95% CI 0.24, 4.13. p=1) and again no protective effect was observed. The number of children in these groups was small and so this may have made statistical analysis inaccurate.

Table 2.13 Distribution of New Carious Lesions Mayron Pilot Study (Mayron *et al.*, 1975)

Number of new lesions	Exposed to CW (cool white light) Jan- May 1973		Exposed to FS (full spectrum light) Jan- May 1973	
	Exposed to CW Sep 1973- May 1974 N=20	Exposed to FS Sep 1973- May 1974 N=8	Exposed to CW Sep 1973- May 1974 N=8	Exposed to FS Sep 1973- May 1974 N=20
0	12	5	6	15
>0	8	3	2	5

2.5.2.2 Hargreaves Study

The Hargreaves study (Hargreaves and Thompson, 1989) published in 1989 was conducted in 1982-84, before the Hathaway study, but with similar methodology. The two studies were clinically very similar, with the exposure source in both provided by full spectrum Vita-Lite lights. For the Hargreaves study, children in Grades 5 and 6 (mean age of approximately 10.5 years) were placed in classrooms which had either full spectrum lighting, or cool white fluorescent lighting. They were observed over 22 months. The outcome measures differed slightly from the Hathaway study, as Hargreaves measured missing teeth and not just extracted teeth. Hargreaves also reported on oral hygiene and gingivitis indexes. Furthermore, the control and the exposure groups were in two different schools, rather than two different classrooms within the same school. This meant increased likelihood of differences between the participants area of residence and potentially the schools, for example environmental features, socioeconomic status, which may have decreased homogeneity between the experimental and control groups.

The examiners subdivided the caries into caries 1-3. Teeth could be classified as sound or caries 1 (minimal enamel defect detected by a catch with a sickle probe or a decalcification without enamel penetration), caries 2 (marked involvement of enamel and/ or dentine with detection by a sticking sickle probe or obvious tooth loss from caries), or caries 3 (severe tooth loss from caries with probably tooth death). Results were presented with and without the inclusion of caries 1 lesions, as seen in Table 2.14. Caries 1 lesions are very early enamel lesions, which have the potential to remineralise and repair, as described earlier in the chapter. As can be seen when caries 1 lesions are included the mean number of new carious lesions in the UV treatment group is negative as lesions present at the start of the study were no longer recordable at the end. With regards to measures of oral hygiene, Hargreaves used the modified debris index of Greene and Vermillion (Greene and Vermillion, 1964) as an Oral Hygiene Index and the index of Loe and Silness (Silness and Loe, 1964) as a gingivitis index.

The Modified Greene and Vermillion index is a simplified version of the original index, which examines a reduced number of teeth, being six rather than twelve. In the posterior portion of the dentition, the first fully erupted tooth distal

to the second premolar is examined, usually the first molar, but the second or third molar can be used if this is absent. The buccal surfaces of the selected upper molar and the lingual surfaces of the lower molar are inspected for plaque. In the anterior portion of the mouth, the labial surfaces of the upper right and the lower left central incisors are used. In the absence of either of these anterior teeth, the central incisor on the opposite side of the midline can be substituted instead. The plaque is graded as 0 (no plaque), 1 (soft debris/plaque covering up to a third of the tooth, or presence of staining), 2 (plaque covering up to 2/3rds of the tooth) and 3 (plaque covers more than 2/3rds of the tooth). The final score is the total of each of the individual surface scores, divided by 6 which is the number of teeth examined. The process is repeated for calculus using the same scoring system as plaque. The two are then added together to give the final debris score as a measure of oral hygiene.

The modified Loe and Silness score is used to record the presence of plaque on 6 teeth, the 16, 12, 24, 44, 32 and 36. If these teeth are missing no alternative teeth are used. All four surfaces of the teeth are examined and scored according to plaque present as 0 (no plaque), 1 (a film of plaque adhering to the free gingival margin and adjacent area of the tooth), 2 (moderate accumulation of soft deposit) or 3 (abundance of plaque). The scores for each tooth are added together and divided by 4, the number of surfaces and then all 6 scores are totalled and divided by 6 to give the average per tooth and the final score for the patient. The process is repeated for the gingival index using the same teeth but with the criteria for gingival inflammation. That Is 0 (normal gingivae) , 1 (mild inflammation, slight change in colour, bleeding on probing), 2 (moderate inflammation, redness, oedema and glazing, bleeding on probing), 3 (severe inflammation, marked redness and oedema, ulceration, spontaneous bleeding).

It can be seen the children in the exposure group developed less new caries than those in the control group and both groups showed a small improvement in oral hygiene measures (Table 2.15). There were, however, differences between the groups at baseline level with the control group having worse levels of oral hygiene measures initially, which would have potentially given them a higher risk of developing new carious lesions. When the oral hygiene index was measured again at the end of the study the oral hygiene of

both groups had improved, with a greater improvement in the control group. Clinically a score of 1 on each tooth in the OHI index would give an overall score of 2 (debris score 1 + calculus score 1) so in clinical terms the mean scores are reasonably low to start with in both groups. Likewise, the baseline DMFT and DMFS in the control and exposure groups was (DMFT) 2.56 control group and 3.56 experimental group, and (DMFS) 3.86 control group and 5.03 experimental group respectively giving the experimental group higher previous caries experience than the control group at the start of the experiment. At the end of the experiment the DMFT and DMFS was 3.43 and 4.83 for the exposure group and 5.00 and 6.92 for the control group. It could be that the exposure group, having a higher DMFT and DMFS, were already in active treatment with their dentists and this may have influenced both the development of new lesions and their oral hygiene, which was better than the control group.

There was a difference in the number of new carious lesions between the groups, with the UV exposure group appearing to show repair of early lesions over the 22-month period. When caries 1 lesions were included (minimal enamel defect without enamel penetration) the mean decayed, missing, filled surfaces of the treatment group decreased slightly (-0.2) over the 22 months suggesting there was remineralisation of some early lesions. In contrast, the DMFS for the control group increased by nearly three surfaces during the same period.

The exposure group also developed less new cavities when the caries 1 early lesions were excluded from the analysis. When only caries that had marked enamel or dentine loss and cavitation were included (excluded caries 1) the control group developed a mean of 2.14 more carious surfaces and the exposure group showed an increase of 0.56 surfaces, again showing a distinct reduction in the exposure group in the caries incidence compared to the control group. Hargreaves reports that the differences between the two groups are significant ($p<0.001$) although the raw data and details of the analysis are not provided. These results may have been due to the effect of the UV exposure but could also have been caused by having better oral hygiene and increased exposure to fluoride topically causing remineralisation of early lesions. The limitation of the DMFT score is that the individual elements were not recorded. Children with higher missing and filled scores may have been more likely to

have been under the care of a dentist whilst those with higher caries scores may not necessarily be receiving treatment if these were asymptomatic. Therefore, it is the changes including the caries 1 lesions that are most interesting in this study as this is disease/ remineralisation process that is active during the exposure period.

When considering the secondary outcomes of this review, this study was part of a larger study (Wohlfarth, 1986) that also compared the number of days absence due to illness and classroom behaviours between the two groups. Full spectrum light in classrooms was also associated with reduced absence and no negative effects were reported during the study period.

Despite scoring strongly in four categories used for rating in the quality assessment tool, this study was marked moderate overall due to there being one weak rating in the consideration of confounders section. This still makes this study one of the stronger studies in the review

Table 2.14 Caries incidence: Mean Number of New Carious Lesions over the Study Period

Study (Hargreaves and Thompson, 1989)	Including caries 1				Excluding caries 1			
	Non-UV Control Group		UV Treatment group		Non-UV Control Group		UV Treatment group	
	DMFT	DMFS	DMFT	DMFS	DMFT	DMFS	DMFT	DMFS
Mean baseline	2.56	3.86	3.56	5.03	1.49	2.32	1.77	2.67
Mean at final review	5.00	6.92	3.43	4.83	3.17	4.46	2.40	3.23
Difference in means	2.44	3.06	-0.13	-0.20	1.68	2.14	0.63	0.56
Standard Deviation +/- (difference in means)	2.43	3.39	2.02	2.89	1.90	2.59	1.28	1.64
Number n	52	52	31	31	52	52	31	31

Table 2.15 Mean Change Oral Hygiene Indices.

Study (Hargreaves and Thompson, 1989)	Non-UV Control Group N=52 +/-SD		UV treatment group N=31 +/-SD	
	OHI	GI	OHI	GI
Mean Baseline score	1.02 +/- 0.37	0.74 +/- 0.33	0.84 +/- 0.37	0.60 +/- 0.35
Mean 22-month score	0.82 +/ - 0.41	0.65 +/- 0.57	0.72 +/ - 0.41	0.49 +/- 0.30
Mean Change	-0.2	-0.09	-0.12	-0.11

Table 2.16 Quality Assessment Rating .(Hargreaves and Thompson, 1989)

Domain	QA Rating	Justification
Selection Bias	Moderate	The children came from the local region of the school. Likely to be representative of the target population. 100% initial participation
Study design	Strong	Controlled trial
Confounders	Weak	Confounders not accounted for
Blinding	Strong	Participants and examiners blinded
Data Collection Method	Strong	Previously published methods to record data. Examiners underwent calibration
Withdrawals and Dropouts	Strong	Withdrawals reported. 83/102 finished study Controls 52/63 Experimental 31/39
Overall rating	Moderate	One weak rating

2.5.2.3 Hathaway Study

The last of the later 20th century studies is the Hathaway study (Hathaway, 1993; Hathaway, 1995), which ran for two years in schools in Alberta, Canada. The study was a large study over five sites, each with different lighting systems emitting varying amounts of UVA and UVB light spectra. Several health outcomes were measured, including dental caries. Dentists, who had undergone calibration training, which are not described in the paper, used a previously published examination technique suitable for epidemiological studies, to perform the dental assessment. No significant differences between the sites were reported for age, sex, daily nutrition, sugar intake or calorie intake at the start of the study. The average age of study participants was 12.2 years and the sex ratio was male: female 47.7%: 53.3%.

The five different sites had the different lighting systems installed and averages of light spectrum were recorded taking measurement in the participating classrooms. The UV measurements were taken using a UVX-36 broadband ultraviolet meter manufactured by Ultraviolet Products Ltd and an EL-791 Spectroradiometer System manufactured by International Light (a NBS traceable calibrated system was used to record the narrow band spectrum between 200-400 nanometers). As can be seen only three of the sites had any significant UVB present in the environmental lighting and site 4 only had very small amounts. If any effects of exposure to different class lighting were recorded, in order for vitamin D production to be a potential cause, the presence of UVB spectrum would be necessary. The study methodology described recording of oral hygiene using the Loe and Silness score however, only the results of caries outcomes were presented in their published report. Students at site 4 were found to have a high proportion of fissure sealants and the authors then decided to exclude site 4 from the results, these individuals therefore have missing data.

The results extracted from the paper are summarised in Tables 2.17, 2.18 and 2.19. The outcomes used were DEFT (decayed, extracted and filled teeth) and DEFS (decayed, extracted and filled surfaces). This was measured in the same way as decayed, missing, filled indices, but only extracted teeth not naturally exfoliated teeth were recorded.

The review also looked for reports of any negative outcomes associated with full spectrum lighting. Full spectrum light was reported as being associated with a positive effect on attendance rates, height gain and weight gain. In the context of secondary outcomes of this review, no negative effects were reported and the amount of UVA and UVB exposure given from the lighting did not approach recommended safety limits.

This study scored well overall in the quality assessment tool ratings; however, some drawbacks and limitations were still present (Table 2.20). The researchers could not randomise the students to each of the sites. This was not practicably possible as students could not be randomly assigned to schools or classrooms, rather the students' families and the school determined this. The researchers noted this point and stated they tried to accommodate for it, although did not explicitly describe how. Steps they took to reduce bias included the students and teachers being blinded to the research question and the dentists recording the oral health outcomes, being blinded to the type of lighting the children had been exposed to. Students at site 4 were found to have a high proportion of fissure sealants and the authors then excluded site 4 from the results after the study had started.

The overall trend again appears to be that exposure to full spectrum lighting reduces the occurrence of new cavities forming and that this effect is even greater in students who have not had fissure sealants placed. However, in Table 2.17 the number of students in each group was not reported in the original paper and analysis to determine statistical significance was not possible. It should also be noted that, although it appears that full spectrum lighting with UV spectra reduced the incidence of dental caries, the full spectrum light included increased amounts of both UVA and UVB. It is not possible to say therefore if either or both could be responsible for the differing outcomes.

Table 2.17 Mean Incremental Change in Caries Indices (Hathaway, 1993)

	UVA $\mu W/cm^2$	UVB $\mu W/cm^2$	Mean incremental increases in caries 1987-89 (students with fissure sealants (FS) included in the analysis)		Mean incremental increases in caries 1987-89 (students with fissure sealants (FS) not included in the analysis)	
Site			DEFT	DEFS	DEFT	DEFS
1. High pressure Sodium Vapour Lamps	0.21	0.0	1.13	1.43	1.3	1.72
2. Full Spectrum UV inhibited	1.01	0.0	0.68	1.18	0.7	1.33
3. Full Spectrum UV enhanced	7.2	0.3	0.42	0.4	0.22	0.14
4. Cool White Fluorescent UV enhanced (excluded due to FS)	0.87	0.07	*	*	*	*
5. Full Spectrum Fluorescent UV enhanced	5.18	0.18	0.21	0.34	0.19	0.19

Table 2.18 Effects of UV Lighting on Caries Outcomes, Individuals with Fissure Sealants Included. Incremental Increase in Caries 1987-1989 (Hathaway, 1993)

	Non-UV Control Group including (Sites 1,2)		UV treatment group (Site 3,5)	
	DEFT	DEFS	DEFT	DEFS
Mean	0.91	1.32	0.32	0.37
Standard Deviation	1.29	2.19	1.08	1.75
Number n	120	120	110	110

Table 2.19 Effects of UV Lighting on Caries Outcomes, Individuals with Fissure Sealants Excluded. Incremental Increase in Caries 1987-1989 (Hathaway, 1993)

	Non-UV Control Group including (Sites 1,2,)		UV treatment group (Site 3,5)	
	DEFT	DEFS	DEFT	DEFS
Mean	1.00	1.53	0.21	0.17
Standard Deviation	1.85	3.24	0.92	1.81
Number n	99	99	92	92

Table 2.20 Quality Assessment Rating Hathaway Study (Hathaway, 1995; Hathaway, 1993)

Domain	QA Rating	Justification
Selection Bias	Strong	Somewhat representative of population- not randomised but systematically chosen- all students in the year chosen. Consent for study not reported. All students in the year appear to have been included.
Study design	Strong	Controlled trial
Confounders	Weak	Author states tried to overcome lack of randomisation by statistical methods but control of confounders is not fully described. Not all relevant confounders discussed
Blinding	Strong	Participants and examiners blinded
Data Collection Method	Strong	Previously published methods used to record data. Examiners trained
Withdrawals and Dropouts	Strong	Withdrawals reported. 71.3% finished study 233/327
Overall rating	Moderate	One weak rating

2.5.3 General Characteristics of the Studies

Unfortunately, the studies did not provide enough statistical information or uniform methodology to assess heterogeneity and conduct a meta-analysis, therefore only a narrative review can be undertaken. However, a summary of the overall direction of findings is presented in Table 2.21 along with a summary of the quality assessment ratings of the papers in Table 2.22.

A total of 11,157 study titles and abstracts were initially identified, but after removal of duplicates and those papers that did not fit the review criteria, only six studies were left. Of these studies, five related to artificial exposure and one to sunlight exposure. In the studies that used artificial light sources, three studies used Vita-lite bulbs and two used mercury vapour lamps. The studies that used Vita-lite bulbs exposed the children every day to environmental lighting, whilst those using mercury vapour lamps gave the light as increasing doses twice a week to the child's back or chest. The latter was provided by health care workers. The doses given ranged from a starting does of 30 seconds, up to 12 minutes for a period of 24 weeks or in one study, with a starting dose of 2 minutes up to 30 minutes over the course of six weeks. The settings for the studies included two in a dental hospital, one in government institutions (orphanages) and three in state schools. Four were set in the USA and two were set in Canada. The three studies that used environmental lighting took place in areas with fluoridated water supplies. All the studies were cluster studies and did not present individual data, only group data and means. None of the studies included were RCTs, five were non-RCTs and one was a before and after study. Their publication dates ranged from 1929 to 1995 and the number of participants in the studies ranged from small cohorts of 18 to large studies with 120 children in some groups. All the artificial light studies included in this review covered populations of children, with ages in the studies ranging from 4 to 16, whilst the natural light study was conducted on an adult population recruited from a dental hospital. Four studies included only permanent teeth and three included mixed dentitions All the studies included some measure of caries incidence but only one study reported any oral hygiene measures. The measures used included DMFT, DMFS, CSI and new lesions over time but only one study differentiated caries types and included early enamel lesions separately.

2.6.4 Quality of the Studies

The quality of the studies ranged from weak to moderate (Table 2.21) as measured by the Hamilton Quality Assessment Tool for Quantitative studies (Hamilton, 2008, (Updated 13 April, 2010)) . None of the studies were randomised controlled trials and overall, four studies were scored as weak, two moderate and none as strong. One frequent limitation in the studies was the consideration of confounders, with no studies scoring strongly in this component, and the category of blinding, where only two studies scored strongly. Three studies did not report the study dropout rate. All the studies scored strong or moderate for data collection techniques as all but one used outcome measures that had been previously recognised and published.

Table 2.21 Summary of Quality Assessment Ratings

Study	Selection Bias	Study Design	Confounders	Blinding	Data Collection Method	Withdrawals and Dropouts	Global rating
(Erpf, 1938)	Weak	Weak	Weak	Weak	Strong	Weak	Weak
(Schoenthal and Brodsky, 1933)	Moderate	Moderate	Weak	Moderate	Strong	Weak	Weak
(McBeath, 1937; McBeath, 1934)	Strong	Moderate	Weak	Weak	Strong	Weak	Weak
(Mayron *et al.*, 1975)	Moderate	Strong	Weak	Weak	Moderate	Strong	Weak
(Hargreaves and Thompson, 1989)	Moderate	Strong	Weak	Strong	Strong	Strong	Moderate
(E. Hathaway, 1992; Hathaway, 1995; Hathaway, 1993)	Strong	Strong	Weak	Strong	Strong	Strong	Moderate

2.7. Discussion

2.7.1 Summary of Findings of the Systematic Review

All the studies included in the review consistently showed a trend of a protective effect for oral health associated with UVB exposure. A summary of the direction of evidence is shown in Table 2.22.

2.7.2 Strengths and Limitations

Whilst all of the papers in the review concluded that increased exposure to UVB, either from natural or artificial sources, decreased the incidence of new carious lesions forming, none applied robust statistical analysis or provided enough primary collected data to allow this to be undertaken. It was possible in some cases to undertake limited statistical analysis of data provided in the original paper, and while these analyses supported the protective trend, sometimes showing statistical significance, the sample sizes were often small and reduced accuracy, as previously discussed. It is also recognised that all the eligible studies were completed in the last century, with the most recent publication only in 1993 and the earliest in 1937. Since they were conducted there will have been many changes in the socioeconomic and ethnic make-up of the populations, as well as changes such as education, diet, healthcare provision and availability of fluoride to name a few. Therefore, it would be with caution that the protective trend shown in the review could be translated to modern society in the UK and assumed to persist in this environment.

2.7.3 Findings in the Context of Proposed Hypotheses

The findings if these papers can be considered in the context of the hypotheses presented earlier in the chapter on the possible mechanisms of action of UVB on oral health. For example, these studies can inform theories suggesting that changes in the oral environment occur with increased UVB exposure, in turn reducing formation of caries. The results of the Hargreaves study presented results showing remineralisation of early lesions when exposed to increased amounts of UVB in full spectrum light. Vitamin D sufficiency is required for optimum calcium and phosphate metabolism, therefore could

increases in vitamin D, due to exposure to UVB, lead to optimal levels of these minerals in saliva. Calcium and phosphate are the main constituents of hydroxyapatite, which is the basic crystalline unit of teeth, and can help remineralise areas of demineralisation, when present in saliva, to reduce risk of caries (Shaw *et al.*, 1983; Pearce *et al.*, 2002).

As previously discussed, teeth present in the mouth form several years before they erupt into the mouth, in the case of primary teeth this is before birth. The UVB in the intervention studies could not be affecting the development of already erupted teeth, or modifying their structure as they form. Therefore, evidence from these studies cannot provide support for the theories that relate to proposed pre-eruptive effects of vitamin D, such as increased mineralisation during tooth formation. These studies also do not provide enough evidence to support theories that exposure to UVB resulting in an increase in vitamin D, then leads to production of antimicrobial substances in the mouth, saliva or gingival crevicular fluid. If this was the case, it could be expected to see changes in the quality or quantity of plaque. Only one study provided data on oral hygiene and although the study did show an improvement in oral hygiene measures, such as plaque and gingivitis, the change was small and no conclusions could be drawn as insufficient data was presented to allow analysis of the effect.

There are other possible explanations for the differences in caries development when exposed to different levels of UVB and these also need to be considered. It is possible that seasonal changes in caries rates are due to changes in diet or intake of fluoridated water supplies, due to the changes in temperature that accompany the increase in sunshine hours. However, this would not explain the differences in the studies where the exposure is artificial, as the full spectrum lighting did not increase temperature in the way natural sunlight would. There is also evidence that exposure to light in general may increase saliva production (Shannon and Suddick, 1973). As saliva has protective buffering, antimicrobial and remineralising effects this would lead to a decrease in caries formation. Again, in studies showing seasonal change the longer daylight hours could support this alternative explanation. This would not however explain the improved oral health in the studies with artificial exposure, where all groups received the same number of hours exposure to light, either

with or without a UVB component in it. There is also evidence that teeth do show a maturation and an increase in mineralisation of their surface enamel shortly after eruption. This could partly explain the resolution of the early carious lesions (white spot lesions) seen in some of the studies (Cardoso *et al.*, 2009). Interestingly this decrease in porosity demonstrates a seasonal cyclic pattern with more mineralisation occurring in the summer months (Ten Bosch, Fennis-le and Verdonschot, 2000). If this were the explanation for the different formation of carious lesions however, it would be expected to be seen in both groups, not just the group exposed to UVB. Finally, it could be that the full spectrum light has antimicrobial properties unrelated to its vitamin D forming properties. UV light is known to be antimicrobial against species of oral bacteria (Takada *et al.*, 2017) and could have caused a decrease in environmental bacteria. This does not hold true for those children exposed to the mercury vapour lamps. These exposures would not have caused a change in the environmental conditions on a day-to-day basis, but would have led to an increase in vitamin D produced cutaneously. In the two studies which used the UVB in this way, the greatest improvement was seen in those children who were also on a poor diet, whilst less contrast is seen between those on a good diet. It is suggested therefore that perhaps UVB exposure enhances the effects of a good diet and reduced the effects of a poor one.

2.7.4 Is There A Difference in The Effects of Natural Sunlight Exposure and Artificial UVB Exposure?

The evidence synthesised in this review did not explicitly compare the relative impacts of natural sunlight and artificial UVB. Although both exposure types appeared to exhibit a protective effect, there was not enough quantifiable data in this review to compare natural sunlight and artificial exposure.

2.7.5 Suggested Future Work

Completion of this review highlighted the lack of good quality studies investigating the relationship between UVB exposure, from natural or artificial sources, and dental caries, gingival and periodontal health and dental plaque. There was not only a lack of RCTs but also a lack of good quality ecological

studies which used accurate exposure measures and consistent oral health outcomes. Further studies using more modern statistical analysis methods, consideration of confounding factors, as well as use of more robust and accurate data sets are advised.

2.8 Conclusions

Within the limits of this review, it is concluded that there is an inverse relationship between UVB exposure and dental caries. Namely, exposure to increased levels of UVB, either artificially or via exposure to sunlight, is associated with a decrease in the formation of new carious lesions. No evidence was found to support associations between UVB exposure and gingivitis, periodontal disease or plaque deposits and no adverse effects were reported to teeth, individuals or populations from the UV exposure during the course of the studies. However, there was a limited number of good quality trials and no RCTs included in this review, and these would be required to obtain strong evidence about a causative relationship. It is also unclear if this association would be reproduced in populations that did not have a fluoridated water supply, or in other geographical locations and latitudes. None of the studies were completed in this century, and the age of the studies explains the limitations in the methodologies and statistical analysis. Further studies are required, incorporating these factors, to inform the evidence base further.

Table 2.22 Summary of Conclusions of Studies with Reference to the Hypothesis “Human Populations Exposed to Higher Levels of UVB Radiation have Better Oral Health than those Exposed to Lower Levels of UVB”?

Study	Exposure type	Outcome measure	Results	Does/ does not support hypothesis (+/-)
(Erpf, 1938)	Natural sunlight	Mean CSI	Spearman’s Coefficient	+
(Schoenthal and Brodsky, 1933)	Mercury vapour lamp	Mean new carious lesions/ child over the study period		+
(McBeath, 1937; McBeath, 1934)	Mercury vapour lamp	Mean new lesions/ 100 days per mouth Mean percentage carious surfaces / mouth		+
(Mayron *et al.*, 1975)	Artificial full spectrum lighting “Vita-lite”	Mean new carious lesions/ child over the study period	RR 0.095 (95% CI 0.014 to 0.660, p<<0.0012)	+
(Hargreaves and Thompson, 1989)	Artificial full spectrum lighting “Vita-lite”	Mean DMFT/DMFS		+

(E. Hathaway, 1992; Hathaway, 1995; Hathaway, 1993)	Artificial full spectrum lighting “Vita-lite”	Mean DMFT/DMFS	Statistically significant difference between group means P<0.005	+

Chapter 3: General Methodology for the Ecological Studies.

3.1 Background Summary

The results of the previous literature review and systematic review showed that further research is needed to inform the discussion on the effect of UVB/Solar irradiation on oral health. The overall direction of evidence consistently supports a positive association between increased sunshine or UVB exposure and reduced incidence of caries in children. However, contemporary ecological studies using more accurate exposure data and outcome measures, would be informative to see if such associations persist in these models. The ecological studies highlighted previously were conducted prior to the introduction of research methods which considered confounders and also lacked sufficient data and contemporary methods for in-depth statistical analysis. Therefore, three empirical studies using secondary data were undertaken. The general methodology of these, including discussion of the selected exposure and outcome data sets, will be initially covered here, whilst the details of the individual studies will be explained in later chapters.

3.2 Oral Health Datasets

In order to complete further empirical studies, three datasets were identified as being freely available to provide outcome measures of oral health. It was from these that the secondary data used in the empirical studies were obtained.

The first two datasets used were the Adult Dental Health Survey (ADHS) and the Child Dental Health Survey (CDHS), which are commissioned by the Department of Health. The third data set was the Oral Health Survey of Five-year-old Children 2014-15 (OHS) conducted by Public Health England as part of their Dental Public Health Epidemiology programme. Sections 3.2.1 to 3.2.3 briefly describe key aspects of the design of each survey based on their respective study documentation.

3.2.1 The Adult Dental Health Survey 2009

The Adult Dental Health Survey 2009 (Office for National Statistics, 2012) was commissioned on behalf of the Department of Health and covered England, Wales and Northern Ireland, although only the English survey is considered in this thesis due to availability of consistent co-variate data. The aims of the survey, as stated in the study documentation, were to "establish the condition of the natural teeth and supporting tissues; investigate dental experiences, knowledge about and attitudes towards dental care and oral hygiene; determine the state and use made of dentures worn in conjunction with natural teeth; examine changes in time in dental health, attitudes and behaviour and monitor the extent to which dental health targets set by the government are being met" (O'Sullivan *et al*, 2011) .

The sampling procedure, described in the Foundation Report ADHS: Technical Information document (O'Sullivan *et al*, 2011) used a two-stage cluster sample technique comprising 253 primary sampling units across England and Wales and 15 in Northern Ireland. Each primary sampling unit consisted of two postcode sectors with 25 addresses sampled from each, giving a total of 13,400 addresses. Sampling design and size was designed to be representative at national and SHA level. The survey consisted of an interview and dental examination, both of which were conducted in participants' homes, by clinicians who had undergone calibration training. The questionnaire enquired about dental history, oral and general health behaviours and socio-demographic questions. There were 77 clinicians examining, with the majority from NHS salaried dental services. Ethics for the survey was submitted for the whole survey, and all areas it covered, via the NHS Research Ethics System and was approved in June 2009. A dress rehearsal to improve planning and development of the survey was undertaken in July 2009. The household sample for the survey was drawn from the postal address finder and in total 13,400 addresses were sampled. Only 12,054 (90%) were eligible as other addresses were unoccupied, businesses or second homes. Letters were sent out on two occasions in the two weeks before the fieldwork began and interviewers called on multiple occasions on different days and times to maximise participation. The final response rate was 60% with 7,233 households taking part. From these households 13,509 individuals were invited to take part and 11,380 (84%)

participated. Only 10,567 of this group were eligible as 813 were edentate, and of that number 6,469 (61%) were also examined. The analysis in this study only considered the data from the England cohort where 11,500 addresses were initially selected and 10,416 were eligible. Of these, 6,157 households responded. When considering individuals in England 11,477 were contacted, 9,663 were interviewed (84%), 9,017 were eligible for examination and 5622 (62%) consented for and underwent an examination. For the examination in the home the participant was seated in a comfortable chair with good head support, with lighting supplied by a standard Daray lamp often used for dental surveys. Instruments used were a dental mirror, CPITN probe and root probe to examine permanent teeth only. The amount of information recorded about each participant's oral health was extensive and the codes, criteria and information recorded are summarised in Appendix 1. In the 2009 survey enough addresses were sampled in each SHA to ensure SHA level estimates, however a consequence of this was that differential sampling rates were used in the English SHAs, Wales and Northern Ireland. To compensate for this a survey weighting was applied along with weighting to compensate for risk of possible bias due to non-response rates at the interview and examination stages.

3.2.2 The Children's Dental Health Survey 2013

The Children's Dental Health Survey 2013 (PHE, 2014) is undertaken every 10 years. In 2013 it was commissioned by the Health and Social Care Information Centre (HSCIC) on behalf of Public Health England, the Department of Health in England, the Department of Health, Social Services and Public Safety Northern Ireland and the Health and Social care department in the Welsh Government. It was carried out by a consortium, led by the Office for National Statistics, and covered England, Wales and Northern Ireland. Only data released for England was used in the analysis for this study due to availability of co-variate data. The aims of the survey were to provide statistics to establish the state of dental health of children in 2013; to explore relationships between oral health, experiences, attitudes and behaviour and to monitor changes in children's dental health and related behaviour over time.

The target population of the survey was five, eight, twelve and fifteen-year-old children in mainstream education, which included state and independent schools. Excluded groups were home schooled children, special schools and pupil referral units. The choice of these age groups meant that primary, mixed and permanent dentition states were represented in the survey population. Full details on the survey methodology are described in the CDHS 2013 Technical Report (*CDHS*, 2015) but are summarised here. For the selection of the participating schools, a list of schools and a list of eligible pupils in those schools, was obtained for each region. The English sample was stratified by the nine regions, which were North East, North West, Yorkshire and Humber, East Midlands, West Midlands, East of England, London, South East and the South West. The schools were clustered into groups nested within regions using Geographical Information Systems and then school groups selected at random included in the study. In England 71% of Primary schools and 42% of secondary schools selected took part in the survey. A separate sampling frame selected children for each cohort from within each school. Each year group from each school represented a separate sampling frame and had its own sampling interval and random start point on the list of pupils in a year group. The lists were sorted by sex, date of birth and numbered sequentially from one. The pupils were selected randomly using a random start and interval method and treating each sample frame as circular, thereby returning to the beginning of the list and continuing to select pupils until the number required to be sampled had been reached. The preferred sample size chosen was 10,000 dental examinations overall, 2500 in each age group. However, to be correctly geographically distributed, have a representative sample of children from deprived schools (33%) and allow analysis of each age group, a larger set sample size was used of 20,922 with oversampling of groups as required.

The survey methodology consisted of a dental examination, a parent questionnaire and a self-completed questionnaire for older children (12- and 15-year-olds) and a questionnaire for parents only for younger children (5- and 8-year-olds). Inclusion in the survey was by positive consent, given by the parents for the two younger cohorts, or by the pupils themselves for the two older cohorts. This led to 70% of five-year-olds, 63% of eight- year-olds, 84% of twelve-year-olds and 72% of fifteen-year-olds, who were eligible for the study,

taking part in the examination in England. The response rates for the questionnaires were 99.7% and 99.5% response rates from the twelve- and fifteen-year-old pupil questionnaires and 45%, 46%, 36% and 31% of productive parent questionnaires for the five-, eight-, twelve-, and fifteen-year-old cohorts respectively. The examination was completed by clinicians who had undergone calibration training and clinical information was recorded for each participant. A summary of the data collected in the examination is shown in Appendix 2.

In order to make the sample representative, weighting was applied to the data collected. The final examination data weighting was produced using design weighting, non-response adjustment and calibration to population totals. Detailed equations on how weighting was achieved are found in the technical report for the survey (*CDHS*, 2015). The final data set is published online by the Office for National Statistics via the UK Data Service (Office for National Statistics, 2015). Individual level oral health outcome data is given but place of residence is provided at Strategic Health Authority level.

3.2.3 The Oral Health Survey of Five-Year-Old Children 2014-15

The Oral Health Survey of five-year-old children 2014-15 (OHS) (PHE, 2016) was conducted to align with diagnostic criteria previously described by the British Association for the Study of Community Dentistry (BASCD) for caries prevalence surveys (Pitts, Evans and Pine, 1997). These criteria also cover sampling techniques suitable for epidemiology studies, which in this case is sampling at lower tier Local Authority (LA) level (districts and unitary authorities). The sample frame consisted of all five year olds in mainstream state funded education (Pine, Pitts and Nugent, 1997a). The aim of the survey was to “measure the prevalence and severity of dental caries among five-year-old children within each lower tier local authority” with the information being used to help Local Authorities fulfil their public health responsibilities (PHE, 2014).

The protocol (PHE, 2014) for the survey advised at least 250 children were needed from a minimum of 20 schools per local authority. It also recognised that this would not produce a sufficient sample size for some LAs and in some cases, larger samples, properly weighted would be required. A

two-stage stratified sampling technique was used to select the participating children for the sampling frame. The first stage acquired a list of all primary schools in the LA and the second stage determined how many children were to be sampled in each school. Ultimately, the sampling processes were agreed after discussion with dental public health consultants, taking into account factors such as the size of schools and number of pupils in each. As positive consent was required for participation, more children than the minimum sample size required were invited to take part, to compensate for cases when consent was not given. To gain consent a letter was sent home to the parents, which required signing and returning. If this was not returned, a second letter was sent to try to maximise participation. It is recognised this opt in approach to consent leads to a response bias and a possible change in the overall clinical findings of the survey.

The summarised results are presented in the document “National Dental Epidemiology Programme for England: Oral Health Survey of five-year-old Children 2015, A report on the prevalence and severity of decay” (PHE, 2016). It is these results which were then used to provide the secondary data used in the ecological studies described in chapters four and five of this thesis. In response to the invitation to participate 4.5% of parents stated they did not want their child to be examined, 0.5% of children refused to be examined on the day, 3.8% were absent on the day and 28.9% of parents did not return the consent forms which was the main reason for non-participation. Of the children sampled 63.1 % were examined which translated to 111,500 clinical examinations being included in the final analysis. Overall, this represented 16.5% of the target survey population of 5-year-old children attending mainstream state schools.

The children selected were examined at their schools by clinicians who had undergone training and calibration in accordance with BASCD guidelines (Pine, Pitts and Nugent, 1997b). The fieldwork examinations of the surveys were basic in their approach and involved a visual examination only. This reflected that they were conducted in a non-clinical environment, although their basic nature meant standardisation of the methodology could potentially be achieved through examiner calibration training. Children were examined, either by lying on a table or in a fully reclining chair, and lighting was provided by a standard Daray X100 light with Halogen bulb with PivotD desk mount or

Brandon Medical MT608BASCD, yielding approximately 4000 lux at one metre. The instruments used were the Number 4 plain mouth mirror and ball ended CPITN probes or blunt/ball ended probes. No suction was used, but excess moisture and food debris was removed by cotton wool rolls. The data was collected on paper and transferred to computer, or recorded directly onto computer, before being processed using the Dental Public Health Epidemiology Programme format 5YR2015 with the Dental Survey Plus 2 version 2.1 release 3 software. The full list of data collected are described in the 2014-15 national protocol document, version 2 (PHE, 2014). The personal and clinical information recorded for each child consisted of the following: child identity number, date of birth, home postcode, ethnicity, examination status, plaque measurement and tooth codes representing the clinical state of the teeth. When the data were collected the Dental Public Health team assigned Index of Multiple Deprivation scores to the participants based on their home postcode. Only primary teeth were recorded, however given the sample population age group the number of permanent teeth present would generally be expected to be low. The data recorded and the coding for the teeth is summarised in Appendix 3.

It was reported in the technical report that the score of “sound” was given not only to teeth with no decay but also to teeth with white/chalky spot lesions, discoloured or rough spots, stained fissures and dark shiny pitted lesions with signs of fluorosis. It is noted therefore that white spot lesions, a sign of early demineralisation of the tooth surface, were not recorded separately in this programme and were therefore not recorded as potential decay. Furthermore, decay was only recorded if it was obviously into dentine which suggests the true prevalence and severity of disease would be higher. The Oral Health Survey of five-year-old children also recorded an assessment of plaque levels using the modified Silness and Loe index. The traditional unmodified Silness and Loe Index was described in 1964 (Silness and Loe, 1964) and was a measure of soft debris and mineralised deposits on teeth, as well as a proxy for tooth brushing. As it is used as a screening tool, not all teeth are included in the index. Instead, only 6 teeth, 3 upper (upper right 6, upper right 2, upper left 4) and 3 lower (lower right 4, lower left 2 and lower left 6) are examined. The 4 surfaces of each tooth, buccal, lingual, mesial and distal, are given a score

between 0 and 3. The scores from each area of the tooth are added together and divided by 4 to give the plaque index for that tooth. To calculate the plaque index for the patient the scores for the 6 teeth are added together and divided by 6 to give the patient plaque index. In the case of this dental health survey the index was further modified to include only the 6 upper front teeth, but the principal overall remained the same. Additionally, a probe was not used in this survey for the modified index and no disclosing of plaque was done. In the dental health survey, the examiner visually examined upper canine to upper canine and only easily visible plaque was recorded. This index was included as a proxy for tooth brushing.

Although individual data were recorded at the time of examination, the survey only released the results aggregated at Local Authority level. It is from these released results that the outcome measures were selected. These included the mean decayed, missing (due to caries), filled primary teeth (dmft) for each LA, the mean dmft for children whose dmft>0 in each LA and the percentage of children in each LA with caries in their incisor teeth. The dmft is a measure of disease that is widely used in dental epidemiology studies and is a measure of past and present disease. DMFT is used to denote permanent teeth and dmft is used for primary teeth. In the dmft teeth not counted include unerupted and congenitally missing teeth, supernumerary teeth and, as only primary teeth are included, the maximum score is twenty. Limitations of the dmft index include, a lack of indication as to the number of teeth at risk or an estimation of treatment needs, and that the indices give equal weight to missing, untreated decay, or well-restored teeth. The index also does not account for teeth lost for reasons other than decay and it does not account for fissure sealants, as these did not exist when this method was designed.

The prevalence of caries in an LA was presented by PHE on their dental health survey website (PHE, 2015) as mean dmft, or mean dmft in children with dmft>0 and the severity was indicated by the percentage of children with decay in their incisor teeth, which can be an indicator of aggressive caries. The results for mean dentinally decayed teeth present at the time of examination was not included in this analysis. This project is focusing on the effects of exposure to sunshine over the children's lifetime and not just at one time point so dmft, which included information about past disease, was felt to be more appropriate.

The counting of decayed teeth only does not account for teeth already filled or extracted. To summarise, this dataset provided information about caries prevalence and severity at lower tier local authority level, but did not provide individual level data.

3.3. Selection of Exposure Datasets

Having identified datasets containing oral health data it was necessary to identify measures that could be used to estimate environmental UVB exposure from solar irradiance in different geographical areas, and therefore be used to estimate the relative potential for vitamin D synthesis in those regions. In order to justify the use of different meteorological measurements for exposure estimation, the relationship between sunshine, UVB and vitamin D needs to be explored.

3.3.1 Relationship Between Sunshine Hours and Vitamin D Levels

In the UK, the main source of vitamin D is from sunlight exposure (Webb *et al.*, 2010) and it is recognised that a direct relationship exists between increasing hours of sunshine per month and increased vitamin D status (Brot *et al.*, 2001). A study in Denmark, latitude 54° -58°, investigated the relationship between vitamin D and sun exposure in healthy Danish peri-menopausal women and showed there was a seasonal fluctuation of their vitamin D levels (Brot *et al.*, 2001). These data on number of hours of bright sunshine were obtained from the Danish Institute of Meteorology although, as in the UK, there are several months of the year, October to April, when no vitamin D production is possible. The serum vitamin D status of the participants was measured and information on behaviour patterns was collected. This allowed dietary vitamin D intake and supplements, use of sunbeds and exposure to sunshine through outdoor activities to be accounted for. In all groups there was a seasonal variation of serum vitamin D levels, even those who avoided sun exposure, although the effect was less marked in that subgroup. The group as a whole showed greatest serum vitamin D between June and October and this was related to the hours of sunshine with a 2-month time lag, although the authors suggested this was because the temperatures in April and May were still too

low to allow regular sunbathing. Active sunbathing was associated with 27.6% increase in mean annual vitamin D status and on average, those who had regular sun exposure, had mean serum levels 17 nmol/l higher than those who did not. It was concluded hours of sunshine are related to mean annual serum vitamin D levels, sun exposure is the predominant contributor to vitamin D status and this is true even in subgroups who avoid regular sun exposure. Given Denmark's similar latitude, climate and culture to the UK, it is reasonable to assume that these relationships are relevant to the setting of this study and sunshine hours are an acceptable proxy for vitamin D serum levels and therefore vitamin D status.

3.3.2 Measurement of Sunshine Duration

The World Meteorological Organisation defines sunshine duration as the period during which direct solar irradiance exceeds a threshold value of 120 watts/m^2 (WMO., 1989). This is approximately the level of solar irradiance just after sunrise or just before sunset in cloud free conditions. There are several methods for measuring sunshine duration but one of the most established is the Campbell-Stokes recorder. The Campbell-Stokes recorder (CS) consists of a glass sphere set into metal housing, with cardboard placed behind the sphere. It was first developed by Campbell in 1853 and modified by Stokes later in the 1870s (Sanchez-Lorenzo *et al.*, 2013). Bright sunlight is focused onto the card, by the sphere, and chars a line into it. If the sun is obscured the line is interrupted. The total length of the line, minus the gaps due to interruption, on the calibrated card measures the daily total of bright sunshine that day. It is a simple device, although it only measures bright sunshine and measurements may be reduced when there is weaker light or increased cloud cover. Its benefits include having no moving parts and requiring no electricity, which makes it suitable for remote locations. Limitations include differences in interpretation by those observing the length of the burnt line and the need to replace the paper after each day of use. When correct methods and procedures for use are followed it is thought to be accurate to within 0.1 sunshine hours (Stanhill, 2003). A network of Campbell-Stokes recorders has been in operation across the UK for many years, run by the Met Office, generating data relevant to this study (MetOffice, 2018). Modern sunshine sensors produce more

accurate data, but are limited in geographical distribution and have only been implemented recently in the UK.

3.3.3 Relationship of Sunshine hours to Erythemal UV

A study by McGrath et al (2001) investigated correlations between the readings of a Campbell-Stokes recorder and an erythemal UV meter (spectroradiometer). The erythemal UV meter is an electronic device that measures solar UV in the range 295-385 nm, which includes the range required for production of vitamin D. The study concluded that there was a statistically significant positive correlation ($r=0.67$, $p<0.001$) between duration of sunshine as recorded by the Campbell-Stokes and the erythemal UV measure. Sunshine hours, as recorded by a Campbell-Stokes recorder, is therefore positively correlated with UV of sufficiency to cause cutaneous vitamin D production and is an acceptable exposure proxy measure for the three studies.

3.3.4 Exposure Measure Data

The key measure of sunshine exposure for this analysis was obtained from The Medical and Environmental Data Mash-up Infrastructure Project (MEDMI). This project aimed to improve research on the effects of climate, weather, the environment, and health by linking environmental and health databases together to create a new resource. The Met Office databases are contained within MEDMI and it was these that were used to obtain measures of sunshine. Raw Met Office sunshine data were downloaded from MEDMI for weather stations across the UK. The longitude and latitude of the UK was used to select the area and time range of interest, in this case a five-year period between 01.01.2010 and 31.12.2014. Data for this time period were selected in order to calculate long-term estimates of population exposure to environmental UVB with relevance to the time periods of the oral health datasets.

A number of exposure measures were available from the Met Office data, however the most appropriate data was that from the Campbell-Stokes monitor network. This had been recorded at the greatest number of meteorology stations around the country over the time period of interest. This was an important factor in creating the most accurate average long term

exposure models for different areas of the UK that could then be linked to the oral health datasets. It was also deemed to be a valid proxy for estimating population UVB exposure and potential vitamin D production as discussed above. In terms of alternatives, there were some measures of UV surface radiation available, however measurement of surface UV can be difficult. Only a small proportion reaches the earth's surface and it can be easily affected by atmospheric conditions, such as cloud cover or pollution, or ground conditions such as snow causing scattering (WMO., 2008). Only a small number of UK meteorology stations had UV data available meaning it would have reduced the accuracy of estimates for smaller areas, such as lower tier local authorities, many of which were far from the nearest recording site. In contrast the Campbell-Stokes measurements were available from 78 stations spread across the UK, meaning spatial interpolation across the study area could be more accurately undertaken. These data were used by the author to create the exposure measures using the processes described in the following sections.

The raw daily data from the 78 stations were selected and aggregated into monthly total bright sunshine hours, however only months where there were data available for every day in the month were included in the analysis to increase accuracy. Following this, the data were averaged by calendar month and station across the five years. These data were transferred to Geographic Information System (GIS) software to enable mapping and transfer to areas for linking to oral health survey datasets (ArcGIS 10.5, ESRI, Redlands, USA). The mean monthly data were mapped using the meteorological station British National Grid References (easting and northing, see Fig 3.2a which shows the location of the CS meters). Once plotted geographically the station data were interpolated to create 5 km square grids with estimates of mean monthly hours of sunshine using inverse distance weighting interpolation (ArcGIS, 2016). This produced an estimate for every cell of a 5km grid across the study area (England), based on nearby monitoring stations, with more weight being given to measurements that were closer to the grid square. These gridded estimates (Fig 3.2b) were then overlaid with the geographical boundaries of Lower Tier Local Authorities (LA, for linkage to Oral Health Survey data, Fig 3.2c), Strategic Health Authorities (SHA, for Adult Dental Health Survey) and regions (Child Dental Health Survey). Boundary data for the appropriate time period for each

dataset were obtained from the Office for National Statistics (ONS). The GIS was used to calculate the mean of the estimated 5 km grid sunshine hours data within each LA, SHA and region. This provided an estimate of the five-year monthly mean hours of sunshine for each geographical area over the period 2010-2014. These were summarised to a five-year mean for the whole period and also a subgroup summary mean for the months April to October, when vitamin D production is possible. Although sunshine hours may occur in the winter, the sun is not of sufficient strength to produce cutaneous vitamin D in winter months at UK latitudes (Webb, Kline and Holick, 1988). The correlation coefficient between the two exposure measures (all year and April-October only) at LA-level was 0.99 (see Figure 3.1), meaning the two measures were highly correlated to each other. As this study was looking at the associations between vitamin D, sunlight exposure and oral health it was decided to use the second exposure measure only for the analysis (mean monthly hours of sunshine April to October only). It was this exposure only that could have produced vitamin D cutaneously in the population of interest, making it a more appropriate proxy for sunshine-generated population vitamin D levels.

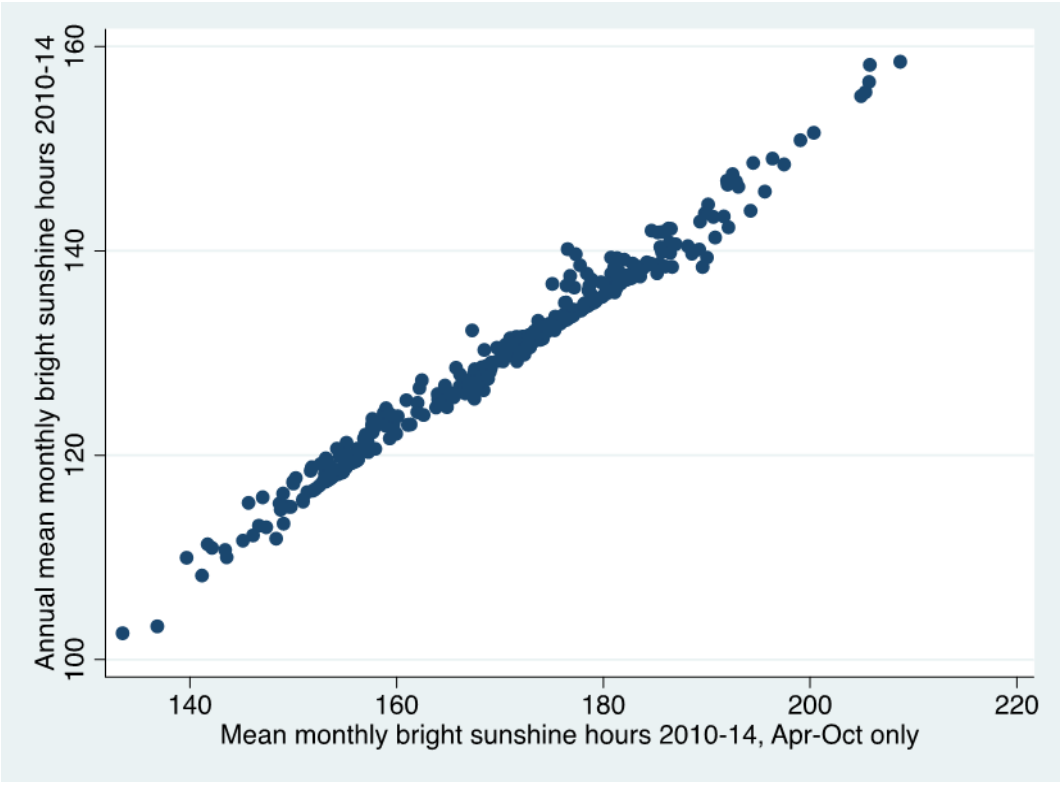

Figure 3.1 Scatter Plot Showing Correlation of Two Sunshine Exposure Variables (All Year Vs April-October Only) Aggregated at Local Authority Level

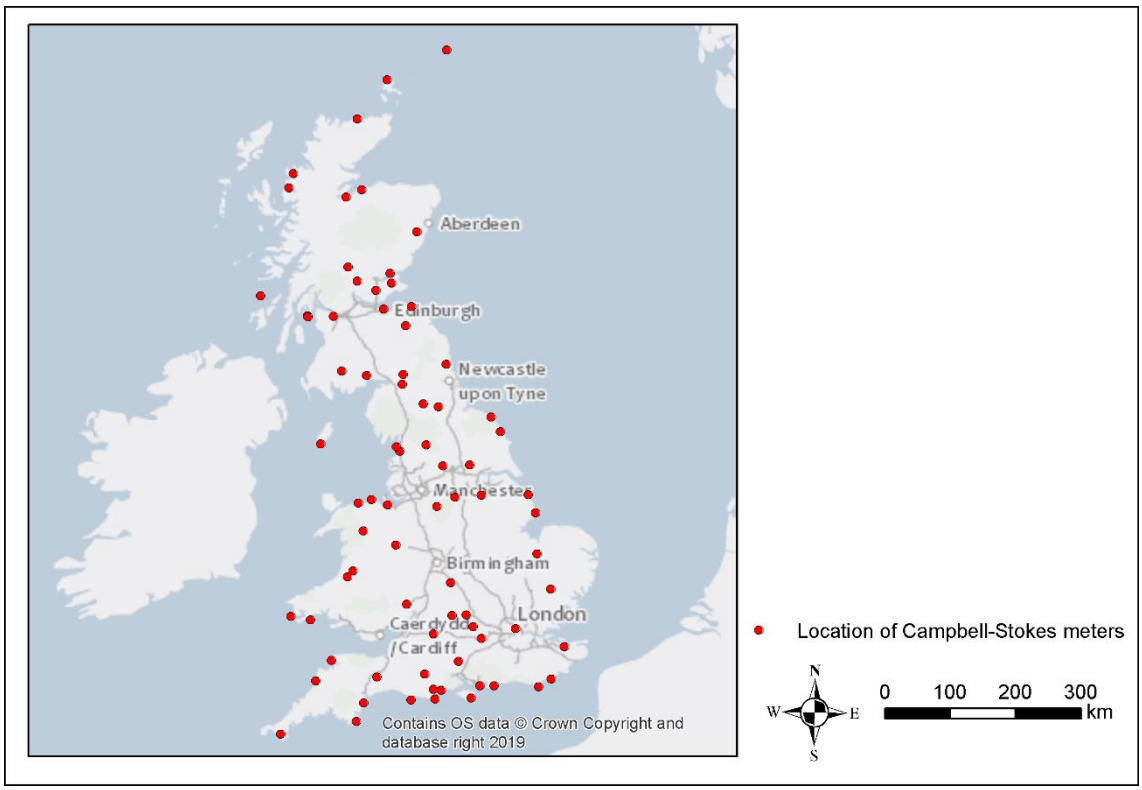

Figure 3.2a Map Showing Location of Campbell-Stokes Meters. 78 Stations Across the UK Used for The Spacial Interpolation Technique.

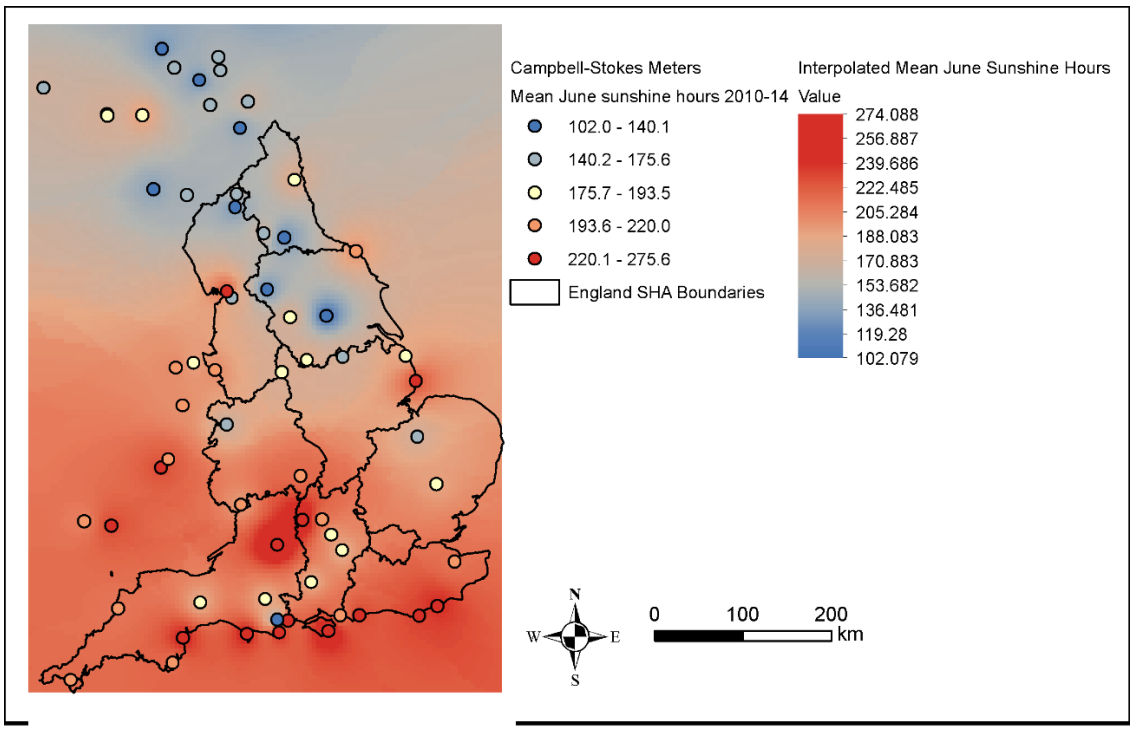

Figure 3.2b Map Showing Interpolated 5 Km Grids of Mean June Sunshine Hours 2010-2014

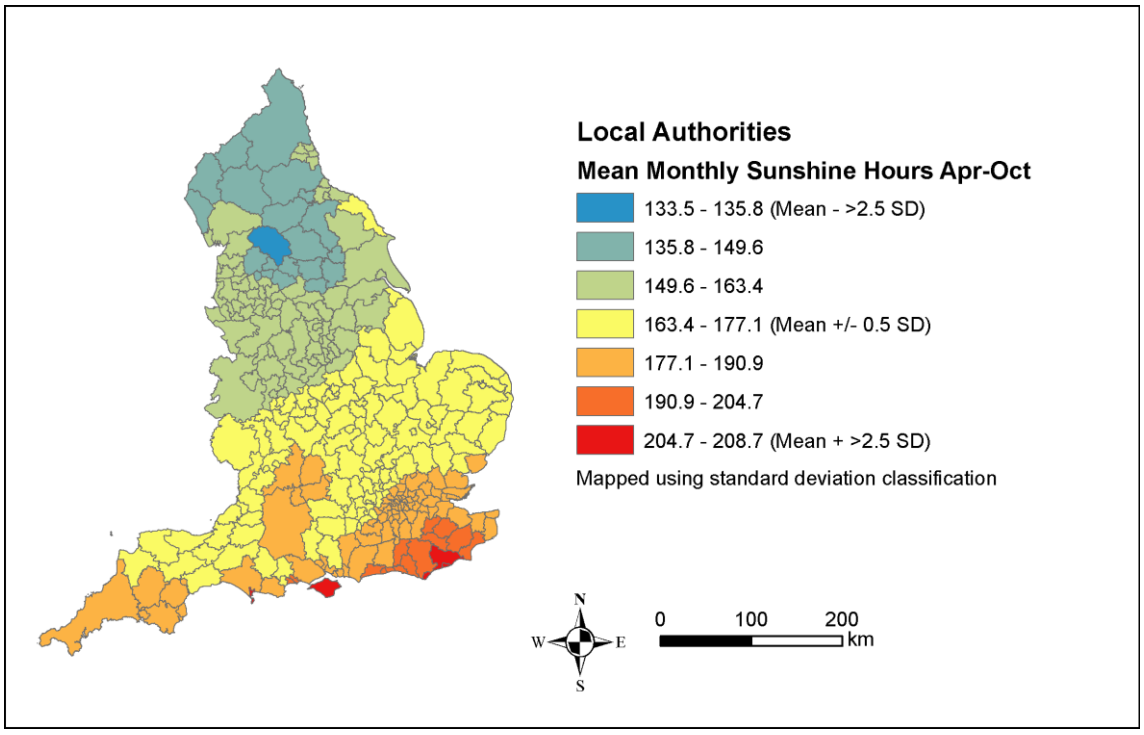

Figure 3.2c Map Showing Variation of Mean Monthly Sunshine Hours Per LA from Apr-Oct Averaged Between 2010 And 2014

Figures 3.2a-c Maps built by the author demonstrating the stages completed when deriving sunshine exposure measures for the empirical studies in chapters four and five.

3.4 Analysis Plan

In the empirical studies conducted here, the outcome measures of interest selected for analysis were:

- Study 1: Adult Dental Health Survey (individual): 1) presence of bleeding on probing and 2) presence of pockets deeper than 3.5mm.
- Study 2: Child Dental Health Survey (individual): 1) Any decay experience in primary teeth, any decay experience in permanent teeth
- Study 3: Oral Health Survey (local authority (LA) aggregate data): 1) mean dmft per child per LA, 2) the mean dmft for children whose dmft > 0 per LA and 3) the percentage of children in each LA with caries in their incisor teeth.

The two studies of children's teeth complement each other as one (Child Dental Health Survey) includes individual level data, but only has an exposure estimation for large areas (SHA), and the other (Oral Health Survey) includes aggregated population level data, but has exposure measures for smaller areas (LA). The limitations of each design will be considered in the appropriate chapters. The literature review and the results of the systematic review included studies finding relationships between sunshine/UV exposure and dental cares for child populations, so the choice of caries-related outcome measures such as decay experience in primary or permanent teeth were selected as appropriate to address the study hypotheses.

The Adult Dental Health Survey study investigated gingival and periodontal inflammation, as determined by the proxy of the presence of bleeding on probing around any teeth and the presence of periodontal pocketing, as defined by the presence of pockets deeper than 3.5mm. The initial literature review identified evidence showing both positive and absence of associations between vitamin D and periodontal health in adults (Dietrich *et al.*, 2005; Lee *et al.*, 2015). One RCT did show a dose-dependent response to

vitamin D supplementation on gingival inflammation (Hiremath *et al.*, 2013b). As discussed previously one possible hypothesis for this apparent relationship is that increased UVB exposure modifies the quantity or quality of plaque in the mouth. This may be seen clinically by changes in plaque scores and signs of gingival inflammation such as bleeding on probing. No studies in the systematic review provided data on gingival bleeding or inflammation. Therefore, the presence of bleeding on probing was chosen as an outcome measure to investigate the proposed association further.

3.4.1 Potential Relationships Between UV Exposure and Health Outcomes

The potential mechanisms for vitamin D interacting with oral health have already been discussed in chapters 1 and 2. These mechanisms can be used to inform the hypothesised relationships, which are described as direct or indirect, and were used to develop an analysis plan for the empirical studies. Figures 3.3a - c demonstrate possible proposed mechanisms of action explaining the relationships between exposure and outcome.

A direct relationship (Figure 3.3a) would be observed when high or sufficient vitamin D levels directly act on the teeth or oral environment via physiological processes of optimal calcium and phosphate metabolism within the body. An example would be the increase in minerals in saliva and/or serum during the formation of the teeth, increased mineralisation of the developing dentition, increased laying down of tertiary dentine or increased remineralisation of early lesions. Increased vitamin D may cause a change in the quantity or quality of plaque (Figure 3.3b), for example through the increased production of cathelicidins and defensins. These antimicrobial substances which can be found in saliva may change the plaque biofilm to contain less cariogenic species. The effect could be partly or wholly explained by confounding with plaque and oral hygiene measures (Figure 3.3c). A confounder in epidemiology is defined as a pre-exposure variable associated with exposure and associated also with the outcome conditional on the exposure, possibly conditional also on other covariates (Miettinen, 1974) .

Figure 3.3a Direct Relationship (via other Mechanisms)

Figure 3.3b Plaque as a Mediator

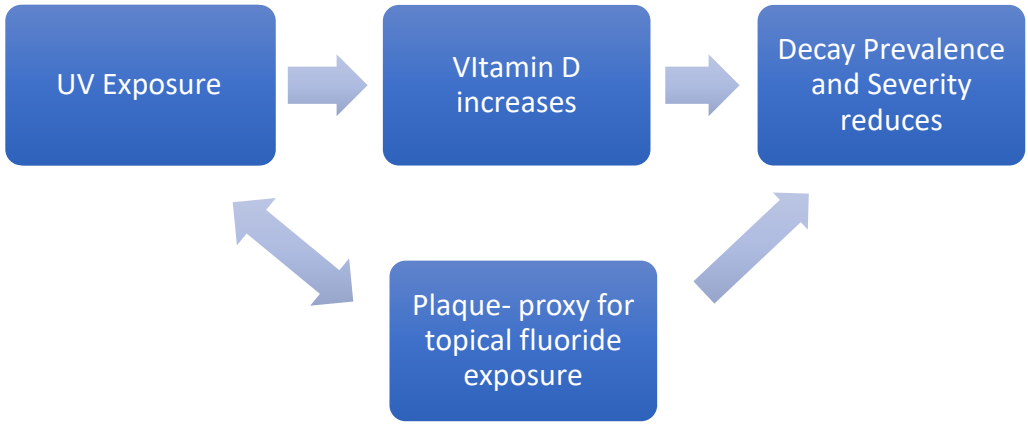

Figure 3.3c Plaque as a Potential Confounder

Figures 3.3a –c. Potential Pathways Between UV Exposure and Tooth Decay, With Consideration of The Role of Plaque

In the ecological studies previously discussed there was an overall association of residence in an area with increased sunshine exposure and reduced caries outcome measures. It may be that this relationship is explained by confounding factors influencing oral hygiene and plaque levels. For example, in England the more northerly regions with lower solar sunshine hours are also regions with greater areas of deprivation, and lower socio-economic status is associated with poorer diet, education and health (Foster *et al.*, 2018; Bower *et al.*, 2007).

3.4.2 The Value of Environmental Studies and Oral Health Epidemiology

The possibility of a naturally occurring environmental element that may have caries protective properties has an analogy with fluoride and natural and artificial water fluoridation. It was first noticed by Frederick McKay, a dentist in Colorado Springs in 1901, that residents of the area had a strange staining of their teeth which was also associated with lower decay rates when compared to adjacent regions where the staining was not present. In the UK in 1925 an Essex dentist, Dr N Ainsworth, also noticed a similar occurrence of the staining and associated reduced decay levels in the populations affected. At this time the cause of the staining and the reduced incidence of decay was unknown but it was suspected it was an environmental factor, and suspicion fell on the drinking water supply. Several studies then took place where the content of the water supply was analysed and the presence of the staining and rates of decay were recorded. These included work by Dr Trendley Dean in the US (1942), and Dr Robert Weaver in the UK (1944). It was Dean's work that established that mottling of the teeth occurs at fluoride levels of 1 ppm or below in the water, but the optimum caries preventive effect was seen at 1ppm. This has been established again since with estimates of the occurrence of fluorosis at levels of aesthetic concern at 1ppm between 1- 12.5% (Iheozor-Ejiofor *et al.*, 2015; McDonagh *et al.*, 2000; Treasure *et al.*, 2002). The levels of 1ppm are still used in artificial fluoridation schemes today. Following the ecological studies that showed the association between natural fluoridation, mottling and reduced levels of decay the possibility of a public health intervention was considered and several artificial water fluoridation pilot schemes were set up starting with Grand Rapids Michigan in 1945. Schemes were also trialled in the UK and in 1955 three sites in Watford, Kilmarnock and Anglesey were selected to have their water artificially fluoridated. When compared to neighbouring areas after five years, five-year-old children in the fluoridated areas had less decay.

Originally it was thought that the main effect of fluoride was systemic but it is now known this is not the case and the incorporation of fluoride into developing teeth, whilst producing some fluorapatite in the tooth structure, is unlikely to have a clinical effect on the prevention of caries. It is the systemic effect however that is responsible for the appearance of fluorosis mottling on the teeth. The main caries protective effect of fluoride is topical by three main

actions. These are inhibition of demineralisation, encouraging remineralisation and provision of a fluoride reservoir. If fluoride is present in solution surrounding the enamel prisms it is adsorbed to the surface of the carbonated apatite crystals. As the fluoride containing crystals dissolve at a lower pH than hydroxyapatite this protects against acid dissolution. Fluoride in saliva leads to supersaturation with respect to fluorohydroxyapatite and encourages remineralisation to this form, which is more resistant to acid dissolution. Finally, in the presence of fluoride there is formation of Calcium Fluoride which acts as a reservoir for fluoride and supports the other two processes. It is now determined that fluoride is most effective when delivered topically as frequent low-level doses rather than systemically or intermittently (Buzalaf *et al.*, 2011; ten Cate, 1999). Once the actions of fluoride had been determined other forms of fluoridation were introduced including community measures such as supplementing food and both systemic and topical individual exposures in the form of tablets, lozenges, toothpastes, mouthwashes and varnishes. The availability of fluoride from other sources has meant that the impact of water fluoridation, whilst still important, has been comparatively reduced.

When comparing the story of fluoride to that of vitamin D several comparisons and lessons can be made showing the importance of ecological studies in identifying possible health interventions. Whilst the quality of these studies is considered to be low due to their numerous limitations discussed, the existence of several studies all showing the same direction of evidence can be used to generate hypothesis about the possible reasons for the health inequalities seen.

3.5 Analysis Plan Summary

3.5.1 Research Question

Does being resident in an area of higher sunshine hours lead to improved oral health? Specifically, is being resident in an area with increased sunshine exposure associated with:

a) decreased presence of bleeding on probing and periodontal pocketing in adults?
b) decreased presence of caries in children?

3.5.2 Specific Hypotheses

Specific *a priori* hypotheses tested in the three empirical studies were:

Study 1: The prevalence of plaque, bleeding on probing and pockets deeper than 3.5 mm, in adults, will be lower in SHAs with higher sunshine hours than in SHAs with less sunshine hours.

Study 2: The prevalence and severity of dental decay in children will be lower in Regions with higher sunshine hours than in Regions with less sunshine hours

Study 3: The prevalence and severity of dental decay of child populations will be lower in LAs with higher sunshine hours than in LAs with less sunshine hours

3.5.3 Key Exposure Data Linkage

The key exposure measure for the empirical studies was the mean monthly hours of sunshine for the months April to October over the period 2010-2014 for the geographical area available for each dataset. The exposure estimates produced as described above were linked to each dataset using the appropriate geographical unit identifier (SHA or region of residence for each participant in the ADHS and CDHS respectively, and LA for each LA reported in the Oral Health Survey dataset).

3.5.4 Key Outcome Measures

As discussed, in the ADHS survey the key outcome measure was the presence of bleeding on probing. This was derived from the “bleeding” variable in the source data. The other outcome measure considered was the presence of pockets deeper than 3.5mm.

For the CDHS survey binary outcomes measures were used including the presence or absence of decay in primary or permanent teeth. The outcome measures were differentiated by classifying for the presence of shallow or deep decay, for example visual caries or cavitated caries, or by the presence of an increased number of decayed teeth, for example more than five primary teeth with obvious decay.

For the OHS the key outcome measures were the mean dmft per child per LA, the mean dmft in children who had dmft greater than 0 per LA and the percentage of children with incisal caries per LA.

Key outcome measures are discussed in more detail in the relevant chapters.

3.5.5 Key Confounders

Key potential confounders considered, where possible or appropriate in the ADHS and CDHS analyses, were age, sex, socio-economic status and oral hygiene status. These confounders were available within the ADHS and CDHS data sets. For the analysis using the OHS the potential confounders used in the analysis were fluoridation, index of deprivation and plaque. Two of these, fluoridation and index of deprivation, were taken from separate sources and applied to the Local Authorities as appropriate.

The fluoridation status for each local authority (LA) was taken from Public Health England's (PHE) document "Health monitoring report for England 2014". The report summarises the evidence of the effects of fluoridation of water supplies in England. Artificial fluoridation schemes of water supplies in England have been operating for nearly 50 years and cover approximately 6 million people. To assess whether a local authority was considered to be fluoridated or not PHE obtained the boundaries of English drinking supply water quality zones (WQZs) from the Drinking Water Inspectorate. This was provided in digital format with a binary variable attached indicating whether they were subject to fluoridation schemes in 2012. PHE's analysis involved using geographic information systems (GIS) to assign the population weighted centroid for each 2001 Lower Layer Super Output Area (LSOA) in England with a fluoridation status depending on the WQZ it was located in. These centroids represent the spatial distribution of the population in each LSOA, as recorded in the 2011 Census, as a single summary reference point on the ground. The LSOAs located within a WQZs that was naturally fluoridated to a level of 1 ppm were also identified. A fluoridation status for 2012 was assigned by PHE to all 32,482 LSOAs (2001 boundaries) in England: 28,433 (87.5%) were not fluoridated; 3,991 (12.3%) were fluoridated; 58 (0.2%) were considered naturally fluoridated to levels of 1ppm. It was PHE's results that were used to determine the

fluoridation status of the LAs in study three. To be considered fluoridated a lower-tier local authority needed more than half of its constituent LSOAs to be fluoridated. Derived from this it meant that PHE determined that out of 326 lower-tier local authority areas in England, one was naturally fluoridated and 34 (10.7%) were considered to be artificially fluoridated. In the fluoridated LAs 25 (8.0% total) received 100% LSOA coverage and nine (2.8%) had more than 50% of LSOAs fluoridated but less than complete coverage. Of the 291 lower-tier local authorities considered non-fluoridated, 280 (85.9% total) received no fluoridation. There was however 11 (3.4%) LA that received some fluoridation to 50% or less of their LSOAs. For the purposes of the empirical Oral Health Survey study completed for this thesis these LAs were considered to be non-fluoridated.

The measures of social deprivation for each LA for the Oral Health Survey of 5-year old children analysis was taken from a report from the Department for Communities and Local Government, part of the English Indices of Deprivation 2015 (Smith, 2015). The report is produced by drawing information from several resources and government offices, for example the Department of Work and Pensions and Office of National Statistics, to calculate the indices to measure deprivation at small area level. The Index of Multiple Deprivation is the official measure of relative deprivation for small areas, lower layer super output areas (LSOAs), in England. It ranks every small area, relative to each other LSOA, from most deprived (1) to least deprived (32,844). Local authorities are likewise ranked from most deprived (1) to least deprived (326) and are also further divided into deciles of most to least deprived (1-10). For the OHS survey the plaque measure came from the percentage of children who had substantial plaque on their teeth in that LA and this was derived from the published dataset. The technique for recording the plaque and the examination technicalities have been described in previous sections. Therefore, the fluoridation status, index of deprivation measures and plaque measures were derived as described above and merged with the Oral Health of 5-year-old children data set for analysis.

In the ADHS and CDHS confounders used in the final model were socio-economic status, as measured by index of deprivation, smoking and plaque. These were all recorded at an individual level as part of the survey and so those

measures were used for the analysis of those studies and the recording of this data either through questionnaire or examination has been discussed earlier in the chapter. There was no record of whether the participant received fluoridated water and it was not possible to determine at SHA or region level. Details of the potential confounder variables are provided in the relevant empirical chapters.

3.5.6 Statistical Analysis

The data was analysed using the statistical software package Stata (15.1 College Station, USA). For each of the three studies, regression models were designed to investigate the possible pathways, proposed in Figures 3.3a-3.3c, between hours of sunshine exposure and oral health measures. The full models adjusted for socioeconomic status, water fluoridation and oral hygiene for studies using data at LA level, or socioeconomic status, oral hygiene, smoking and plaque for studies using data at Regional or Strategic Health Authority level. Further details of model development and statistical analysis is provided in the description of each study, which will now be discussed in the following chapters.

3.6 Summary

In summary the empirical studies of this thesis will use secondary data, to investigate the association between gingivitis and periodontal pocketing in adults and dental caries in children, with mean sunshine hours in area of residence. The oral health data are taken from the 2009 ADHS, 2013 CDHS and 2014 OHS of Five-year-old children and the sunshine hours data is derived from Met Office Data, recorded from 78 weather stations around the UK during 2010-2014 and available through the MEDMI project. The resulting ecological studies will be discussed further in the following chapters.

Chapter 4: Study 1: Association between Gingival Inflammation and Sunshine Exposure in Participants of the UK 2009 Adult Dental Health Study.

4.1 Introduction

As discussed in Chapter One, vitamin D is a secosteroid hormone that is essential for bone metabolism and immuno-modulatory functions (Hewinson, 2012; Prietl, 2013), and has antimicrobial properties (Youssef, 2011). Although some vitamin D is obtained from diet, in the UK most of an individual's vitamin D is obtained during exposure to sunshine (Macdonald and et al., 2008; Hypponen and Power, 2007). When UVB from solar radiation between the wavelengths of approximately 290-315nm is absorbed into the skin a photolytic process involving the cholesterol derivative 7-dehydrocholesterol (7-DHC) occurs (Holick, 1981). These UV wavelengths convert the 7-DHC to pre-vitamin D3, and once produced it can be isomerised within the skin, in a heat dependent process, to vitamin D3. Vitamin D levels are measured in ng/ml or nmol/L (1 ng/mL = 2.5 nmol/L) serum levels of the metabolite 25-Hydroxyvitamin D (25(OH)D) which is the main circulating form of vitamin D, and has a half-life of approximately two to three weeks (Jones *et al.*, 2012). Whilst there is variation in what may be considered deficiency, the Institute of Medicine reported that serum 25(OH) D concentrations of less than 30 nmol/L is deficient , 30-50 nmol/L may be inadequate in some people, and sufficiency is considered higher than 50 nmol/L (IOM, 2011b).

In the UK vitamin D deficiency is thought to be common, with levels of vitamin D being highest in September and lowest in February. It is suggested that levels of 80 nmol/L are required post summer in order to remain vitamin D sufficient (≥50nmol/L) year round, and as such the majority of the population in the UK becomes vitamin D insufficient during the winter (Webb and et al., 2010). Nearly half of the population of middle-aged white adults in the UK have 25(OH) D concentrations of less than 40 nmol/L, and 90% have less than 75 nmol/L, over winter and spring in the UK, and the problem is not restricted to high-risk groups (Hypponen and Power, 2007). Vitamin D production also decreases with increasing age, increasing skin pigmentation and the use of sunscreen (Holick, 1981; Faurschou *et al.*, 2012).

4.1.2 Gingivitis and Vitamin D

A previous randomised double blind placebo control trial reported a dose dependent improvement in gingival health with vitamin D supplements (Hiremath *et al.*, 2013b). In a study of 84 participants, four groups were given daily supplements of 2000, 1000, 500 IU and placebo respectively for 3 months. Measurements of serum vitamin D, calcium, and the Silness and Loe index of gingival inflammation (Loe, Theilade and Jensen, 1965), were taken at baseline and at 30, 60 and 90 days. A statistically significant increase in vitamin D was seen in groups given 1000 IU and 2000 IU, but the greatest increase was in 2000 IU/day group. All groups showed an improvement in gingival scores, although the placebo group was extremely small compared to the other three groups and was not statistically significant. At the end of the trial all groups taking vitamin D supplements showed significant improvements in gingival health, but the 500IU/day group did not reach this until the final examination. The mean gingival score for gingival bleeding in the group taking 2000IU/ day decreased from a baseline score of 2.4 to a final mean score of 0.4. The Silness and Loe system has been described previously in a prior chapter and it can be seen that a decrease of this magnitude would be clinically relevant. The maximum score is 3 and reducing the mean score from 2.4 to 0.4 would suggest the gingival inflammation of the group overall reduced from moderate to mild. In contrast, a Finnish study investigated an association between serum vitamin D and presence of gingival bleeding in a low-risk population of 1262 adults aged 30-49, never smokers with no evidence of diabetes mellitus, obtained from the Health Survey 2000 in Finland. They found no association between lower serum levels of vitamin D and gingival bleeding (Antonoglou *et al.*, 2015).

4.1.3 Sunshine hours and Vitamin D

As discussed previously in chapter three, sunshine hours exposure is correlated with vitamin D status, even in individuals who avoid sun exposure (Brot *et al.*, 2001) and in the UK the main source of vitamin D is from sunlight exposure (Webb *et al.*, 2010). Therefore, for the purpose of this study, sunshine hours in area of residence are used as a proxy for vitamin D status as individuals and populations resident in areas with higher average sunshine

hours have higher levels of vitamin D than those living in areas with lower average sunshine hours (Cherrie *et al.*, 2015).

4.2 Aims

As stated in Chapter 3 specific *a priori* hypotheses tested in this empirical study were:

Study 1: The prevalence of bleeding on probing and pockets deeper than 3.5 mm, in adults, will be lower in SHAs with higher sunshine hours than in SHAs with less sunshine hours.

Despite the known links between sunshine and blood vitamin D levels, no recent previous studies have analysed the evidence in an attempt to link sunshine exposure to specific markers of oral health. Therefore, the primary aim of this study was to investigate a possible association between environmental UVB exposure (sunshine) and the presence of:

i) plaque
ii) gingival inflammation

A secondary aim was to investigate the relationship with regards to:

iii) periodontal disease

Specifically measuring

i) presence of plaque at individual level
ii) presence/ absence of bleeding on probing at individual level

and for the secondary aim

iii) presence/absence of pocketing greater than 3.5 mm depth at individual level

4.3 Methodology

4.3.1 Exposure Dataset

The exposure of sunshine hours was obtained from MEDMI, which utilises Met Office data from meteorological stations around the UK. It included

sunshine hours, as recorded using a Campbell-Stokes meter, from 78 stations around the UK. The process of deriving the sunshine hours data has been fully discussed in Chapter 3.

4.3.2 Exposure Measure

As described in Chapter 3 inverse distance weighting interpolation was used to create 5km squares with estimates of mean monthly sunshine hours. For Study 1 these squares were then overlaid with the geographical boundary data of the UK Strategic Health Authorities (SHA). For analyses the SHA areas were firstly treated as ten categories ordered by sunshine hours. Secondly, they were grouped according to high, medium and low estimated exposure in order to undertake sensitivity analysis. The average sunshine hours exposures are shown in Table 4.1, which also demonstrates the 3 groupings of the 3 highest, 4 middle and 3 lowest UVB regions.

Table 4.1 Mean Monthly Hours of Sunshine (April - October) 2010-2014 per Strategic Health Authority

Strategic Health Authority (SHA number)	Mean Sunshine Hours per Month	Sunshine Hours Category	N (9,663)
North East (1)	149.14	3 lowest	992
North West (2)	149.87		970
Yorks and Humber (3)	150.78		1,021
West Midlands (5)	161.77	4 middle	876
East Midlands (4)	165.69		1,130
East of England (6)	172.60		1,033
South West (10)	176.23		1,012
South Central (9)	177.17	3 highest	968
London (7)	181.77		762
South East Coast (8)	191.87		899

4.3.3 Oral health Dataset

This study utilised secondary data in the form of published data from the 2009 Adult Dental Health Study (ADHS). The technical aspects of this study have been discussed previously in detail in Chapter 3, so will be only summarised here for context. The ADHS is commissioned on behalf of the Department of Health for England, Wales and Northern Ireland and conducted approximately every 10 years by the Office for National Statistics (Office for National Statistics, 2012). The survey aimed to establish the oral health status of the nation, investigate participant's dental experiences, knowledge and attitudes towards dental care and oral hygiene and examine changes in dental health, attitudes and behaviour over time. The statistical analysis employed for the ADHS survey used cross tabulations and logistic regression to produce the tables and data presented in the ADHS reports, which were used by the author for the analysis in this thesis.

4.3.4 Oral Health Outcome Measure

During the ADHS the presence of plaque was recorded for each tooth. A positive outcome for the presence of plaque was recorded if any plaque or calculus was visible on any tooth surface on visual inspection. Following the initial visual assessment, a clinical examination was undertaken. The presence of bleeding on probing was recorded by using a C type Basic Periodontal Examination (BPE) periodontal probe to examine around the gingival margins. If examination of the buccal surfaces of the upper teeth, or the lingual surfaces of the lower teeth, exhibited bleeding from any of the pockets in a sextant, the sextant was recorded positive for bleeding on probing (BOP). If any sextant had bleeding on probing then that participant was classified as having gingival bleeding and therefore a binary outcome measure was provided from the original data. The periodontal status of participants was also reported using the outcome measure of periodontal pocketing. This was measured using the same sextant system as for the gingival bleeding. In the case of pocket depth measuring the probe was inserted into the gingival sulcus and down the side of the tooth into the pocket if one was present. A score of 0 was given for pockets less than 3.5mm depth, a score of 1 for pockets 4-5.5mm, a score of 2 for pockets 6-8.5mm, a score of 3 for pockets over 9mm and a score of 9 if the

sextant was unscorable. A healthy periodontal pocket depth is between 1 and 3 mm (Lang and Bartold, 2018). A pocket depth of more than 3.0 mm may be indicative of periodontal disease, either active or past alveolar bone loss. This information from the ADHS data was used by the author to derive a binary outcome to be used in the analysis, where a score of 0 was given to patients who had no sextants with pockets greater than 3.5 mm and a score of 1 for individuals who had one or more sextants with pockets depths above or equal to 3.5 mm.

4.3.5 Analysis

It was hypothesised that participants living in areas with higher mean sunshine hours would be less likely to have visible plaque, and consequently less likely to have BOP. Logistic regression was used to compare the odds of the presence of plaque amongst participants from the three different groups of SHAs by estimated UVB exposure. The analysis was completed initially without any confounders being taken in to account providing the simple model. Analysis was then completed for a second full model which adjusted for age, the index of deprivation and smoking status (Table 4.3). These models were then repeated using bleeding on probing as the outcome measure, and included a third model which adjusted for age, the index of deprivation, smoking status and also the addition of plaque. Finally, the analyses were repeated using the outcome measure of pocket depths above 3.5 mm. In each analysis population weighting, as derived from the dataset, was applied.

4.4. Study 1: Results

4.4.1 Demographics of the Dataset

The original data set included 11,380 individuals from the whole of the study which included England, Wales and Northern Ireland, however this study limited the analysis to the subgroup of England only. Furthermore, for the logistic regression models, only individuals with no missing data from any of the covariates were included to ensure accuracy consistent analysis sample. The demographics of the datasets are shown in Table 4.2. Females formed a greater percentage of the dataset at all stages making up approximately 55% of

the groups. Older people were better represented, with age groups 35-54 and 55 and over making up 35% and 41% in the whole survey and 38% and 37% in the subgroups respectively. Younger people (16-34) were less well represented, making up only 22% of the whole data set and 24% in the subgroups. The proportion of current smokers was 21% in the whole group, 20% in England and 19% in the subgroups, meaning the vast majority of the cohort were not current smokers. The index of deprivation was not available for the whole group, as the dataset was only presented by country and the methodology for recording the index of deprivation was different in England, Wales and Northern Ireland, so could not be combined. Those from the five most deprived deciles of England were less represented at only 45%, when compared to the five least deprived deciles who made up 55% of the sample. In order to account for the study sampling design, weighting was applied to the regression models analysis. The frequency weightings for both the examination and interview data were included in the original ADHS dataset. As the outcome measures of interest for this study were derived from the clinical examination, the examination weighting value was applied to the regression models.

Table 4.2 Study 1: ADHS. Demographics of Individuals Analysed in the Logistic Regression Models

N (%)	Full Sample	England Sample N (%)	Analysis Model 1: Presence of Plaque and Bleeding on Probing	Analysis Model 2: Pocketing > 3.5mm
Number of people	11,380 (100)	9,663 (100)	5,601 (100)	5,552 (100)
Male	5,086 (44.69)	4,314 (44.64)	2,557 (45.65)	2,538 (45.71)
Female	6,294 (55.31)	5,349 (55.36)	3,044 (54.35)	3,014 (54.29)
Age				
16-34	2,539 (22.31)	2,182 (22.58)	1,346 (24.03)	1,339 (24.12)
35-54	4,090 (35.94)	3,464 (35.85)	2,131 (38.05)	2,116 (38.11)
55 and over	4,751 (41.75)	4,017 (41.57)	2,124 (37.92)	2,097 (37.77)
Current Smoker	2,404 (21.12)	2,019 (20.89)	1,079 (19.26)	1,066 (19.20)

Non smoker	8,962 (78.85)	7,633 (79.08)	4,522 (80.74)	4,486 (80.80)
English Index of Deprivation	-	9,657 (100)	5,601 (100)	5,552 (100)
Deciles 1-5 (most deprived)	-	4,361 (45.16)	2,364 (42.21)	2,344 (42.22)
Deciles 6-10 (least deprived)	-	5296(54.84)	3,237 (57.79)	3,208 (57.78)

4.4.2 The Results of Logistic Regression Models Study 1: Objective 1: Sunshine hours, Plaque and Bleeding on Probing

The initial unadjusted model demonstrated an overall reduction in the presence of plaque as the categories of sunshine exposure increased (Odds Ratio (OR)=0.88 95%CI 0.86-0.91, p<0.001), and in the full model, accounting also for age, sex, IMD and smoking status this association persisted (OR=0.90 95% CI 0.87-0.92 p<0.001). These results are shown in Table 4.3. When sensitivity analysis was undertaken with categorical grouping of sunshine hours, high, medium and low, as shown in Table 4.4 the association became more pronounced. In the fully adjusted model, the middle and highest category of sunshine regions had reductions in the presence of plaque of OR=0.69, CI 0.59-0.80 p<0.001 and OR=0.56, CI 0.47-0.66, p<0.001 respectively, when compared to the lowest category.

In the second analysis, in the unadjusted model, each increase in sunshine hour per region from 1 to 10 was associated with a 3% decrease in bleeding on probing (OR=0.97 95% CI 0.95-0.99 p=0.009, n=5601). However, when the model was adjusted for sex, age, smoking, IMD this small association disappeared (OR=0.98 95% CI 0.96-1.01 p=0.125, n= 5601) and the addition of plaque to the model had minimal effect (OR=1.01, 95% CI 0.99-1.04 p=0.30, n= 5601). In the unadjusted model, individuals living in the SHA with the highest sunshine hours had a 31% reduced risk of positive bleeding on probing (OR=0.62 95% 95% CI 0.52-0.91, p=0.008) when compared to the SHA with the lowest sunshine hours, but ORs did not decrease in a linear fashion across categories (Table 4.5). Sensitivity analysis was also completed using the three-level classification of sunshine hours exposure (Table 4.6). Unadjusted results suggested that the odds of reported gingival bleeding were lower in high and medium UVB regions compared to low UVB regions, OR=0.82 (95% CI 0.71-1.03 p=0.004) and 0.88 (95% CI 1.17-1.46 p=0.122) respectively. After adjustment for sex, age, smoking and index of deprivation, the odds ratio for medium versus low and high versus low UVB attenuated to 0.86 (95% CI 0.74-0.99 p=0.035) and 0.96 (95%CI 0.81-1.14 p=0.651) respectively. In a fully adjusted model including plaque, these ORs fully attenuated to 0.94 (95 % CI 0.81-1.09 p=0.42) and 1.14 (95 %CI 0.95-1.37 p=0.15) and the relationship disappeared.

Plaque had the largest effect on risk of gingival bleeding, with the presence of plaque on the teeth strongly associated with an increased risk of gingival bleeding (OR=3.75, 95% CI 3.25-4.34 p<0.001). As gingivitis is caused by the accumulation of plaque this is expected.

4.4.3 The Results of Logistic Regression Models Objective 2: Sunshine Hours and Pocketing Greater than 3.5mm

The logistic regression models were repeated, substituting the outcome measure of gingival bleeding for the outcome measure of pocketing greater than 3.5mm (Tables 4.7 and 4.8). This analysis showed no correlation between sunshine hours and pocketing depths in the simple model. When the full model was analysed living in an SHA with higher sunshine hours was associated with an increased risk of having pockets greater than 3.5mm anywhere in the mouth. The results indicated a trend of increased odds ratio of 4 % (1.04, 95% CI 1.02-1.07, p=0.002) per category. The trend was confirmed by sensitivity analysis showing living in the three highest versus three lowest SHAs in terms of sunshine hours associated with increased odds of 28% in the full model (OR=1.28 95% CI 1.07-1.54, p =0.006).

Table 4.3 Study 1: Association of Presence of Visible Plaque with Mean Annual Sunshine Hours/SHA of Residence (N=5601)

Sunshine hours 1=low, 10=high	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE		
n=5601	OR	95% CI	p	OR	95% CI	p
1	1.00	-	-	1.00	-	-
2	1.80	1.34-2.41	<0.001	1.93	1.41-2.62	<0.001
3	0.91	0.69-1.21	0.527	0.95	0.71-1.26	0.710
4	1.81	1.32-2.47	<0.001	1.87	1.35-2.61	<0.001
5	1.49	1.13-1.96	0.005	1.59	1.19-2.12	0.002
6	0.32	0.25-0.41	<0.001	0.36	0.27-0.47	<0.001
7	0.86	0.67-1.12	0.270	0.92	0.70-1.21	0.556
8	0.63	0.49-0.82	0.001	0.88	0.66-1.17	0.368
9	0.87	0.65-1.16	<0.001	0.89	0.66-1.20	0.436
10	0.35	0.27-0.47	<0.001	0.40	0.29-0.54	<0.001
p for trend	0.88	0.86-0.91	<0.001	0.90	0.87-0.92	<0.001

Sex	M	-	-	-	-	-	-
	F				0.62	0.55-0.71	<0.001
Age	16-24	-	-	-	1.00	-	-
	25-44				1.20	0.95-1.51	0.123
	45-54				1.31	1.02-1.69	0.035
	55-64				1.60	1.24-2.07	<0.001
	65-74				1.50	1.14-1.96	0.004
	75+				1.61	1.17-2.22	0.003
IMD Quintile							
1 Lowest		-	-	-	1.00	-	-
2					1.04	0.71-1.54	0.831
3					0.97	0.67-1.42	0.883
4					0.74	0.51-1.06	0.101
5					0.66	0.47-0.94	0.020
6					0.82	0.58-1.17	0.280
7					0.56	0.40-0.79	0.001

8					0.43	0.31-0.62	<0.001
9					0.54	0.38-0.76	<0.001
10					0.40	0.28-0.56	<0.001
Current Smoke:	No	-	-	-	1.00	-	-
	Yes				1.60	1.32-1.93	<0.001

Table 4.4 Study 1: Sensitivity Analysis: Association of Presence of Visible Plaque with Mean Annual Sunshine Hours/SHA of Residence (N=5601). Categorical Data

	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE		
N=5601	OR	95% CI	p	OR	95% CI	p
Sunshine hours						
Low	1.00	-	-	1.00	-	-
Medium	0.66	0.57-0.77	<0.001	0.69	0.59-0.80	<0.001
High	0.50	0.42-0.60	<0.001	0.56	0.47-0.66	<0.001

Table 4.5 Study 1: Association of Bleeding on Probing Presence with Mean Annual Sunshine Hours/SHA of Residence (N=5601).

Sunshine hours 1=low, 10=high	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE			Model 3 Model 2 + Adjusted for plaque		
N=5601	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
1	1.00	-	-	1.00	-	-	1.00	-	-
2	0.65	0.50-0.84	0.001	0.65	0.50-0.84	0.001	0.54	0.41-0.71	<0.001
3	1.02	0.79-1.34	0.837	1.01	0.77-1.33	0.984	1.03	0.77-1.37	0.835
4	0.99	0.76-1.30	0.963	1.00	0.76-1.32	0.978	0.86	0.64-1.14	0.291
5	0.94	0.73-1.21	0.629	0.97	0.75-1.25	0.801	0.85	0.66-1.11	0.231
6	0.29	0.23-0.38	<0.001	0.32	0.25-0.41	<0.001	0.40	0.30-0.53	<0.001
7	0.84	0.65-1.07	0.165	0.89	0.69-1.14	0.356	0.90	0.70-1,17	0.444
8	1.12	0.87-1.45	0.382	1.38	1.06-1.82	0.019	1.51	1.14-2.00	0.004
9	0.59	0.45-0.78	<0.001	0.59	0.45-0.78	<0.001	0.58	0.44-0.78	<0.001
10	0.69	0.52-0.91	0.008	0.78	0.59-1.03	0.077	1.03	0.77-1.37	0.849

p for trend		0.97	0.95-0.99	0.009	0.98	0.96-1.01	0.125	1.01	0.99-1.04	0.300
Sex	M	-	-	-	-	-	-	-	-	-
	F				0.86	0.76-0.97	0.015	0.97	0.85-1.10	0.596
Age	16-24	-	-	-	1.00	-	-	1.00	-	-
	25-44				1.31	1.07-1.63	0.011	1.29	1.03-1.60	0.025
	45-54				1.59	1.27-2.01	<0.001	1.55	1.22-1.97	<0.001
	55-64				1.47	1.17-1.86	0.001	1.34	1.05-1.70	0.017
	65-74				1.09	0.85-1.41	0.491	0.99	0.76-1.29	0.925
	75+				1.27	0.95-1.70	0.103	1.13	0.84-1.53	0.410
IMD Quintile										
1 Lowest		-	-	-	1.00	-	-	1.00	-	-
2					0.92	0.66-1.28	0.609	0.90	0.64-1.27	0.549
3					0.83	0.60-1.14	0.254	0.82	0.58-1.15	0.245
4					0.71	0.52-0.98	0.036	0.75	0.54-1.04	0.082
5					0.65	0.48-0.88	0.006	0.70	0.51-0.96	0.025
6					0.64	0.47-0.87	0.004	0.64	0.47-0.88	0.006

7 8 9 10					0.57 0.56 0.55 0.50	0.42-0.78 0.41-0.75 0.41-0.74 0.37-0.68	<0.001 <0.001 <0.001 <0.001	0.64 0.67 0.61 0.62	0.46-0.87 0.49-0.92 0.45-0.84 0.45-0.85	0.005 0.012 0.002 0.003
Current Smoke:	No Yes	-	-	-	1.00 1.15	- 0.98-1.35	- 0.089	1.00 1.03	- 0.87-1.22	- 0.728
Current Plaque:	No Yes	-	-	-	-	-	-	1.00 3.75	- 3.25-4.34	- <0.001

Table 4.6 Sensitivity Analysis: Association of Bleeding on Probing with Mean Annual Sunshine Hours/SHA of Residence (N=5601). Categorical Data

	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE			Model 3 Model 2 + Adjusted for plaque		
N=5601	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine hours									
Low	1.00	-	-	1.00	-	-	1.00	-	-
Medium	0.82	0.71-1.03	0.004	0.86	0.74-0.99	0.035	0.94	0.81-1.09	0.42
High	0.88	0.75-1.03	0.122	0.96	0.81-1.14	0.651	1.14	0.95-1.37	0.15

Table 4.7 Study 1: Association of Pocket Depths of > 3.5 mm with Mean Annual Sunshine Hours/SHA of Residence (N=5601).

Sunshine hours 1=low 10=high	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE			Model 3 Model 2 + Adjusted for plaque		
N=5601	OR	95% CI	p	OR	95% CI	p	OR	95% CI	P
1	1.00	-	-	1.00	-	-	1.00	-	-
2	1.04	0.81-1.34	0.744	1.04	0.80-1.36	0.744	0.96	0.73-1.25	0.746
3	0.96	0.74-1.25	0.768	0.98	0.75-1.29	0.895	0.99	0.75-1.29	0.911
4	1.50	1.15-1.96	0.003	1.62	1.22-2.14	0.001	1.50	1.13-1.99	0.005
5	1.01	0.80-1.30	0.884	1.11	0.85-1.44	0.443	1.04	0.80-1.35	0.783
6	0.63	0.49-0.81	<0.001	0.62	0.48-0.81	<0.001	0.73	0.56-0.95	0.020
7	1.86	1.46-2.38	<0.001	1.82	1.41-2.35	<0.001	1.86	1.43-2.41	<0.001
8	0.86	0.67-1.10	0.235	0.87	0.66-1.13	0.294	0.88	0.67-1.16	0.369
9	1.15	0.87-1.51	0.323	1.26	0.94-1.68	0.118	1.28	0.96-1.72	0.093
10	1.27	0.97-1.66	0.088	1.31	0.98-1.75	0.066	1.55	1.16-2.07	0.003

p for trend	1.01	0.99-1.04	0.198	1.02	1.00-1.05	0.089	1.04	1.02-1.07	0.002
Sex M	-	-	-	-	-	-	-	-	-
F				0.83	0.73-0.94	0.004	0.89	0.78-1.01	0.074
Age 16-24	-	-	-	1.00	-	-	1.00	-	-
25-44				2.96	2.29-3.82	<0.001	2.96	2.29-3.83	<0.001
45-54				5.05	3.85-6.62	<0.001	5.05	3.84-6.65	<0.001
55-64				7.73	5.88-0.17	<0.001	7.59	5.76-10.02	<0.001
65-74				7.92	5.92-0.61	<0.001	7.87	5.86-10.59	<0.001
75+				7.31	5.30-0.09	<0.001	7.18	5.21-9.91	<0.001
IMD Quintile									
1 Lowest	-	-	-	1.00	-	-	1.00	-	-
2				0.94	0.66-1.32	0.704	0.94	0.66-1.32	0.709
3				0.82	0.59-1.14	0.239	0.82	0.59-1.14	0.242
4				1.05	0.75-1.47	0.778	1.10	0.78-1.53	0.593
5				0.88	0.64-1.21	0.434	0.93	0.68-1.29	0.679
6				0.79	0.58-1.09	0.150	0.80	0.59-1.10	0.176

7 8 9 10					0.79 0.65 0.63 0.96	0.58-1.08 0.48-0.89 0.46-0.86 0.70-1.32	0.143 0.007 0.003 0.806	0.85 0.73 0.68 1.10	0.62-1.17 0.53-1.00 0.50-0.93 0.80-1.52	0.318 0.050 0.014 0.546
Current Smoke:	No Yes	-	-	-	1.00 1.70	- 1.44-2.01	- <0.001	1.00 1.62	- 1.37-1.92	- <0.001
Current Plaque:	No Yes	-	-	-	-	-	-	1.00 2.06	- 1.79-2.39	- <0.001

Table 4.8 Sensitivity Analysis: Association of Pockets > 3.5mm with Mean Annual Sunshine Hours/SHA of Residence (N=5601). Categorical Data

	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE			Model 3 Model 2 + Adjusted for plaque		
N=5601	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine hours									
Low	1.00	-	-	1.00	-	-	1.00	-	-
Medium	1.14	0.99-1.31	0.059	1.17	1.01-1.36	0.033	1.25	1.07-1.44	0.004
High	1.08	0.93-1.28	0.298	1.16	0.97-1.38	0.095	1.28	1.07-1.54	0.006

4.5 Study 1: Discussion

In summary for objective 1, these analyses demonstrate a clear association between decreased presence of plaque and being resident in an area of increased sunshine hours. Whilst an initial small association was found between exposure to higher UVB levels and a lower risk of positive bleeding on probing, this disappeared when confounding factors were considered. With regards to objective 2, the presence of pockets greater than 3.5 mm was associated with an increase in sunshine hours, in SHA of residence. Therefore, the association whilst clear and consistent for plaque is not clear or present for the other outcomes, and is unexpectedly inverse with regards to pocketing. Possible explanations for this could be due to the limitations of the study, such as the crudity of the geographical exposure area, or the sensitivity of the outcome measure. The outcome measure of positive bleeding on probing has limitations. In the ADHS patients were recorded as positive for bleeding on probing if any of the sextants had bleeding on probing giving only a binary outcome accounting only for presence, not severity. It is recognised that the sensitivity of this outcome is therefore reduced by treating those with one or multiple positive sextants equally, and this is also true for the interpretation of the outcomes of plaque and periodontal pocketing. There is also recognition that the ADHS shows variation between the outcome measures between SHAs. This could be true differences in prevalence of disease, but could also be variation in recording between examiners in different SHAs. The clinical examination, whilst following a recognised protocol and undertaken by trained and calibrated examiners, is done in a sub optimal non-clinical environment and inaccuracies may occur. It is suggested this is more likely to occur when measuring pocket depths, than when recording simpler binary outcomes such as the presence or absence of plaque or gingival bleeding. This residual confounding cannot be accounted for with the data available. Furthermore, bleeding on probing only measures the presence of active inflammation in the gingival sulcus or pocket. It does not distinguish between the diagnosis of gingivitis or periodontitis. Gingival bleeding and inflammation are a prerequisite for the diagnosis of gingivitis, however it is possible to have periodontal pockets with no active inflammation. Therefore, bleeding on probing can only measure current inflammation of the superficial gingival tissues and is not a measure of

past disease. The presence of plaque and presence of gingival bleeding are reversible conditions which can develop quickly over a few days. The causal pathway from increased sunshine, to increased vitamin D and then to changes in immunomodulatory function which could potentially change the quality or quantity of plaque or reduce gingival bleeding, would occur over a short time period. This is supported by the literature where a previous randomised clinical trial found gingivitis decreased in a dose dependent response to vitamin D supplementation over a study period of three months (Hiremath *et al.*, 2013a). In contrast periodontal disease is not reversible and from the outcome measure used it is not possible to differentiate between past and active current bone loss. A more chronic disease process over the course of an individual's lifetime is more likely to have a more complex causal pathway and it may be that the model used here is not sensitive enough to account for the multifactorial cumulative process. Recent literature reviews of vitamin D and periodontal disease have not found a clear association between the two factors, as discussed in previous chapters.

In the current study we did not look at vitamin D supplementation, but instead at UVB exposure to the area of residence of the individual. No recent studies looking at sunshine hours in area of residence versus oral health outcome measures for caries and periodontal health currently exist for comparison or reference. In England, it is recognised that most people get the majority of their vitamin D from endogenous production, however there will be a complex relationship between living in an area with higher UVB levels, personal vitamin D levels and oral health. It is recognised that a principal limitation of the study is that this is an ecological analysis and no information is presented at the individual level, either directly regarding serum vitamin D status, or indirectly via lifestyle factors such as outdoor activity or dietary vitamin D. It is not known how long an individual has lived in the area they were examined and so the sunshine hours allocated to them may not be representative of their history. Further research is needed to obtain this data in order to confirm if the findings of this analysis are transferrable to an individual exposure level. Finally, the sample population is large and the model also includes individual data on various co-variables, which are strengths of the study.

4.6 Study 1: Conclusion

In this study residing in an area of increased sunshine hours was associated with reduced presence of plaque in all regression models. Initially, a weak association was found between increased sunshine hours and gingival bleeding but this disappeared when confounders were considered. Being resident in an area of increased sunshine hours was associated with increased risk of pocketing greater than 3.5mm.

4.7 Further Work

The analysis described here focused on an adult population, plaque levels and gingival health outcomes. After completing the literature review in chapter 1 the evidence suggested that vitamin D may influence the immune response and the inflammatory response. Changes in the immune system could modify the quantity or quality of plaque, whilst changes in the inflammatory response would most likely be seen in periodontal outcomes, such as bleeding on probing. Previous studies, as discussed in chapter 2, had also suggested an association between caries and sunshine /full spectrum light exposure in children and it was felt it would be useful to repeat the epidemiological studies using more comprehensive modern datasets. Periodontitis is uncommon in children but childhood caries is an important childhood health issue. Therefore, the following chapter focuses on caries outcomes in a child population.

Chapter 5: Association of Dental Caries with Sunshine Exposure in Participants of Study 2: The Children's Dental Health Survey 2013 and Study 3: The Oral Health Survey of Five-year-old Children 2014-15

5.1 Introduction

Vitamin D can be obtained from both dietary sources and sunlight exposure. In the case of sunlight exposure vitamin D3 is formed in the skin when a person is exposed to UVB light causing 7-dehydrocholesterol (7-DHC) to be converted to vitamin D. It is known that vitamin D deficiency in the UK, defined here as the level at which risk of rickets is increased, can occur in some children and is indicated by serum concentrations less than 25 nmol/L (10 ng/mL) (SACN, 2016). The latest SACN report concluded the proportion of UK children with plasma 25(OH)D concentration < 25 nmol/L(10ng/mL) in the winter was 31% in children (four to ten years old) and 40% in adolescents (11-18 years). In the summer these figures were lower but persisted in 2% and 13% of individuals in the two groups respectively. For the UK population the SACN report recommends that children aged four to eleven have a daily intake of 10 µg/d (400 IU/d). It also advises a safe intake range of 8.5-10 µg/d (340-400 IU/d) for infants aged zero to eleven months and 10 µg/d (400 IU/d) for infants and children aged one to three years.

5.1.2 Vitamin D Supplementation and Dental Caries in Children: A Summary of the Relevant Literature

Vitamin D supplementation has been associated with decreased incidence of dental caries in children (Hujoel, 2013) but no randomised controlled trials exist showing the effect of solely vitamin D supplements or sunshine or UVB exposure. As discussed in Chapter 2, some of the first reports discussing caries and vitamin D occurred in the 1920s and 1930s, including those by May Mellanby, who looked extensively at diet, vitamin D supplements, formation of teeth and caries (Mellanby and Pattison, 1928; Mellanby and Pattison, 1932; Mellanby, Pattison and Proud, 1924). Along with other researchers at the time, she published reports which supported a role for vitamin D rich diets, or vitamin D supplements, in the prevention of childhood

caries (McBeath, 1934). More recently, in 2013 a systematic review was published which looked at the relationship between vitamin D and dental caries (Hujoel, 2013). The review included many of these older studies which considered dietary intake of vitamin D, vitamin D supplements in several forms and UV supplements. Many of the studies were decades old and had limitations in the study design and variation in exposures and outcome measures, however the review concluded that the pooled relative risk estimate of supplemental vitamin D was 0.53 (RR 0.53, 95% CI 0.43 to 0.65; p<0.001) in relation to reducing dental caries, with subgroup analysis of UV light supplementation studies showing an even greater impact with RR of 0.36 (RR 0.36, 95% CI 0.17 to 0.78, p=0.009). Since then, a number of studies have used data collected in national health surveys to investigate the relationship between vitamin D and dental caries in children, and for context on where the analysis described in this chapter sits, they will be discussed further here.

Schroth *et al*. assessed the relationship between vitamin D status and dental caries in 1,017 Canadian school-aged children who participated in the Canadian Health Measures Survey 2007-2009 (Schroth *et al.*, 2016b). This was a national cross-sectional survey that was representative of 97% of the Canadian population between 6-79 years, although the age group considered in this study was 6-11 years. The survey collected both clinical and interview data. Vitamin D status was determined from serum measurements of 25(OH)D and caries outcomes were measured by the presence or absence of caries in both the primary or secondary dentition, and the dmft/DMFT score of the primary or secondary dentition. Levels of vitamin D above 75 nmol/L (30 ng/mL) and 50 nmol/L (20 ng/mL) were considered in the analysis. The results of logistic regression models suggested that children with serum vitamin D above 75nmol/L had 39% lower odds of having dental caries or caries experience than those who were deficient (OR 0.61 95%CI 0.46-0.79, p=0.002). Those children who had serum levels above 50 nmol/L had 47% lower odds of dental caries than those who had serum vitamin D below 50 nmol/L (OR 0.53, 95%CI 0.34-0.84, p=0.007). The same researcher previously conducted a case–control study from 2009 to 2011 in the city of Winnipeg, Manitoba, Canada, determining the association between serum concentrations of 25(OH) D and severe early childhood caries (S-ECC) in preschool children (mean age 40.8 ± 14.1 months).

Mean 25(OH)D levels were significantly lower among children with S-ECC than caries-free controls with multiple regression for 25(OH)D concentrations (p=0.006) revealing that levels were significantly and independently associated with S-ECC (Schroth *et al.*, 2013). A German study utilised data from the ongoing LISAplus birth cohort study which collected a large amount of lifestyle and health data, including supplement use, from healthy full term babies born in Germany enrolled in the study between 1995 and January 1999 (Kühnisch *et al.*, 2017). Follow up examinations were performed at six months, one year, eighteen months, two years, four years, six years and ten years, when questionnaires collecting data on health status and supplement use were completed. Fifty percent of children were lost to follow up and at ten years of age the remaining 406 children, who had complete data on vitamin D and fluoride supplementation, underwent a dental examination and were included in the study. The dental examination was consistent with those used in other epidemiological studies and caries experience was determined using the WHO DMFS index for the permanent and primary dentition. Data on molar-incisal hypomineralisation (MIH) were also recorded. Logistic regression models, adjusted for age, sex, BMI, parental education and equivalent net income, were used to analyse the association between supplement use and caries and MIH. For vitamin D supplementation, a caries preventive effect was observed in the primary dentition only when it was administered past the age of 12 months (p=0.001). There was no association found with MIH or between vitamin D and caries prevention in the permanent dentition. A Swedish study of eight-year-old children, who had previously participated in an intervention study on milk-based vitamin D supplementation at six years of age, evaluated associations of vitamin D status with caries (Gyll *et al.*, 2018). The dental study was conducted 2 years after the intervention study as a later addition and so only 85 of the 206 children in the original study underwent a dental exam. Of the 85 children in the dental analysis, 37 had been given 25 micrograms (1000 IU), 38 had 10 micrograms (400 IU) and ten had 2 micrograms (80 IU, placebo dose) of vitamin D daily, and caries experience was measured using the combined Decayed Filled Surface (DFS) measurement for permanent and primary teeth, excluding the missing component as most teeth had not been lost from caries. Logistic regression models were used to analyse the relationships with the full model accounting for number of teeth, tooth brushing, presence of *S. mutans* bacteria,

father's education level, region of residence, BMI, current vitamin D supplement and skin type. Vitamin D status had been recorded from serum samples taken at the end of the three-month intervention study. In the univariate analysis increasing vitamin D status showed a statistically significant protective effect of higher vitamin D levels and less dental caries, however in the full corrected model the relationship attenuated and was no longer significant statistically. No relationship was found regarding enamel defects and vitamin D status. Similarly a further study used data from the 2005-6 NHANES study, which is a national stratified, multistage, probability sample survey conducted annually in the United States (Herzog *et al.*, 2016). Herzog aimed to determine the association between serum vitamin D levels and dental caries in noninstitutionalised children who participated in the study between the ages of five and twelve years. The children had undergone a dental examination and in Herzog's study overall caries experience was defined as the presence of at least one restoration or one tooth with untreated caries. Vitamin D status was derived from serum measurements of 25(OH) D from venous blood samples and the children were grouped according to their status. The grouping used the Institute of Medicine 2011 guidelines and defined vitamin D deficiency as serum 25(OH) D less than 30 nmol/L (12 ng/ml), vitamin D inadequacy as serum 25(OH)D between 30 (12 ng/ml) and 49 nmol/L, sufficiency as serum 25(OH)D between 50 and 125 nmol/L (20-50 ng/ml) and serum 25(OH) D greater than 125 nmol/L (50 ng/ml) as potentially harmful levels. A multivariate logistic regression model for age, sex, race and ethnicity, ratio of family income to poverty threshold, and sugar consumption was used for the analysis of the data. In this study no significant association was found between vitamin D deficiency, 25(OH) D less than 30 nmol/L (12 ng/ml), and caries experience. The direction of evidence, although not significant, trended towards deficiency being associated with more caries experience. The authors suggested therefore that there may have been limitations to their study, which explained why their findings did not align with some other study outcomes. These limitations included a lack of information on the season of measurement of 25(OH) D levels, sun exposure, fluoride exposure, water fluoridation status, tooth brushing habits, detailed geographic data, and location of participants' home. The method of recording caries in the NHANES study may have led to an underestimation of the true caries prevalence.

Overall, there appears to be inconsistent evidence about the relationship between vitamin D and childhood caries. Although the direction of evidence often points towards a protective effect with increased serum vitamin D levels no definitive conclusions can be drawn.

5.2 Aims

As stated in Chapter 3 specific *a priori* hypotheses tested in these empirical studies were:

Study 2: The prevalence and severity of dental decay in children will be lower in Regions with higher sunshine hours than in Regions with less sunshine hours

Study 3: The prevalence and severity of dental decay of child populations will be lower in LAs with higher sunshine hours than in LAs with less sunshine hours

Therefore, these studies aim to investigate the relationships between sunshine hours in area of residence and prevalence of dental caries in children.

Specifically

i) Prevalence of decay in the primary dentition

ii) Prevalence of decay in the permanent dentition

5.3 Methodology

5.3.1 Exposure Dataset

As per ADHS analyses presented in Chapter 4, estimated exposure in terms of sunshine hours was obtained from MEDMI, which utilises Met Office data from meteorological stations around the UK. It included sunshine hours, recorded using a Campbell-Stokes meter, from 78 stations around the UK. The process of quantifying and assigning sunshine hours to specific areas and regions is described fully in Chapter 3.

5.3.2 Exposure Measure

The exposure measures used were derived using the same weighted interpolation process described previously for the ADHS study in Chapter 4. Sunshine data were allocated to residence area boundaries in order to be matched to Child Dental Health Survey (Government Office Regions) or Oral Health Survey of Five-year-old Children (Local Authorities) participant or area data. In the CDHS analysis the gridded data were overlaid with geographical boundary data of the nine Government Office Regions of England (GOR). Again, this was used to obtain the average estimate of the five-year monthly mean hours of sunshine for each region over 2010-2014 and then summarised to a five-year monthly mean for the whole period for the months April to October. The results are shown in Table 5.1. The process was repeated to create an exposure measure for the Dental Epidemiology Programme for England, Oral Health Survey of Five-year-old Children, based on the boundaries of the 326 Lower Tier Local Authorities (LA).

Table 5.1 Monthly Mean Hours of Sunshine (March – October) 2010-2014 per Government Office Region

Government Office Region	Mean Sunshine Hours	Number of children
North East	149.0	692
North West	150.0	855
Yorkshire and Humber	150.4	594
West Midlands	161.6	591
East Midlands	165.4	488
East of England	172.5	520
South West	176.2	583
London	181.5	685
South East	184.2	634

5.3.3 Oral Health Datasets

Data for this study were obtained from two oral health surveys. The first was the Child Dental Health Survey (CDHS) 2013, which included data from 5-, 8-, 12- and 15-year-olds in mainstream education in England, Wales and Northern Ireland. The second was The National Dental Epidemiology Programme for England, Oral Health Survey of Five-year-old Children (OHS), 2015. Both data sets were national epidemiological surveys and their full descriptions have been covered in Chapter 3 but are summarised again for context here.

5.3.3.1 Study 2: Children's Dental Health Survey (CDHS) 2013

CDHS is a cross sectional survey consisting of a dental examination, a parent questionnaire and a self-completed questionnaire for older children (12- and 15-year-olds) and a parent questionnaire only for younger children (5- and 8-year-olds). According to the technical documentation for the selection of the participating schools, a list of schools and a list of eligible pupils in those schools, was obtained for each region. The English sample was stratified by the nine GORs, which were North East, North West, Yorkshire and Humber, East Midlands, West Midlands, East of England, London, South East and the South West. The schools were clustered into groups nested within regions using Geographical Information Systems and then school groups selected at random included in the study. In England 71% of Primary schools and 40% of secondary schools selected took part in the survey. Positive consent was required and in the final survey 70% of five-year-olds, 63% of eight-year-olds, 84% of twelve-year-olds and 72% of fifteen-year-olds, who were eligible for the study, underwent clinical examination in England. The response rates for the questionnaires were 99.7% and 99.5% from the twelve and fifteen-year-old pupil questionnaires and 45%, 46%, 36% and 31% of productive parent questionnaires for the five, eight, twelve, and fifteen-year-old cohorts respectively. For the purposes of this study only children from England were considered in the analysis conducted by the author, and in total 1,526 five year olds, 1,369 eight year olds, 1,434 twelve year olds and 1,313 fifteen year olds, were included in this analysis (*CDHS,* 2015). The data from the CDHS included individual level oral health data but area of residence was reported at GOR

level. The outcome measures for oral health in this study were derived outcomes provided in the original dataset obtained from the UK Data Service, referring to permanent and primary teeth (NHS Digital, 2015).

These included primary outcome measures, chosen to investigate the prevalence of caries:

- the presence of any clinical decay experience in primary teeth (including cavitated enamel caries)
- the presence of any clinical decay experience in primary teeth (including cavitated and visual enamel caries *)

- the presence of any clinical decay experience in permanent teeth (including cavitated enamel caries)
- the presence of any clinical decay experience in permanent teeth (including cavitated and visual enamel caries)

* Visual enamel caries is early caries seen in enamel but without cavitation.

Secondary outcome measures were chosen to investigate severity. These included:

- the presence of more than five primary teeth with obvious decay
- the presence of more than three primary teeth with decay into dentine

- the presence of more than five permanent teeth with obvious decay
- the presence of more than three permanent teeth with decay into dentine

- the presence of any enamel opacities (12-year-olds only)

Most of the outcomes related to caries, active or/and past disease, however the presence of enamel defects was also included as a secondary outcome as it is

a sign of disrupted enamel mineralisation and formation. The term clinical decay experience refers to evidence of tooth decay in the enamel, dentine or pulpal layers of the crown of the tooth. All teeth with cavitated or visual dentine caries, restorations with cavitated or visual dentine caries, teeth with filled decay (otherwise sound), teeth extracted due to caries and teeth with visual or cavitated enamel caries were included under this terminology in the CDHS survey. However, the outcome measures chosen for use in the analysis differentiate between including visual caries or not. The visual enamel caries includes early demineralisation, also known as white and brown spot lesions. In one of the papers included in the systematic review of Chapter 2 it was suggested that exposure to full spectrum lighting encouraged remineralisation of these early non cavitated lesions (Hargreaves and Thompson, 1989). It was therefore useful to consider the outcomes with and without these lesions as it is hypothesised their occurrence may be less in areas with more sunshine. As described previously, the primary teeth are formed *in utero* and before weening in the months after birth, so the vitamin D available at the time to the children would have been influenced by the maternal vitamin D levels, as well as potentially sunshine exposure. Optimum vitamin D at this time, it is hypothesised, would lead to better mineralisation during the formation of the teeth and also better tooth surface remineralisation from mineral in the saliva once the teeth were erupted. The same hypothesis applies to the permanent teeth except that these teeth formed after birth and so vitamin D would be from sunshine exposure and the diet. The outcome measures for five or more decayed primary/permanent teeth and three or more primary/permanent teeth with decay in to dentine were included as a measure of the number of children who had more extensive dental caries at the time of the survey. It was proposed this would be higher in areas with less sunshine. Enamel opacities can be a sign of disruption of the amelogenesis process during tooth formation. Vitamin D influences amelogenesis and mineralisation of tissues as discussed in previous chapters, therefore, it was hypothesised these opacities would be higher in areas of lower sunshine exposure. Other factors considered for use in the logistic regression models included age, sex and index of deprivation (quintiles only provided with dataset). The variable for presence of plaque was also used as a proxy measure for oral hygiene. With regards to tooth brushing, the frequency of this was recorded in the questionnaire but the use of fluoride

toothpaste was not. Although most toothpastes do contain fluoride, the amount is variable, therefore this measure was not considered useful as an indicator of an exposure to fluoride and the clinical examination measure of the presence of plaque was considered to be more accurate as to the effectiveness of oral hygiene measures.

5.3.3.2 Study 3: Dental Epidemiology Programme for England, Oral Health Survey of Five-year-old Children, 2015 (OHS)

The Oral Health Survey of Five-year old Children is a cross sectional epidemiological survey which aims to measure the prevalence and severity of dental caries among five-year-old children. It uses caries diagnostic criteria and examination techniques based on those agreed by the British Association for the Study of Community Dentistry (BASCD) and although it collects individual level data, only data at lower tier local authority level (district councils and unitary authorities) is released. The survey population is defined as all five-year-old children attending state funded primary schools of all classifications within the local authority (boundaries at unitary, metropolitan borough or lower tier level). According to the technical documentation the sampling is via a two-stage technique. The first stage obtained a list of all state maintained primary schools within each local authority area, and the numbers of pupils attending at each. The second stage was a stratified sampling method taking school size into account. The primary sampling unit was the lower tier local authority (LA) and in total the survey covered 324 out of 326 lower-tier local authorities. Consent to be examined was gained by positive written consent from parents. This resulted in 111,500 (representing 95.7% of the consented sample) clinical examinations in the final analysis, which represented 16.5% of the population of this age cohort attending mainstream state schools.

The primary outcome measures, chosen to investigate the prevalence of caries, from this dataset was *:

- Mean number of decayed missing filled teeth (dmft)**

The secondary outcome measures chosen to investigate severity of caries were:

- Mean dmft in children with dmft>0

- Percentage of children with incisor caries

* all outcome measures are weighted

**missing status only recorded if due to caries, decay only recorded if into dentine.

The decay recorded in this survey was decay which had reached dentine. Uncavitated lesions, white spot lesions and early caries were not recorded, therefore the actual amount of decay present may have been greater than recorded levels. Only teeth missing, or those assumed to be missing, due to caries were included as missing in the decayed, missing, filled teeth score. The presence of substantial amounts of plaque was also used to provide a proxy measure for toothbrushing. Poor oral hygiene increases the risk of caries, as plaque levels would be high and exposure to topical fluoride would be low.

5.4 Analysis

5.4.1 Study 2: Child Dental Health Survey Analysis

As with the analysis of the ADHS in Chapter 4, for the CDHS logistic regression was used to investigate the association between sunshine hours and the various outcome measures across the nine GORs. The basic models consisted initially of no confounders being taken into account. Analysis was then completed for a second model which adjusted for sex, age, the index of multiple deprivation for England (IMDE) quintile and smoking status (where applicable in the older age groups) and a third full model which adjusted for sex, age, the IMDE quintile, smoking status and also plaque. Plaque was added in separately and lastly to the third and final model to explore whether mediation by plaque was potentially taking place, as discussed in chapter three. As with the ADHS and due to the complex sampling approach of the survey, weighting as provided from the CDHS dataset was applied to the regression analyses in order to make the sample representative. The frequency weightings for the examination data were used as the outcome measures of interest for this study were all derived from the clinical examination.

5.4.2 Study 3: Oral Health Survey of Five-Year Old Children Analysis

As oral health data was available at lower tier local authority level and not at individual level the Oral Health Survey of Five-Year Old Children data was completed using linear regression. The basic model included sunshine hours and the oral health outcomes of interest, with no additional factors considered. The second model included water fluoridation and index of deprivation at lower tier local authority level. The fluoridation status for each LA was taken from Public Health England's (PHE) document "Health monitoring report for England 2014" (Public Health England, 2014) and the measures of social deprivation from a Department for Communities and Local Government report, part of English Indices of Deprivation 2015 (Smith, 2015). These data sources were discussed in detail in Chapter 3. Percentage of children with substantial plaque was added in to the third and final model. All the outcome measures were presented in the published report as weighted prevalence data, so no weighting factor had to be applied in this case.

5.5 Results

5.5.1 Study 2: CDHS Results

5.5.1.1 Demographics of the CDHS Analysis Population

The unweighted demographics of the CDHS population are described in Table 5.2. In total across all age groups 5,642 children participated in a clinical examination, with slightly more females (51.51%) than males. Across the quintiles of deprivation, overall, the largest percentage of children fell into the most deprived quintile, 39.24% and only 11.50% of children in the least deprived quintile. The CDHS technical document explains oversampling of deprived schools at country, LA and school level was undertaken in order to achieve a target of a third of pupils included being from schools classed as deprived. Deprived was defined at school level as 30% or more of pupils being eligible for free school meals. Other social information, such as smoking, was not relevant to most children, which is expected given the ages of the population. Five- and eight-year-olds were not asked about smoking and in the older age groups of twelve- and fifteen-year-olds only 155 (2.75%) children

reported being smokers currently and most of these, 139, were age fifteen. However, it is recognised that children may not have wished to self-report and this may be an under representation of true numbers of smokers. Over half the children (60.85%) had visible plaque, which can be considered an indicator of poor oral hygiene.

Overall, in England nearly 25% of children presented with signs of current or past decay in their primary teeth. This was highest in the group of eight-year-olds where 49.23% had some clinical decay experience of their primary teeth. When uncavitated enamel lesions were also included these figures rose to 31.67% and 60.41% respectively. It was also noted that in the five-year-old age group 7.4% of children already had more than five primary teeth with obvious decay and 13.5% had more than three with decay extending into dentine. In the eight-year-old year group these figures rose to 10.08% and 17.02 %. Once in to the age twelve and fifteen age groups the number of decayed primary teeth reduced, as expected when these teeth exfoliated.

When considering the permanent dentition, overall, 27.06% of children were noted to clinically have had experience of decay in their permanent teeth and this rose to 40.39% if non cavitated enamel lesions were included. As expected, the group with the most clinical experience of decayed permanent teeth were the fifteen-year-old age group, who had the most permanent teeth erupted into the oral environment for the longest period of time. In that group 52.70% had teeth with clinical signs of active or past decay and 67.48% if uncavitated enamel caries was counted also. Worryingly by the age of fifteen in this cohort, 12.19% had more than five decayed permanent teeth and 9.06% had at least three teeth with decay into dentine. Finally, the prevalence of enamel opacities, which were only recorded in the upper eight front permanent teeth of twelve-year olds (UR1-4, UL1-4), was 31.45%.

Table 5.2 Demographics of the CDHS Population

	England Sample N (%)	Age 5	Age 8	Age 12	Age 15
Number of children	5,642 (100)	1,526 (100)	1369 (100)	1434 (100)	1313 (100)
Male	2,736 (48.49)	763 (50.00)	661 (48.28)	689 (48.04)	623 (47.45)
Female	2,906 (51.51)	763 (50.00)	708 (51.72)	745 (51.95)	690 (52.55)
SES- IMDE QUINTILES 1-5 ENGLAND					
IMDE 1 (most deprived)	2,214 (39.24)	591 (38.72)	495 (36.16)	597 (41.63)	531 (40.44)
IMDE 2	1,073 (19.02)	283 (18.55)	286 (20.89)	260 (18.13)	244 (18.58)
IMDE 3	756 (13.40)	236 (15.47)	194 (14.17)	169 (11.79)	157 (11.96)
IMDE 4	724 (12.83)	184 (12.06)	175 (12.78)	193 (13.46)	172 (13.10)
IMDE 5 (least deprived)	649 (11.50)	173 (11.34)	163 (11.91)	157 (10.95)	156 (11.88)
Missing data	226 (4.01)	59 (3.87)	56 (4.09)	58 (4.04)	53 (4.04)
Smoking					
Current smoker	155 (2.75)	N/A	N/A	16 (1.12)	139 (10.59)
Non-Smoker	2,544 (45.09)	-	-	1390 (96.93)	1154 (87.89)
Missing data	48 (0.85)	-	-	28 (1.95)	20 (1.52)
N/A	2,895 (51.31)	-	-	-	-
Plaque					
Plaque visible	3,433 (60.85)	732 (47.97)	1000 (73.04)	955 (66.60)	744 (56.66)
No Plaque	2,196 (38.92)	7895 (51.70)	365 (26.66)	475 (33.12)	567 (43.18)

Missing Data	13 (0.23)		5 (0.33)	4 (0.29)	2 (0.14)	2 (0.15)
	England Sample N (%)		Age 5	Age 8	Age 12	Age 15
Number of children	5,642 (100)		1,526 (100)	1369 (100)	1434 (100)	1313 (100)
Any clinical decay experience in primary teeth (*incl. cavitated enamel caries)	Yes	1,413 (25.04)	563 (36.89)	674 (49.23)	168 (11.72)	8 (0.61)
	No	4,229 (74.96)	963 (63.11)	695 (50.77)	1,266 (88.28)	1,305 (99.39)
Any clinical decay experience in primary teeth (*incl. cavitated and visual enamel caries)	Yes	1,787 (31.67)	768 (50.33)	827 (60.41)	183 (12.76)	9 (0.69)
	No	3,855 (68.33)	758 (49.67)	542 (39.59)	1251 (87.24)	1304 (99.31)
>5 primary teeth with obvious decay	Yes	256 (4.54)	113 (7.40)	138 (10.08)	5 (0.35)	0 (0)
	No	5,386 (95.46)	1,413(92.60)	1,231 (89.91)	1,429 (99.65)	1,313 (100)
>3 primary teeth with decay into dentine	Yes	457 (8.10)	206 (13.50)	233(17.02)	18 (1.26)	0 (0)
	No	5,185 (91.90)	1,321(86.57)	1,136 (82.98)	1,416 (98.74)	1,313 (100)
Any clinical decay experience in permanent teeth (*including cavitated enamel caries)	Yes	1,527 (27.06)	37 (2.42)	232 (16.95)	566 (39.47)	692 (52.70)
	No	4,115 (72.94)	1,489 (97.58)	1,137 (83.05)	868 (60.53)	621 (47.30)

Any clinical decay experience in permanent teeth (*inch, cavitated and visual enamel caries)	Yes	2,279 (40.39)	64 (4.19)	481 (35.14)	848 (59.14)	886 (67.48)
	No	3,363 (59.61)	1,462 (95.81)	888 (64.86)	586 (40.86)	427 (32.52)
>5 permanent teeth with obvious decay	Yes	245 (4.34)	9 (0.59)	10 (0.73)	66 (4.60)	160 (12.19)
	No	5,397 (95.66)	1,517 (99.41)	1,359 (99.27)	1,368 (95.40)	1,153 (87.81)
>3 permanent teeth with decay into dentine	Yes	243 (4.31)	2 (0.13)	20 (1.46)	102 (7.11)	119 (9.06)
	No	5,399 (95.69)	1,524 (99.87)	1,349 (98.54)	1,332 (92.89)	1,194 (90.94)
Any enamel opacities (12yrs only)	Yes	451 (7.99)	0 (0)	0 (0)	451(31.45)	0 (0)
	No	983 (17.42)	0 (0)	0 (0)	983 (73.80)	0 (0)
	N/A	4,208 (74.58)	1,526 (100)	1,369 (100)	0 (0)	1,313 (100)

It is important to look at these figures within the context of the children having a primary, mixed and permanent dentition. Figures 5.1- 4 show the teeth that are present, on average, in the mouth at ages five, eight, twelve, and fifteen years of age (Welbury R). Using the Palmer notation system children have 20 primary teeth, defined here using the letters A to E in each quadrant, and adults have 32 permanent teeth numbered 1 to 8 in each quadrant. The 8s, or wisdom teeth, usually do not erupt until late teens and were not recorded in this survey. At age twelve the dentition can be very varied, with some children having only permanent dentition and others still with a mixed dentition and retained primary teeth. These figures are an average representation as the eruption of teeth into the mouth is variable and it is not considered unusual for teeth to erupt six months either side of the average eruption dates. The actual numbers of children in the CDHS dataset and the number of primary and permanent teeth present are displayed in Tables 5.3 and 5.4. This demonstrates not only the loss of primary teeth with age, but also the mixed dentition phase aged twelve. As it can be seen that eight-year-old children had both primary and permanent teeth in significant numbers they were included in the analysis for both primary and permanent teeth. Therefore, regression models for outcome measures for primary teeth were completed on age groups five and eight years and models for permanent teeth were completed on age groups eight, twelve and fifteen.

Maxillary															
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
			E	**D**	**C**	**B**	**A**	**A**	**B**	**C**	**D**	**E**			
			E	**D**	**C**	**B**	**A**	**A**	**B**	**C**	**D**	**E**			
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
Mandibular															

Figure 5.1 Age 5 Primary Dentition Example

Maxillary															
8	7	**6**	5	4	3	**2**	**1**	**1**	**2**	3	4	5	**6**	7	8
			E	**D**	**C**	B	A	A	B	**C**	**D**	**E**			
			E	**D**	**C**	B	A	A	B	**C**	**D**	**E**			
8	7	**6**	5	4	3	**2**	**1**	**1**	**2**	3	4	5	**6**	7	8
Mandibular															

Figure 5.2 Age 8 Mixed Dentition Example

Maxillary															
8	**7**	**6**	**5**	**4**	3	**2**	**1**	**1**	**2**	3	**4**	**5**	**6**	**7**	8
			E	D	**C**	B	A	A	B	**C**	D	**E**			
			E	D	C	B	A	A	B	C	D	**E**			
8	7	**6**	**5**	**4**	**3**	**2**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	7	8
Mandibular															

Figure 5.3 Age 12 Mixed Dentition Example

Maxillary															
8	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	8
			E	D	C	B	A	A	B	C	D	E			
			E	D	C	B	A	A	B	C	D	E			
8	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	8
Mandibular															

Figure 5.4 Age 15 Permanent Dentition Example

Figures 5.1-4 Most Common Teeth Present in Each Age Group of CDHS Survey. Teeth Most Commonly Present in Bold

Table 5.3 Number of Children and Number of Primary Teeth Present in CDHS Dataset.

	No. of children				
No. of primary teeth present	5 yrs.	8 yrs.	12 yrs.	15 yrs.	Total
0	0	9	1,068	1,265	2,342
1	0	6	117	29	152
2	1	13	67	12	93
3	0	7	36	3	46
4	2	25	40	2	69
5	1	19	24	0	45
6	3	28	21	0	53
7	2	39	19	0	60
8	2	64	16	0	82
9	1	57	5	0	63
10	7	109	10	0	126
11	2	152	4	0	158
12	12	656	7	0	675
13	8	66	0	0	74
14	34	75	0	0	109
15	29	19	0	0	48
16	58	14	0	0	72
17	69	8	0	0	77
18	291	2	0	0	293
19	126	0	0	0	126
20	878	1	0	0	879
Total	1526	1369	1434	1313	5642

Table 5.4 Number of Children and Number of Permanent Teeth Present in CDHS Dataset

	No. of Children				
No of permanent teeth present	5 yrs.	8 yrs.	12 yrs.	15 yrs.	Total
0	814	1	0	0	815
1	100	0	0	0	100
2	183	1	0	0	184
3	77	0	0	0	77
4	117	2	0	0	119
5	56	1	0	0	57
6	95	8	0	0	103
7	29	12	0	0	41
8	24	28	0	0	52
9	13	31	0	0	44
10	14	147	0	0	161
11	1	118	0	0	119
12	2	692	7	0	701
13	0	117	8	0	125
14	0	83	10	1	94
15	0	36	8	0	44
16	0	30	22	0	52
17	0	15	17	0	32
18	0	15	17	0	32
19	0	12	28	0	40
20	1	7	34	3	45
21	0	3	32	1	36
22	0	0	39	2	41
23	0	1	61	10	72
24	0	6	100	106	212
25	0	1	73	26	100
26	0	0	153	121	274
27	0	0	184	126	310
28	0	2	641	917	1,560
Total	1,560	1,369	1,434	1,313	5,642

5.5.1.2 Results of the CDHS Analysis for Different Outcome Variables

5.5.1.2.1 Results for Outcome Measures for Primary Teeth

The analyses were only completed on individuals who had data for all the variables in the model. If data were missing regarding either the outcome or confounding variables the individual was removed from the analysis. Children who were 12 or 15 were also excluded as they had very few or no remaining primary teeth, as previously discussed (Table 5.3). After those with missing data were removed 2771 individuals remained. The exclusion of individuals without full data did not lead to large discrepancies in the groups. Slightly more of the children were aged 5 rather than aged 8 (52.8% versus 47.2%), and there were marginally more girls than boys (51% versus 49%). Almost two thirds of children had the presence of plaque recorded clinically and the largest difference was in the representation between the different quintiles of deprivation. Nearly 40% of children were in the most deprived quintile and only approximately 12% in the least deprived quintile. This mirrors the full sample population and is indicative of oversampling in the most deprived areas to ensure representation in the final analysis.

Table 5.5 Demographics of the Analysis Sample for the Primary Dentition

	No. of children N (%) = 2771
Age 5	1462 (52.76)
Age 8	1309 (47.24)
M	1357 (48.97)
F	1414 (51.03)
1 Most deprived	1083 (39.08)
2	567 (20.46)
3	427 (15.41)
4	359 (12.96)
5 Least Deprived	335 (12.09)
Visible plaque	1664 (60.05)
No visible plaque	1107 (39.95)

5.5.1.2.2 Presence of any clinical decay experience in primary teeth, primary outcome measures

Tables 5.6 and 5.7 demonstrate the association firstly between the presence of any decay experience and cavitated lesions only in primary teeth, and secondly between the presence of any decay experience and both visual caries and cavitated lesions in primary teeth, with sunshine hours. Cavitated lesions occur when the enamel surface has been broken down by the decay process and a definite cavity occurs. Visual lesions include lesions before cavitation occurs. Therefore, if including visual lesions as well as cavitated lesions, an increase in the number of children who have signs of decay or decay experience present would be expected, as both early and established decay are recorded, as opposed to cavitated lesions only. However, one of the hypotheses for the actions of increased vitamin D due to increased sunshine exposure, is that higher levels of mineral are found in saliva and more remineralisation of early lesions occurs. This would mean that in areas of higher sunshine exposure, there would potentially be a slower progression to cavitated lesions, compared to areas with less sunshine. There would also potentially be a higher ratio of visual caries to cavitated caries.

In the first simple model (Table 5.6), considering only sunshine hours and the presence of any decay experience (excluding visual caries), for every one extra hour of sunshine there was a small reduction in the presence of any decay experience in primary teeth of 1% (OR 0.99 CI 0.98-1.00 p=0.019). In the full model, which considered sex, age, IMDE and the presence of plaque this relationship persisted (OR 0.99 CI 0.98-1.00 p=0.024). There was clear increased risk of any decay experience associated with being aged 8 rather than 5 (OR 1.46 CI 1.25-1.75, p<0.001) and clear decreased risk associated with living in a less deprived area (OR 0.35, 0.24-0.53, p<0.001 least deprived IMDE quintile) and the absence of plaque (OR 0.45, CI 0.36-0.58, p<0.001). The associations with these variables would be expected as 8-year-olds would have had their primary teeth for longer and therefore there would have been more time for decay experience, a cumulative measure, to occur. It has also been discussed how lower socio-economic status is a risk factor for tooth decay and the relationships with IMDE seen in this model support this. Furthermore, plaque bacteria are a prerequisite for decay therefore having no visible plaque,

a proxy for good oral hygiene, leads to a reduced risk of tooth decay as expected. To give an idea of the potential scale of sunshine on the population and presence of caries by comparing between the highest and lowest sunshine categories (36 hours difference), there appears to be a 35% (OR 0.65, CI 0.44-0.94, p=0.024) reduction in the number of children with any decay experience (excluding visual caries). The analysis was repeated for sensitivity treating the exposure measure as categorical rather than linear. Although the relationship was less clear the overall trend was still decreasing risk for each increase in sunshine hours category. These categorical analyses are shown in Tables 5.18 and 5.19, Appendix 6.

When the analysis was repeated, including any decay experience *and* visual caries, the relationship was not seen in either the linear model (OR 0.99 CI 0.98-1.00, p=0.082 (Table 5.7) or over the full range of sunshine hours (OR 0.67 CI 0.43-1.05 p=0.082) models. Although the results were similar and suggested a protective trend, they did not quite reach statistical significance. This may be because no relationship existed or the sample size was too small.

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Table 5. 6 Results of Logistic Regression Models CDHS Data for Presence of any Clinical Decay Experience in Primary Teeth (Excluding Visual Caries)

N=2771 Age 5,8		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.99	0.98-1.00	0.019	0.99	0.98-1.00	0.022	0.99	0.98-1.00	0.024
Sex M F		-	-	-	1.00 0.95	 0.79-1.14	 0.559	1.00 0.97	 0.80-1.17	 0.757
Age 5yrs 8yrs		-	-	-	1.00 1.75	- 1.45-2.12	- 0.00	1.00 1.46	- 1.21-1.75	- <0.001
IMD Quintile 1 (Most deprived) 2 3 4 5 (Least deprived)		-	-	-	 1.00 0.82 0.54 0.42 0.36	 - 0.62-1.07 0.38-0.77 0.28-0.61 0.25-0.53	 - 0.144 0.001 <0.001 <0.001	 1.00 0.84 0.56 0.42 0.35	 - 0.64-1.10 0.41-0.78 0.29-0.62 0.24-0.53	 - 0.211 0.001 <0.001 <0.001
Current Plaque:	Yes No	-	-	-	-	-	-	1.00 0.45	- 0.36-0.58	- <0.001

Table 5.7 Results of Logistic Regression Models CDHS Data for Presence of any Clinical Decay Experience in Primary Teeth (Including Visual Lesions)

N=2771 Age 5,8		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.99	0.97-1.00	0.083	0.99	0.98-1.00	0.096	0.99	0.98-1.00	0.082
Sex M F		-	-	-	1.00 0.88	 0.71-1.10	 0.263	1.00 0.90	- 0.71-1.14	- 0.392
Age 5yrs 8yrs		-	-	-	1.00 1.47	- 1.22-1.78	- <0.001	1.00 1.18	- 0.98-1.42	- 0.080
IMD Quintile 1 (Most deprived) 2 3 4 5 (Least deprived)		-	-	-	 1.00 0.77 0.55 0.48 0.46	 - 0.58-1.04 0.38-0.80 0.32-0.71 0.29--0.71	 - 0.083 0.002 <0.001 0.001	 1.00 0.80 0.57 0.48 0.46	 - 0.60-1.07 0.40-0.80 0.32-0.72 0.28-0.70	 - 0.134 0.002 0.001 0.001
Current Plaque:	Yes No	-	-	-	-	-	-	1.00 0.40	- 0.30-0.53	- <0.001

5.5.1.2.3 Presence of severe caries in primary teeth, secondary outcome measures

The secondary outcome measures considered were the presence of more than five decayed primary teeth and the presence of more than three primary teeth with decay into dentine (Appendix 6, Tables 5.16 and 5.17). The primary outcome measures showed the presence or absence of any decay experience; however, this binary outcome did not differentiate between only one decayed tooth or several. Therefore, the secondary outcome measures were chosen as they indicated the severity of caries experience, due to the presence of an increased number of decayed teeth (more than 5) or an increased number of deep carious lesions (more than 3 teeth with caries into dentine).

In the full models no relationship was seen between having more than 5 decayed teeth and increased sunshine hours (OR 0.99 CI 0.98-1.01 p=0.328). There appeared to be a small decrease in risk of having more than three teeth with decay into dentine of 2% (OR 0.98, CI 0.97-1.00, p=0.030) associated with hourly increased in sunshine hours. Again, the factors which had the most effect on the risk of having the secondary outcomes was the index of deprivation and the presence of plaque. Living in the least deprived IMDE category was associated with a 78% reduction in the presence of clinical decay in more than five primary teeth (OR 0.22, 0.10-0.50, p=0.001) and having no plaque present was associated with a 71% reduction in risk (OR 0.29, CI 0.178-0.470, p<0.001). For the presence of decay into dentine in more than three primary teeth living in the least deprived area led to a 73% risk reduction (OR 0.27 CI 0.14-0.51, p<0.001) and having no plaque present on examination led to a reduced risk of 68% (OR 0.32, CI 0.21-0.49, p<0.001)

5.5.1.2.4 Results for outcome measures of permanent teeth

The primary and secondary outcome measures for permanent teeth were the same as for the primary dentition but pertaining to permanent teeth only. As before, the analysis was only completed on individuals who had data for all the variables in the model. If data were missing from any of the outcome or confounding variables the individual was removed from the analysis. As

previously discussed, children in age groups 8 and 12 have a mixed dentition and would generally have a number of permanent teeth, therefore only five-year olds were excluded from the analysis models for permanent teeth.

5.5.1.2.5 Demographics of the analysis population

After those with missing data were removed 3,941 individuals were left for each of the outcome measure analysis models. The exclusion of individuals without full data again did not lead to large discrepancies in the groups (Table 5.8). There were marginally more girls than boys (52% versus 48%). Almost two thirds of children had the presence of plaque recorded clinically, which is the same as for the primary demographics and the largest difference was again in the representation between the different quintiles of deprivation due to oversampling of the most deprived quintile.

Table 5.8 Demographics of the Analysis Population, Permanent Dentition

N= 3941	Age			Sex		Presence of Plaque		Index of Deprivation Quintile 1=most deprived, 5=least deprived				
	8	12	15	M	F	Y	N	1	2	3	4	5
N	1309	1374	1258	1908	2033	2595	1346	1621	788	518	539	475
%	33.2	34.9	31.9	48.4	51.6	65.9	34.2	41.1	20.0	13.1	13.7	12.1

Some further analysis was undertaken only including the 12 and 15 age groups and excluding 5- and 8-year-olds. The two oldest age groups had data collected regarding sugar intake in their diet and smoking. Thus, further regression models were completed for these participants, due to the potential importance of these as possible confounders. The results of these additional analysis are in Tables 5.22 and 5.23 Appendix 6.

5.5.1.2.6 Presence of any clinical decay experience in permanent teeth

The results of the caries models for permanent teeth are shown in Tables 5.9-10. The regression models show that the association between sunshine hours and presence of dental caries is also present when considering permanent teeth. The relationship between the presence of cavitated lesions, or cavitated and visual lesions, and sunshine hours is a reduction of 2% for both outcomes in the simple model (OR 0.98, CI 0.97-0.99, p=0.001 and OR 0.98, CI 0.97-1.00, p=0.010) and persists at this level even in the full model for both outcomes (OR 0.98, CI 0.97-0.99, p=0.001 and OR 0.98, CI 0.97-1.00, p=0.018).

When the variable “eats sugary food more than four times a day” is added into the full model the relationship strengthens slightly for both the presence of cavitated lesions (OR 0.98, CI 0.97-1.00, p=0.009) and both cavitated and visual lesions included (OR 0.97, CI 0.96-0.99, p=0.001). Although the sample size reduces to 2,529, as this variable was only recorded for 12- and 15-year-olds in the patient questionnaire, the confidence intervals do not show a large change and the p values remain below the typical $p<0.05$ threshold (Tables 5.22 and 5.23 Appendix 6). Again, the scale of the relationship between sunshine hours and presence of caries has a potential impact clinically, with the comparable odds ratio between the highest and lowest sunshine categories (36 hours difference) showing a 45% (OR 0.55, CI 0.39-0.78, p=0.001) reduction in the number of children having cavitated any decay experience present and a 44% (OR 0.56, CI 0.35-0.90, p=0.018) reduction when both visual and cavitated caries is considered (n = 3941).

5.5.1.2.7 Presence of severe caries in permanent teeth, secondary outcome measures

The above results suggest a relationship between a decrease in prevalence of decay experience and an increase in sunshine hours in area of residence. Further analysis was undertaken to see if this relationship extended to the severity of dental decay, with more severe decay being found in areas with less sunshine. Again, to check the relationship between severity of caries

and sunshine two outcome measures were chosen. The presence of more than five permanent teeth with clinical decay which was a moderate number of decayed teeth, and the presence of more than three teeth with decay into dentine, which was indicative of deeper decay. Firstly, the models used previously were repeated with the outcome measure of at least five permanent teeth with any clinical decay. In the simple model (OR 0.98, CI 0.96-1.01, p=0.144) and in the full model adjusting for sex, age, IMDE and finally plaque (OR 0.99, CI 0.97-1.01 p=0.274) no relationship was found. For the second outcome measure of at least three permanent teeth with decay into dentine, in both the simple model (OR 0.98, CI 0.96-1.01, p=0.192), and in the full model (OR 0.99, CI 0.96-1.01, p=0.36 respectively) again no association was seen. The results tables for these analyses are in appendix 6, Tables 5.20 and 5.21 respectively.

5.5.1.2.8 Presence of enamel opacities in 12-year-olds, secondary outcome measure

The presence of enamel opacities was recorded in the twelve-year-old age group only. As discussed, lower levels of vitamin D have previously been associated with the occurrence of enamel opacities and may be caused by suboptimal mineralisation of the enamel matrix during its formation (Kühnisch *et al.*, 2015). As this outcome was only recorded in the 12-year-old age group the sample size was greatly reduced. In all models no association between enamel opacities and sunshine hours was found (full model OR 0.99, CI 0.96-1.01, p= 0.35, n=1374) and so this study cannot support this hypothesis. (Table 5.11).

Table 5.9 Results of Logistic Regression Models for Presence of Clinical Decay in Permanent Teeth (Cavitated Lesions Only)

N=3941 Age 8,12,15		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.98	0.97-0.99	0.001	0.98	0.97-0.99	<0.001	0.98	0.97-0.99	0.001
Sex	M	-	-	-	1.00	-	-	1.00	-	-
	F				1.26	1.00-1.60	0.052	1.33	1.05-168	0.021
Age	8yrs	-	-	-	1.00	-	-	1.00	-	-
	12yrs				2.73	2.03-3.67	<0.001	2.84	2.11-3.82	<0.001
	15yrs				4.75	3.57-6.32	<0.001	5.25	3.87-7.13	<0.001
1 (Most deprived)		-	-	-	1.00	-	-	1.00	-	-
2					0.78	0.51-1.21	0.270	0.79	0.51-1.23	0.297
3					0.62	0.43-0.89	0.011	0.63	0.44-0.92	0.017
4					0.42	0.30-0.59	<0.001	0.44	0.32-0.61	<0.001
5 (Least deprived)					0.35	0.24-0.52	<0.001	0.36	0.25-0.53	<0.001
Current Plaque:	Yes	-	-	-	-	-	-	1.00	-	-
	No							0.65	0.53-0.78	<0.001

Table 5.10 Results of Logistic Regression Models for Presence of Clinical Decay in Permanent Teeth (Cavitated and Visual Caries)

N=3941 Ages 8,12,15		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.98	0.97-1.00	0.010	0.98	0.97-1.00	0.011	0.98	0.97-1.00	0.018
Sex	M	1.00	-	-	1.00	-	-	1.00	-	-
	F				1.18	0.95-1.46	0.140	1.24	1.00-1.55	0.054
Age	8yrs	1.00	-	-	1.00	-	-	1.00	-	-
	12yrs				2.43	1.75-3.38	<0.001	2.55	1.83-3.56	<0.001
	15yrs				3.17	2.16-4.64	<0.001	3.59	2.44-5.29	<0.001
1	(Most deprived)	1.00	-	-	1.00	-	-	1.00	-	-
2					0.81	0.55-1.19	0.270	0.82	0.55-1.22	0.327
3					0.72	0.48-1.07	0.105	0.74	0.49-1.13	0.156
4					0.47	0.31-0.74	0.001	0.50	0.32-0.76	0.002
5	(Least deprived)				0.39	0.25-0.63	<0.001	0.40	0.25-0.65	<0.001
Current Plaque:	Yes	1.00	-	-	-	-	-	1.00	-	-
	No							0.60	0.48-0.76	<0.001

Table 5.11 Results of Logistic Regression Models for the Presence of Enamel Opacities in 8 Upper Permanent Teeth: UR4-UL4

N=1,374 Age 12		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.98	0.97-1.01	0.229	0.99	0.97-1.01	0.249	0.99	0.98-1.01	0.35
Sex	M	-	-	-	1.00	-	-	1.00	-	-
	F				0.94	0.68-1.30	0.704	1.01	0.74-1.38	0.121
1	(Most deprived)	1.00	-	-	1.00	-	-	1.00	-	-
2					1.08	0.78-1.50	0.643	1.07	0.76-1.51	0.700
3					0.74	0.50-1.10	0.134	0.77	0.52-1.12	0.166
4					0.78	0.51-1.22	0.275	0.82	0.53-1.25	0.346
5	(Least deprived)				1.03	0.53-2.00	0.928	1.04	0.53-2.04	0.914
Current Plaque:	Yes	1.00	-	-	-	-	-	1.00	-	-
	No							0.63	0.45-0.90	0.012

5.5.2 Study 3: Oral health survey of Five-Year Old Children Results

5.5.2.1 Description of the Analysis Population

The geographical boundaries of outcome measures for the OHS analyses were conducted at Lower Tier Local Authority level. Overall, 324 Local Authorities (LA) were included in the study and in total 111,500 children were examined. As the data was given at LA level characteristics such as sex, IMDE, oral hygiene or caries experience were not available at individual level. The data set reported the mean number of children examined per LA as 344, ranging from 0 to 3156. In this dataset approximately 76% of 5-year-old children were reported as caries free, somewhat more than for the 5yr olds in the CDHS which was 63%. This may have been because the caries experience measure for OHS survey was less sensitive, as it only recorded decay in to dentine, and excluded smaller lesions. It could also have been due methodological variation such as examiner variation, differences in calibration or differences in examination technique. Of the 24% of children with decay the average number of decayed, missing or filled teeth (dmft) was 3.4 teeth, compared to an average overall dmft of 0.8 teeth when children without decay were included. It is noted that in this dataset the geographical areas were smaller at LA level, compared to the larger regional analyses in the CDHS, and this gave a wider range of exposure. In this dataset the mean monthly hours of sunshine per LA is approximately 170, but this ranged by almost 75 hours difference from an LA minimum of 133.48 hours to a maximum 208.71 hours. In the CDHS the difference between the minimum and maximum exposure levels was only 36 hours. This smaller area level data allowed for more accuracy in assessing the exposure measure and outcome, with less averaging out of data across large areas. The summary of the dataset characteristics are displayed in Table 5.12.

Table 5.12 Summary of Oral Health Survey of Five-year old Children in England Dataset

Variable	Mean per LA	Std. Dev.	Min	Max	Inter-Quartile Range	Number of LAs
Number of children examined in each LA	344.136	354.764	0	3156	203.5-337.5	324
Percentage with dmft=0	76.261	8.126	43.9	91.8	71.3-81.9	321
Percentage with dmft >0	23.690	8.126	8.2	55.7	18.1-28.7	321
Percentage with incisal caries	4.960	3.426	0	20.8	2.5-6.7	321
Percentage with substantial plaque	1.772	3.400	0	28.4	0-1.7	321
IMD	19.505	8.012	5.009	41.997	12.87-25.28	324
Mean dmft overall	0.784	0.385	0.1	2.5	0.5-1	321
Mean dmft in those children with decay	3.198	0.618	1.2	5.3	2.8-3.6	321
Mean Monthly Hours of Sunshine	129.73	9.61	102.58	158.50	122.79-136.37	324
Mean Monthly Hours of Sunshine April-October 2010-2014	170.19	13.84	133.48	208.71	158.03-179.25	324

5.5.2.2 Study 3: Results of the Analyses

As described previously, linear regression analysis was completed on the available dataset which was presented at lower tier local authority level with continuous outcome measures. Four linear regression models were used for the analysis of each outcome measure. In Model 1 the association of the outcome measure of interest with sunshine hours only was considered. The complexity of the models was then increased with Model 2, considering both sunshine and fluoride. Model 3 was as for Model 2, but with the addition of IMD. Model 4 considered all potential confounders, plus the percentage of children with the presence of substantial plaque. The results of the different outcomes are shown in Tables 5.13-5.15.

5.5.2.2.1 Primary Outcome Measure: Mean dmft child/ LA and Mean Monthly Hours of Sunshine April – October 2010-2014 among Five-Year-Old Children in England

In the simple model, which considered sunshine hours only (April-October 2010-2014), there was an association between mean monthly hours of sunshine April to October and the mean dmft/ LA, with a reduction in the dmft of approximately 0.01 for each one-hour increase in sunshine (β -0.010, CI -0.013, -0.007, p<0.001). This relationship was attenuated, but persisted, when fluoride and IMD were added to the model as potential confounders (Coef -0.007, CI -0.009, -0.004, p<0.001). The final addition of the prevalence of substantial plaque deposits to the model did not alter the relationship, which suggests that this is not a potential confounder or mediator in this population, mindful that accounting for confounders is less reliable in ecological studies.

Although considered at an hourly interval the effect appears small, when the effect was considered over the full range from the lowest to highest hours of sunshine, that is 75 hours difference*, this represented a potential decrease in mean dmft of 0.5 (Coef -0.50, CI -0.68, -0.33, p<0.001).* The mean dmft per LA is 0.784, therefore in the context of this population a change of 0.5 is of clinical impact and relevance. For context, of the other factors in the model, living in a fluoridated area had the greatest reduction on the mean dmft (Coef -0.182, CI -0.306, -0.059, p=0.004), whilst the percentage of children in an area with

substantial plaque present was positively associated with an increase in mean dmft (Coef 0.016, CI 0.007, 0.025, p<0.001)

5.5.2.2.2 Secondary outcome measure: Mean dmft/ child/ LA and Mean Monthly Hours of Sunshine among five-year-old children with any decay experience (dmft>0)

The regression models were repeated using an analysis sample which was limited to those children who had experience of decay with dentinal involvement, that is had a dmft score of greater than 0. The simple model demonstrated that each hourly increase in sunshine hours was associated with a reduction in mean dmft in this population of 0.011 (Coef. -0.011, CI -0.016, -0.006, p<0.001) and when fluoride was considered this reduction increased to -0.012 (CI -0.017, - 0.007, p<0.001). In the final full model, the protective effect persisted with a decrease in mean dmft of 0.007 (Coef. -0.007, CI -0.011, -0.003, p=0.002) per additional monthly hour of sunshine. If the potential impact over the full range of sunshine hours is considered, 75 hours between highest and lowest LA sunshine hours, this could be a potential reduction of approximately 0.5 in mean dmft in children with dmft greater than 0, per LA (Coef. -0.519, CI -0.844, -0.194, p=0.002).

5.5.2.2.3 Secondary outcome: Percentage of children with Incisor Caries and Mean Monthly Hours of Sunshine

Finally, the analyses were completed using the percentage of children with incisor caries, as an outcome indicative of severity, and mean monthly hours of sunshine per LA. In the simple model each hour increase in sunshine hours was associated with a reduction in the percentage of children with incisal caries of -0.06% (Coef. -0.062, CI -0.089, -0.036, p<0.001). When fluoride was added to the model each hourly increase in sunshine hours was associated with a reduction in the percentage of children with incisor caries per LA of approximately 0.07% (Coef. -0.069, CI-0.095, -0.042, p<0.001). Finally, when IMD and percentage of children with substantial plaque are added successively to the model the relationship attenuates slightly but remains significant and negative. Initially with the addition of IMD the relationship attenuates slightly and

the reduction in percentage of children with incisal caries attenuates to a reduction of approximately 0.04% (Coef. -0.036, CI -0.060, -0.013, p=0.002). It then remains unchanged by the addition of plaque (Coef. -0.036, CI -0.059, -0.013, p=0.002). *This translated across the full range of sunshine hours, from the highest to the lowest, to a reduction of -2.70% (CI -4.41, -0.996, p=0.002) in children with incisor caries.* For context, in this model, fluoridation of an LA, is associated with a reduction in the percentage of children who have decayed front teeth of -1.6% (Coef. -1.575, CI -2.781, -0.369, p=0.011).

Table 5.13 Linear Regression of Mean dmft/ child/ LA and Mean Monthly Hours of Sunshine April-October 2010-2014

	Model 1 Sunshine hours only			Model 2 Model 1 plus Fluoride			Model 3 Model 2 plus IMD			Model 4 Model 3 plus plaque		
	Coef.	P	CI	Coef.	P	CI	Coef.	P	CI	Coef.	P	CI
Mean monthly hours of sunshine Apr-Oct	-0.010	<0.001	-0.013, -0.007	-0.011	<0.001	-0.136, -0.078	-0.007	<0.001	-0.009, -0.004	-0.007	<0.001	-0.009, -0.004
Fluoride				-0.254	0.002	-0.410, -0.097	-0.203	0.002	-0.328, -0.077	-0.182	0.004	-0.306, -0.059
IMD average							0.027	<0.001	0.023, 0.031	0.028	<0.001	0.024, 0.032
Plaque										0.016	<0.001	0.007, 0.025

Table 5.14 Linear Regression Model of Mean dmft / child/ LA and Mean Monthly Hours of Sunshine April- October 2010-2014 (in children with dmft < 0)

	Model 1 Sunshine hours only			Model 2 Model 1 plus Fluoride			Model 3 Model 2 plus IMD			Model 4 Model 3 plus plaque		
	Coef.	P	CI	Coef.	P	CI	Coef.	P	CI	Coef.	P	CI
Mean monthly hours of sunshine Apr-Oct	-0.011	<0.001	-0.016, -0.006	-0.012	<0.001	-0.017, -0.007	-0.007	0.002	-0.011, -0.003	-0.007	0.002	-0.011, -0.003
Fluoride				-0.415	0.002	-0.676, -0.153	-0.346	0.003	-0.574, -0.117	-0.337	0.004	-0.566, -0.107
IMD average							0.037	<0.001	0.030, 0.044	0.037	<0.001	0.030, 0.045
Plaque										0.007	0.396	-0.010, 0.024

Table 5.15 Linear Regression Model of Percentage of Children who had Caries in their Incisor Teeth and Mean Monthly Hours of Sunshine April-October 2010-2014

	Model 1 Sunshine hours only			Model 2 Model 1 plus Fluoride			Model 3 Model 2 plus IMD			Model 4 Model 3 plus plaque		
	Coef.	P	CI	Coef.	P	CI	Coef.	P	CI	Coef.	P	CI
Mean monthly hours of sunshine Apr-Oct	-0.062	<0.001	-0.089, -0.036	-0.069	<0.001	-0.095, -0.042	-0.036	0.002	-0.060 -0.013	-0.036	0.002	-0.059, -0.013
Fluoride				-2.203	0.003	-3.648 -0.757	-1.783	0.004	-3.008, -0.558	-1.575	0.011	-2.781, -0.369
IMD average							0.225	<0.001	0.186 0.264	0.231	<0.001	0.193, 0.270
Plaque										0.168	<0.001	0.079, 0.256

5.6 Discussion

5.6.1 Discussion Study 2: CDHS

5.6.1.1 Primary Dentition

In the CDHS analysis the first primary outcome measure of any decay experience excluding visual caries was chosen to consider the prevalence of caries. In these regression models, an increase in sunshine hours was associated with a small, but consistent, reduction in the presence of dental caries (OR 0.99 CI 0.98-1.00 p=0.024), which potentially scaled to a difference between the areas with the highest and lowest numbers of sunshine hours of 35% (OR 0.65, CI 0.44-0.94, p=0.024). However, when the second primary teeth outcome measure, any decay experience including visual caries, was considered in the regression models however, no clear relationship was seen (OR 0.99, CI 0.98-1.00, p=0.061). As the definition of decay experience got broader the association disappeared. If increased sunshine hours and exposure optimises mineral content of saliva via increased vitamin D levels, one would expect to see an increased protective effect from increased sunshine hours on early decay lesions. For example, this may present as less demineralisation and formation of early lesions, or before cavitation occurred, increased remineralisation of white spot lesions, potentially preventing or delaying the occurrence of a cavitated lesion. Once cavitated however, this effect would be less influential due to the increased susceptibility of dentine to decay and the increased difficulty of removing plaque from a cavity compared to an intact enamel surface.

In the first outcome measure those teeth with white spot lesions and non cavitated caries would be recorded as sound, even though technically there was some demineralisation. In the areas of less sunshine hours as more white spot lesions progressed to cavitation, sound to positive for some decay experience, it would be expected that a clear difference in the number of cavitated lesions would be seen between the areas with more or less sunshine hours. In the second outcome where visual caries was included in the definition of any decay experience all teeth with either early or cavitated lesions were counted as positive for caries experience and there was no differentiation between the two.

The difference here would only be between sound teeth and any decay experience. Here, applying the proposed hypothesis that increased levels of vitamin D increase the ability for remineralisation we may expect to see less decay experience in areas where there are more sunshine hours, however given the small effect it may be that the caries process is slowed rather than completely inhibited and this outcome measure is not sensitive enough to show this. Given these considerations the results that were found in the deciduous teeth analysis support these hypotheses. From a methodology perspective cavitated lesions would have been more obvious to diagnose in a non-clinical setting than visual lesions. Without very good lighting, and the use of radiographs, non cavitated visual caries would be harder to confirm and therefore potentially the caries experience recorded could be less accurate than when visual caries was excluded, leading to over or under reporting of lesions.

With regards to the secondary outcomes relating to severity of dental caries increasing sunshine hours was found to be associated with a decrease in the risk of having three teeth with decay into dentine but not with a decrease in risk of having five or more decayed primary teeth. Therefore, again the protective effect was associated with reduction in the number of deeper/ cavitated lesions rather than with decay measures that included early demineralisation and non cavitated lesions. This supports the hypothesis that there is more remineralisation and less progression of early uncavitated lesions to cavitated caries in primary teeth in areas with more sunshine hours.

5.6.1.2 Permanent Dentition

The outcome measures used for the permanent dentition were the same as for the primary dentition but as expected only considered decay experience in permanent teeth. In the first analysis logistic regression of the presence of any clinical decay, excluding visual caries, and sunshine hours showed a reduction of 2% in risk for every hour increase in sunshine hours (OR 0.98, CI 0.97-0.99, p =0.001) in the simple model. This was not considering any confounders in the model, however even when sex, age, IMDE (model 2), and finally plaque (model 3) were included the reduction in OR and the protective effect remained unchanged. Other factors which increased the risk of having any decay experience in the full model included being female (OR 1.33 CI 1.05-

1.68, p =0.021) and increasing age, which was as expected as the teeth would have been erupted for longer in older children and had more time to be affected by decay (age 15>12>8 associated with OR 5.25>2.84>1.00 all reaching statistically significance). In contrast living in an area in the least deprived IMDE quintile, and not having visible plaque present, were both strongly associated with large reductions in ORs, OR 0.36 CI 0.25-0.53, p<0.001 and OR 0.65 CI 0.53-0.78 p<0.001 respectively.

In the second analysis which included any decay experience including visual caries the relationship persisted (OR 0.98, CI 0.97-1.00, p=0.018, n=3941), which is in contrast to the findings with primary teeth, where the results did not quite reach significance. Permanent teeth have thicker enamel than primary teeth, which means the progression to cavitated cavities may take longer (De Menezes Oliveira *et al.*, 2010). This may have contributed to the different results. The sample size was also larger for the analysis for permanent teeth as it included more age groups. This larger sample size may have meant the results were more accurate and produced a significant result. Alternatively, there may have been no difference clinically, but the practical advantages of examining older children could have made the recording of caries easier and more accurate than for the younger age groups.

Secondary outcomes investigating the severity of the caries and the presence of enamel opacities were also considered for the permanent dentition. No relationship was found between living in an area with more sunshine and more severe caries outcomes of more than five permanent teeth with clinical decay, or more than three permanent teeth with decay into dentine. Whilst there appeared to consistently be a reduced risk in having any type of decay present, the risk of having more severe decay was not reduced. The numbers of children with more severe decay present where small, in comparison to those who had any decay experience. Therefore, either no relationship exists, or it may be that a larger sample size is needed to detect any association.

With regards to the secondary outcome measure of the presence of enamel opacities, our results did not show any relationship between increasing sunshine hours and decreasing enamel opacities. Enamel opacities are due to disruption of the enamel as it is forming, which as discussed previously, is before eruption of the teeth into the mouth. The front upper eight teeth were

examined and in the case of the incisor teeth these would have been formed around the time of birth. A limitation of the study is that only the current area of residence is known and it is not possible to know which area of the country the children lived in during the time of the teeth developing. If the model is correct, it suggests that living in a sunnier area does not lead to improved mineralisation of enamel structure. The findings may also have suffered from a limitation in the methodology. Enamel opacities can also be hard to identify without proper lighting and clinical examination conditions. Indeed, in the statistical model used the only factor associated with reduced enamel opacities was the absence of plaque which suggests some of the enamel opacities recorded may in fact have been white spot lesions and had an aetiology of plaque retention. Enamel opacities can be caused my many different factors, such as childhood illness, excess fluoride intake, indeed anything which can alter the activity of the ameloblasts when they are laying down enamel. Therefore, this outcome measure and method may not have been particularly sensitive in this screening survey to record the complexities of its occurrence.

5.6.2 Discussion Study 3: OHS

In this OHS analysis areas with higher sunshine hours were associated with a reduced mean dmft, a reduced mean dmft in five-year-old children with dentinal caries experience and a reduction in the percentage of children who have incisor caries, which is a measure of caries severity. These associations attenuate, but ultimately persist, even when water fluoridation, Index of Deprivation, and percentage of children with substantial plaque present on their teeth are taken into account. In this survey only one age group was included, 5-year-olds, and so only primary teeth can be considered. It is not possible therefore to see if the results translate and persist in a permanent dentition. Furthermore, in this survey individual participant data was not available, making this a true ecological study, the limitations of which have already been discussed. These include the ecological fallacy and the decreased reliability of adjustment for confounding. It is noted that the protective trend found in Study 3 supports the findings of study 2. Again, the model appeared robust as fluoridation and deprivation indexes were all associated with decrease/increase in dmft as expected.

5.7 Conclusions

In conclusion studies 2 and 3 in this chapter both find that a small, but consistent, statistically significant reduction in the prevalence and severity of caries in primary teeth, is associated with living in an area with increased mean sunshine hours. These findings were supported across studies 2 and 3 showing consistency across datasets. For permanent teeth, it was only possible to investigate in study 2, where there was a small statistically significant reduction in the prevalence of overall caries associated with increased sunshine hours in area of residence, but no association was found regarding severity of caries or the presence of enamel opacities. This could be due to sample size or methodology, or it could be sunshine exposure and vitamin D status having a greater effect in the primary dentition than in the permanent teeth.

The findings of these studies overall are in support of the findings from studies discussed in Chapter 2, in the supporting literature and systematic review, suggesting that the protective effect of sunshine exposure against caries in primary teeth seen in previous ecological and environmental studies persists even when more robust methodology and statistical analysis are applied.

Chapter 6: Summary, Discussion and Conclusion of Results

6.1 Summary of Findings

When formulating a summary of the findings presented in this thesis, it is apparent that the persistent trend in each of the chapters is an association between increased exposure to sunshine and/or UVB light and a small decrease in the occurrence of dental caries, but this relationship is not simple or clear.

In Chapter 1 the review of the literature summarised the existing body of evidence showing vitamin D is necessary for general health, with particular importance in bone metabolism and immunomodulation (DeLuca, 2004; Colotta, Jansson and Bonelli, 2017). Since its discovery in the 1920s it has been known that it prevents rickets and osteomalacia in children and adults (Mellanby, 1919; Hess, 1922; Holick, 2006). As discussed, in the UK vitamin D can only be produced in the skin between approximately March and September each summer and as a result vitamin D deficiency, 25(OH) D less than 20 ng/ml (50 nmol/L), is not uncommon (Hyppönen and Power, 2007; O'Neill *et al.*, 2016). For most people in the UK this sunshine exposure is the main source of vitamin D rather than diet, however several environmental, physical and behavioural factors can influence how much vitamin D is made in this way (Hyppönen and Power, 2007). Once vitamin D is made in the skin from cholesterol, it is absorbed as an inactive prohormone and carried by vitamin D binding protein (VDP) first to the liver and then to the kidney to undergo two hydroxylation steps. These steps create first 25(OH)D, the main circulating form of vitamin D used as the main measurement of vitamin D status, and then the final active form of 1,25(OH)2D. Although vitamin D can also be sourced from the diet in the UK, Public Health England guidelines recommended an intake of 400 IU/day (10 µg) for everyone above the age of 4 years old to achieve positive health outcomes and they recommend the use of supplements over the winter to reduce the risk of deficiency (SACN, 2016). Several studies were discussed which showed associations between increased vitamin D status and improved oral health outcomes including reduced caries in children, reduced incidence of enamel hypoplasia, reduced gingivitis and better periodontal health in adults. There were however few intervention studies amongst the literature and the

extent and direction of the relationship was unclear. The findings of the literature review were used to refine the research question further so a systematic review could be undertaken.

Chapter 2 addressed the systematic review question “Do human populations exposed to higher levels of UVB radiation have better oral health than those exposed to lower levels of UVB radiation”. There were two further sub questions considered which were “what are the effects of sunlight exposure on caries, gingivitis, periodontal disease and plaque deposits” and “what are the effects of artificial UVB exposure on caries, gingivitis, periodontal disease and plaque deposits”? This chapter included a scoping review which summarised the various geographical and environmental studies that informed the systematic review question. These studies again suggested an association between living in areas with more sunshine or hours of daylight and less dental caries, however they mostly used poor quality exposure measures or poor quality geographical data. It was these studies which inspired the empirical studies in the later chapters of this thesis, which used more accurate oral health data and sunshine exposure data. Although the studies included in the systematic review varied in terms of quality, it was concluded that an inverse relationship exists between dental caries and exposure to UVB. This association applies whether the UVB is produced from artificial sources or via exposure to sunlight and manifests as a reduction in the formation of new carious lesions. In this review no evidence was found to support associations between UVB exposure and gingivitis, periodontal disease or plaque deposits and no adverse effects were reported to teeth, individuals or populations from the UV exposure during the course of the studies.

The empirical studies 1-3 of this thesis were discussed in Chapters 3-5 and used Campbell-Stokes sunshine hours data from the Met Office downloaded from MEDMI along with dental health data from the Adult Dental Health Survey 2009, Child Dental Health Survey 2013 and the Oral Health Survey of Five-year-old Children 2014-15 epidemiological surveys. Inverse distance weighting interpolation was used to create 5 km gridded estimates of mean monthly sunshine hours which were overlaid with the relevant geographical boundaries, for example Lower Tier Local Authorities or Strategic

Health Authorities, depending on which study was being undertaken, to create the data for the exposure measures.

Chapter 4 discussed the analysis of Study 1: Adult Dental Health Survey (ADHS) data, where it was found that residing in an area of increased sunshine hours was not associated with reduced gingival bleeding, but was positively associated with increased pocketing depths, which was statistically significant. Analysis for Study 1 in Chapter 4, also showed that increased sunshine hours were associated with a reduced presence of plaque in all regression models.

Chapter 5 described analysis models for Study 2: Child Dental Health Survey (CDHS) and Study 3: The Dental Epidemiology Programme for England, Oral Health Survey of Five-year-old Children (OHS). The CDHS had individual level oral health data on 5, 8, 12 and 15-year-old children, along with low resolution geographical data at HEA level. In contrast the OHS had better resolution in area of residence with geography at lower tier Local Authority level, but also only presented the oral health data at this level with mean outcomes for each LTLA. Analysis models for both studies 2 and 3 demonstrated a persistent small and statistically significant decrease in caries associated with increased sunshine hours.

When looking at these findings in context one method of critiquing, the likelihood of epidemiological associations also being causative is to consider the Bradford Hill Criteria (Hill, 1965).

1. Strength of Association

It is considered that the larger the association between the exposure and disease the more likely it is to be causal. This is considered along with the statistical significance of the association. In study 2 only a small, but statistically significant, association between increasing hours of sunshine and a reduction in the incidence of caries in children was found. This ranged from OR 0.99 (95%CI 0.98-1.00 p=0.024) for any decay cavitated excluding visual lesions in primary teeth to OR 0.98 (95% CI 0.97-0.99, p=0.001) for any carious lesions excluding visual lesions in permanent teeth. Study 3 was an ecological study and while neither study can demonstrate causality, this survey supports the findings of Study 2. Here the linear regression full model showed a reduction of -0.007 in the mean DMFT for each hour increase in mean monthly sunshine hours (-

0.007, CI -0.009- -0.004, p=<0.001). Although the effect seems small, when looked at per hour, the potential effects across the whole range of sunshine hours were larger and therefore also potentially clinically relevant.

2. Consistency

Consistency is demonstrated when a variety of locations, populations and methods show a consistent association between the two variables of interest, that is sunshine hours and an oral health outcome, such as dental caries, gingivitis or periodontal disease.

In regards to dental caries, the systematic review concluded that poor quality evidence existed regarding an inverse relationship between sunshine hours and dental caries. This review contained geographically varied studies, mainly including child populations and were primarily old, outdated in methodology and lacked robust statistical analysis. No such relationship was found for gingivitis or periodontal disease.

The analysis in Study 1 found no evidence to support an association between sunshine hours and gingivitis or periodontal disease.

Study 2 and Study 3 found an inverse association between sunshine hours and dental caries in children.

Therefore, in the systematic review of available evidence, and in the two new empirical studies, the evidence consistently supported an inverse association between sunshine hours and dental caries in children.

3. Specificity

An association is specific when the exposure or intervention only causes one disease. It is accepted that vitamin D deficiency causes rickets or osteomalacia and sufficient vitamin D is necessary for bone health and calcium and phosphate metabolism. Whilst there are specific dental defects associated with rickets (Souza *et al.*, 2013; Goodman *et al.*, 1998), vitamin D deficiency alone is not a direct cause of dental caries, as this is a multifactorial disease. There are however specific mechanisms within vitamin D metabolism, such as regulation of calcium and phosphate availability in plasma and saliva (DeLuca, 2004; Glijer, Peterfy and Tenenhouse, 1985), or related to development of the teeth (Onishi *et al.*, 2008; Nociti *et al.*, 2014), which may offer protection against

caries. However, these mechanisms are not specifically or directly related to caries and so the evidence generated by this thesis does not support specificity in this case. In the literature it is accepted that a group of diseases related to abnormal mineralisation and mineral metabolism are caused by vitamin D deficiency.

4. Temporality

In order for an association to be causative the exposure has to have occurred before the onset of the disease. In the papers included in the systematic review the UV exposure in the studies which used artificial light was before the development of new carious lesions and there was not a long period of latency during the trials.

In the empirical studies the design of the studies assumes prior exposure in the form of lifetime exposure but as they are not longitudinal, does not prove it. It is therefore not possible to know how long the individuals lived in their area of residence or if they had lived somewhere else prior to their exam. We also are limited as the exposure measure assigned to each individual is the same as it is derived from an average over the whole geographical area where they live. The exposure measure does not account for lifestyle factors which would affect their prior exposure.

5. Biological gradient

If a dose response is seen this is considered supportive of a causal relationship. In the case of UV exposure from natural source of sunlight, it is hard to quantify a dose received. Proxy measures can be used, in studies 1 to 3 this is sunshine hours, but this is not an individual level dose. Many factors, discussed in chapter one, affect vitamin D formation from sunlight and the dose of sunlight received. In the systematic review studies those that delivered a “dose” of artificial UV are more controlled in their delivery but do not account for UV light from natural sources and do not measure resultant vitamin D levels. Therefore, in this thesis the findings cannot demonstrate a dose-dependent relationship between sunshine exposure and oral health outcomes.

6 and 7. Plausibility and Coherence

Plausibility and coherence are considered together as they are similar in narrative. The plausibility of a causative relationship between vitamin D deficiency, UVB light exposure and increased incidence of dental caries or periodontal disease has already been extensively explained and discussed in chapter two, where the possible mechanisms of action of vitamin D on oral health were discussed. Coherence is similar to plausibility in that it dictates that a cause-and-effect interpretation for an association should not conflict with what is known of the biology and pathogenesis of the disease. The empirical studies support the theories of a protective effect against dental caries, as their results are what would be expected if this theory is true, and do not contradict what is known about the formation of dental caries as presented in the literature and systematic reviews. They do not present any evidence to support the plausibility of improved periodontal health with increased sunshine exposure.

8. Experiment

Experimental data to support causality refers to *in vitro* or *in vivo* studies which control and then withdraw the exposure resulting in a direct change in the course of the disease, for example resolution. This is hard to show in the case of established caries or periodontal disease, as these diseases are irreversible and they will not resolve, but may stop progressing and become stable. Early caries can be “reversed” as white spot lesions can be remineralised with calcium and phosphate ions present in saliva. In one of the studies included in the systematic review there was reported reversal of white spot lesions in children exposed to the artificial UVB from the intervention of broad spectrum lighting (Hargreaves and Thompson, 1989). More generally the artificial lighting studies included in the systematic review are an example of an exposure being given, controlled full spectrum lighting, and finding a change in the course of the disease, new carious lesions reducing in occurrence, but the poor quality and limitations of these studies has already been discussed. As the empirical studies were not longitudinal none of the studies completed as part of this thesis can be considered of sufficient quality evidence to prove causation.

True experimental studies are rarer in environmental epidemiology as exposing participants to sufficient or insufficient exposures is difficult to implement, control, and can have ethical implications.

9. Analogy

This criterion considers whether analogous examples exist that would make the causation more plausible. We know that exposure to different minerals, metals and chemicals can influence or be associated with the resistance of the teeth to caries and remineralisation (Duggal, Chawla and Curzon, 1991; Lippert, 2012). Of these, fluoride is the most well known. There is also evidence supporting latitudinal associations with other conditions such as allergy or MS, diseases which are dependent on a specific immune response (Osborne *et al.*, 2012; Ponsonby, Lucas and van der Mei, 2005).

Summary: Likelihood of Causality

The evidence from the review and the empirical studies are indicative of a causal association, but with a number of design and data limitations that mean a strong causal inference is not possible. However, some criteria are affected by issues very common in environmental epidemiology that are intractable e.g., relatively weak relationships are often observed with chronic, low level exposures (criterion 1) and very rarely have an exposure that is amenable to robust experimental study (criterion 8).

6.2 Strengths and Limitations of the Empirical Studies

6.2.1 Strengths and Limitations of Ecological Studies

Studies one and two which use regression models incorporating ADHS and CDHS data are individual level cross-sectional studies as although the exposure measure is at large area level the oral health data, the unit of analysis, is at individual level. The model in study three is an ecological study as the unit of exposure and the outcome measure unit of analysis are both at group level. Each of studies have strengths and limitations.

In the ecological study, and when considering the exposure measure of the ADHS and CDHS study, the sunshine hours allocated to each geographical

region is only a proxy for individual exposure. Caution has to be exercised when inferring an individual's exposure from aggregate data as this may lead to "ecological fallacy", assuming an individual's characteristics is the same as the average characteristics of the group. It is also not possible to know in true ecological studies that the individuals who have the disease outcome of interest are the same individuals who had the exposure and it is harder to control for confounding if individual level data is not available. For example, the children in the CDHS who had the lowest amount of dental decay present may have had a level of sunshine hours exposure which was higher or lower than the exposure amount allocated to their area of residence. Ecological studies cannot prove evidence of causation and so, even though there is a persistent association between increased hours of sunshine in an area and reduced incidence of dental caries, this does not confirm that reduced incidence of caries is caused by increased exposure. This is especially true in a multifactorial disease, such as caries or periodontal disease, and the range of confounding factors that need to be considered. Instead, ecological studies are used to allow an initial assessment of the health status of a population and present an overview of disease or need, which can then be used to generate hypothesis for more comprehensive studies. In that respect ecological studies can often highlight potential public health concerns quickly, identifying potential causes of poor health outcomes for further investigation. Ecological studies can be a useful way of interpreting available data when individual data does not exist. Further benefits of ecological studies include the use of secondary data sets, which is efficient in regards to both time and cost, and problems with individuals dropping out or providing inaccurate data do not occur. (Savitz, 2012). Ecological studies also enable research of groups of individuals who may be less likely as individuals to engage with research as individuals, for example lower socioeconomic groups, individuals with language barriers. The studies undertaken in this work have accounted for socio-economic factors within the limits of the data and include data from different socioeconomic groups.

6.2.2 Strengths and Limitations of the Oral Health Datasets

Studies 1, 2 and 3 benefit from the quality of the data from the oral health studies. These datasets are produced from well executed large epidemiological

studies. Data are expressly collected for the purposes of assessing oral health, data collection is standardised and detailed and examiners are trained and calibrated. The ADHS and CDHS also contained individual level data on some demographic and lifestyle factors which allowed these confounders to be considered in the regression models. The sampling numbers and techniques are designed to encourage equal representation of groups, and weighting is also applied accordingly, however the consent process used positive consent, with consent being required by return of written forms and confirming consent verbally on the day. It is possible that the families where caries may have been more prevalent would also have been the families less likely to return the consent forms or the children less likely to agree to be examined. This would lead to bias in the data collection, with this group under represented, and would have implications for the generalisability of the survey findings. The surveys applied weightings to attempt to compensate for participation bias. The method of examination in these studies is per agreed a priori protocols and are designed to allow some comparability between surveys over different years. The techniques are also designed to be compatible with a non-clinical environment however these examination techniques are not as rigorous as a clinical exam in dental practice and it is likely that the amount of disease is under recorded.

6.2.3 Strengths and Limitations of the Exposure Data Set

The exposure measure allocated to the geographical areas for the studies consists of an average sunshine hours exposure measure produced using inverse distance weighted interpolation techniques. As the exposure is estimated from known points and weighted accordingly the quality of the estimate can be decreased by uneven or insufficient points of data collection. Therefore, in the case of the exposure measure in this study, if there were not many Campbell Stokes Meters in an area, then the measurement from one CS Meter influenced a wide area. Accuracy is improved where there are more weather stations in an area. However, the Campbell Stokes Meters were the most widespread of the datapoints and stations available used to measure sunshine hours in the UK, and sunshine hours have been established as an acceptable proxy for vitamin status and UV exposure.

6.3 Findings in Context

The results from the empirical studies one, two and three align with the findings of earlier studies evaluated in Chapters 1 and 2. Repetition of the studies in a more geographically relevant country, England, and using more accurate oral health and sunshine hours data confirmed the association between reduced dental caries in children in regions with higher sunshine hours.

6.4 Comparison with Fluoride and Fluoridation.

The analogy of fluoride as an environmental factor influencing oral health has already been explained, but the precedent of fluoride and eventual water fluoridation as a public health measure can inform future studies for vitamin D and sunshine/ UVB exposure. Once ecological, environmental epidemiology has suggested as association the next step is to explore if any risks or negative health outcomes exist and the ethical feasibility of conducting pilot studies. In the case of fluoride no significant health risks were associated with areas where natural fluoridation was present, and this continues to be the position of the public health authorities today (Public Health England, 2014). There was a risk of fluorosis occurring in teeth at higher fluoridation levels and so the amount of fluoride added to water in pilot trials was balanced to give the greatest caries effect against the lowest incidence of mottling. In the case of UVB and UV light there are risks associated with exposure which are recognised. Vitamin D supplementation however is relatively safe with cases of toxicity and hypervitaminosis rare. Following this there was the introduction of pilot trials with artificial water fluoridation. In the case of UVB and vitamin D an analogy here could be the introduction of full spectrum lighting in schools and hospitals. *In vitro* studies helped to determine the method of action of fluoride and therefore informed further interventions. In the case of UVB and vitamin D more studies are required to determine if effects are due to UV light or the induction of cutaneous vitamin D production. Interventions such as vitamin D supplements and food fortification are already trialled in order to increase vitamin D levels but the existence of RCTs for oral health are still limited. It is also important to determine if the production of vitamin D endogenously is

comparable to supplementation and artificial sources. The feasibility and risks of further research will be discussed.

6.5 Implications of Findings: Oral Health Interventions

Childhood caries is a significant but preventable disease in the UK. A recent position statement by the Royal College of Surgeons Faculty of Dental Surgery described childhood tooth decay as a major public issue, both in the UK and globally (*RCS, England*, 2015). Referencing the most recent PHE Oral Health Survey of Five year old children, 23.3% of five year olds in that survey had visible decay and in a third of local authorities there was an increase in the level of decay (England, 2016). There were marked inequalities in the decay with 33.7% of children in the most deprived areas having visible decay versus only 13.6% in the least deprived areas. Tooth decay is a prominent cause of admissions to hospital for children, when they are admitted due to infection or more often for a general anaesthetic to have decayed teeth removed. The RCS FDS report states “In total there were 102,663 hospital admissions due to tooth decay amongst children under the age of 10 between April 2015 and March 2018, highlighting the need for continued action to address the issue (FDS, 2019)”. Public health interventions in place or suggested so far include a sugar tax to reduce sugar content of food, reduced promotion and advertising of junk food and sugary food especially in schools, supervised tooth brushing and community health interventions to support families and children in making healthy lifestyle choices. There is also a move to educate all healthcare professionals, not just dentists, about the impact of oral health on general health, putting the mouth back in the body, and this is being addressed by campaigns such as Mini Mouthcare Matters, a range of educational and training resources for nursing staff to be used in hospitals to help assess and monitor the oral health of children whilst in hospital (MiniMouthcareMatters, 2019).

The Royal College of Paediatrics and Child Health responded to the FDS position statement in kind stating “We know that tooth decay is the most common reason why children aged five to nine require admission to hospital. Treating it often means extracting the decayed tooth and this is not cheap, costing the NHS around £50m for those under the age of 19. Tooth

decay is preventable and this new analysis sets out a range of actions to help improve the state of children's teeth. Coordinated, targeted interventions that also address the postcode lottery in children's oral health will make a difference"(RCPCH, 2019).

The British Society of Paediatric Dentistry document on improving children's oral health states "The British Society of Paediatric Dentistry supports the fluoridation of public water supplies in communities where the burden of dental decay is severe enough to warrant this public health measure and fluoridation is technically feasible (BSPD, 2016)".

If there is the potential for exposure to UVB to cause a decrease in childhood caries this could be used to develop novel targeted public healthcare interventions in areas of need. The feasibility of developing this further will be discussed here.

6.6 Feasibility, Benefits and Risks of Prescribed UV Exposure

6.6.1 Natural Sunlight Exposure - Feasibility and Risks

Guidelines for recommended sun exposure focus mainly on the risk of sunburn, damage to sight and increased risk of skin cancer associated with excessive sun light exposure. The NICE guidelines for sunlight exposure (NICE, 2016) identify at risk groups who should take extra care to avoid skin damage from sun exposure, of which one group is babies and children. Older adults, those who are institutionalised and those who cover up skin for cultural reasons are also identified as at-risk groups for vitamin D deficiency and are advised to combat this with supplements rather than sun exposure. The guidance encourages recommendations to consider peoples lifestyle and skin type when assessing their risk of sunshine exposure and recommends healthcare professionals tailor advice to individuals considering their specific risk factors. The guidance emphasises that vitamin D cannot be made between October and March but in the summer months between this time, sunscreen should be used, especially when the sun is strongest between 11.00-15.00. Although the document does state that the amount of time in the sun required to make

vitamin D is less than the time required to burn the main emphasis is on sunlight protection and not the benefits of exposure. The WHO guidelines also focus on the negative acute and chronic health effects of UV exposure rather than positive outcomes. Whilst confirming a small amount of sunlight is required for vitamin D exposure WHO guidance again focuses on protective measures and behaviours and the use of the Global Solar UV Index (UVI) for education and guidance on sun exposure and risk (*Global Solar UV Index*, 2002). Given that the emphasis on natural UVB exposure is on protection rather than beneficial exposure it would be difficult to advise or "prescribe" sun for oral or general health exposure at public health guidance level, despite the evidence of the benefits, without giving conflicting messages. It may be possible to give guidance at individual consultation level after properly assessing geographical, biological and lifestyle factors pertinent to the individual patient. However, this would require engagement by the individual and for the individuals engaging with the treatment to be those with the most treatment need.

6.6.2 Full Spectrum Lighting in Schools- Feasibility and Risks

It is possible to install lighting systems that emit UVB radiation and so exposure to UVB and production of vitamin D in this way is feasible. As with any source of UV (UVA, UVB and UVC) there are risks associated with excess exposure. In the case of UV light concerns centre mainly around retinal and ocular damage and skin damage. The International Commission on Non-Ionising Radiation Protection (ICNIRP) guidelines on UV exposure advises radiant exposure to skin and eyes in an eight-hour period does not exceed 30 J/m^2 (joules/metre squared). The ocular exposure is limited to unweighted UVA, 10kJ/m^2 in eight hours, and occupational exposure levels in eight hours are 0.33 Wm^2 (watt/metre squared) for UVA and 0.001 W/m^2 for UVB. The public exposure guidelines are up to 0.174 W/m^2 and <0.0015 W/m^2 over 16 hours for UVA and UVB respectively (International Commission on Non-Ionising Radiation Protection, 2004). Some compact fluorescent lamps and incandescent lamps exceed these levels in UV emissions. One study found that at a distance of two meters the UVB W/m^2 was on average <0.0015 from both types of lamps but in some cases the maximum exceeded these exposure guidelines (Azizi, Golmohammadi and Aliabadi, 2016). Therefore, if artificial

lighting was used as a way to encourage endogenous vitamin D production it would have to be carefully controlled in terms of distance of bulbs from individuals and expected duration in the environment. Monitoring and measurements would be needed regularly at different sites and heights about the room to ensure the recommended exposures weren't breached. Although the lighting of individual classrooms is certainly more targeted than advising sunshine exposure this is still a public health approach where all individuals receive the same exposure regardless of their needs, for example outdoor activity or skin type.

Furthermore, while the risks discussed above are considered low to those with normal skin, some individuals are photosensitive and are at risk from light sources that emit UV radiation, especially when close to the light source. Conditions such as Solar Urticaria, Lupus Erythromatosus and Actinic dermatitis can all be exacerbated by UV exposure (Moseley and Ferguson, 2011). Therefore, the use of UVB emitting light sources in public spaces would be limiting for these individuals. The Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR) estimated that in the EU up to 250,000 people may be at risk of photosensitive reactions from UV emitted from lighting (SCENIHR).

6.6.3 Topical Full Spectrum Lighting to Individuals- Feasibility and Risks

Finally, the use of light to induce vitamin D production could be delivered at an individual level via phototherapy using topical individual light treatments. Phototherapy is a recognised technique to induce vitamin D production. Full body exposure in a UV light cabin, for two minutes three times a week for four weeks during the winter months increased serum vitamin D levels by 77.5% in Finnish adults age 18-65 years (Ala-Houhala *et al.*, 2012). The exposure dose in that study was increased each time over the course of the treatments from an initial exposure equivalent to 0.21 J/cm^2 to 1.25 J/cm^2 and is roughly the equivalent of the dose received on a sunny summer day. This protocol is a recognised therapeutic UV exposure dose and is not associated with increased risk of skin cancer. When compared to a group receiving 20 µg (800 IU) of oral vitamin D daily for 4 weeks the mean serum vitamin D in the supplement group

only increased 37.9%. Comparing the two groups, 85% of patients who had received the phototherapy treatment had serum vitamin D above 75 nmol/L but only 43% of those who had received the oral supplements achieved the same. In both groups vitamin D levels remained raised from baseline levels for two months following the interventions. It is therefore feasible that a short course of UV phototherapy could be given one to three times a year to maintain optimal vitamin D levels depending on an individual's circumstances. Indeed this is reflective of some of the earliest studies where children received UV by exposure of the skin under mercury vapour lamps, however now adhering to good practice, it is possible to control the dose far more accurately (Moseley *et al.*, 2015). This approach would have the benefit of being tailored to the needs and risk status of the individual, however as with many individual level interventions it requires patient compliance. The children most at risk may be the ones least likely to attend for treatment appointments. If approaching from a public health perspective a mobile unit going to schools may be more effective than clinic attendance and appointments.

6.7 Suggested Future Research and Randomised Trials

6.7.1 Short Term Research Proposals

Further potential research could also focus on investigating whether the findings of associations between oral health and sunshine hours can be replicated or made more robust using other oral health datasets. Alternatives to the ADHS, CDHS and Oral Health Survey of 5 year old children would need to be identified. Possible datasets to be considered will be discussed briefly here, with suggestions for further investigation.

1) National Diet, Nutrition and Dental Survey of Children Aged 1 1/2 to 4 1/2 Years, 1992-1993

These data are part of the National Diet and Nutrition Survey (NDNS) which started in 1991 and is a rolling continuous cross-sectional survey. Early studies were organised by the Ministry of Agriculture, Fisheries and Food, and the Department of Health. More recent studies are funded by Public Health England (PHE) and the UK Food Standards Agency. The survey is designed to collect

information on the diet and nutrition of the UK population. Specifically, it aims 'to collect detailed, quantitative information on the food consumption, nutrient intake and nutritional status of the general population aged 1.5 years and over living in private households in the UK (*Office of Population Censuses and Surveys, 1995).*

In this 1991-92 survey participants were selected using a multi-stage stratified random sampling technique. Participants completed a face-to-face interview, parent questionnaire and dietary intake diary. As part of this cohort blood samples were collected and a dental examination by a dentist was also completed. Numerous clinical data were collected but data of relevance to future research in this area included anthropomorphic measurements; socioeconomic indicators; region of residence; diet and use of supplements; serum measurements of vitamin d; and clinical information on the condition of the teeth which could be used to determine dmft. Although the number of participants in the survey is small (1839 children took part in the interview) the relevant data on confounders and outcomes of interest is detailed. Relatively small geographical area references are available (postcode sector), so bright sunshine hours data from the Met Office could be linked using this fairly high spatial resolution. The date of the survey does differ by some years from the exposure measures used in this thesis, however the exposure measure for the relevant decade could be calculated as an alternative to the use of the current exposure measure as a proxy for a long term average.

This dataset would allow a repeat of the cross sectional study similar to the CDHS study in chapter 5, to investigate whether the observed associations were replicated using this survey data.

2) The Avon Longitudinal Study of Parents and Children (ALSPAC)

The ALSPAC study is a longitudinal cohort study started in 1991 following 14,472 children, from before birth, and their mothers (Golding *et al.*, 2001). A subgroup of 10% of the children attended the University of Bristol for clinical appointments at various intervals between 4 and 61 months of age. The children in the sample all came from the 1991 administrative area of Avon, consisting of three district health authorities. Recruitment to the study was

opportunistic recruiting women as early in their pregnancy as possible with recruitment staff visiting community locations to promote participation. The study cohort was felt to be broadly representative but analysis reported that there was a shortfall in inclusion of families from lower socioeconomic households and non-white ethnicities. Whilst this data set has limited geographical variation, meaning the gradients of sunshine exposure would be small, it does include serum vitamin D measurements and oral health data in the form of self-reported dental health (via parent questionnaire), and a dental exam by trained non dental examiners. Whilst geographical variability in sunshine exposure would be minimal, it is likely that vitamin D levels would vary naturally based on behaviours (outdoor activity, diet etc.) and any consumption of supplements. Dental data is longitudinal and dental exams were completed at 31, 43, 61 months of age. Information on confounding factors includes sex, age, socioeconomic data, diet and oral heath regimens, as well as many other health and social history outcomes. As this dataset is focusing on children it should be considered as a resource for further investigation of the relationship between vitamin D and caries and has the potential for a cohort study to be undertaken, potentially tackling some of the design limitations of cross-sectional studies.

3) Biobank

UK Biobank is large-scale prospective study creating a biomedical database and research resource, containing in-depth genetic and health information from half a million UK participants. Data has been collected since 2006. Participants are 40 and 69 years olds living in the UK and provide blood, urine and saliva samples, as well as completing lifestyle questionnaires. Data collected is also linked to their health-related records (Sudlow *et al.*, 2015).

Vitamin D serum measurements, residence location (accurate within around 200m), detailed dietary intake data and self-reported oral health outcomes including dental treatment, presence of loose teeth, painful teeth and extractions are all recorded. The oral health data is limited as there is no clinical examination of the dentition and it is suggested self-reported data may be less accurate. However, strengths of this dataset include the wide range of geographical locations in the participants throughout the UK and high resolution geographical referencing, along with vitamin D serum measurements. The comprehensive data collected also allows for confounding to be considered for

factors such as age, sex, socioeconomic status, and diet. Given the age of the participants this dataset has the potential to investigate the relationship between periodontal outcomes, for example tooth loss and loose teeth, and sunshine hours exposure and vitamin D. The longitudinal nature of the data would make it suitable for cohort and nested case-control studies.

4) UK National Cancer Registration and Analysis Service

This thesis explored the relationship between sunshine and UVB , and the oral health conditions of plaque, caries, gingivitis and periodontal disease. Future research could explore this relationship with other oral health conditions of significant relevance or impact, for example oral cancer. Oral cancer, according to the International Classification of Disease ICD10 C00-C06 includes cancers of the lip, tongue and oral cavity and is commonly caused by squamous cell carcinoma (SCC). The incidence of oral cancer in the UK is increasing (Tataru *et al.*, 2017; G Price) and the State of Mouth Cancer UK Report 2020/2021 reports that in the UK mouth cancer accounted for just over 2% of all cancers, with greater occurrence in men and those over the age of 55. Known risk factors include tobacco and smoking, excess alcohol intake and the HPV virus types -16,-18. Vitamin D deficiency is also known to be associated with the risk of having oral cancer (Udeabor *et al.*, 2020; Anand *et al.*, 2017). One recent systematic review concluded Vitamin D deficiency may be more common in head and neck cancer patients than in the healthy population but there was insufficient evidence to confirm a causal relationship (Mäkitie *et al.*, 2020). Possible mechanisms explaining a potential relationship include the immune moderating and gene regulating properties of vitamin D and its active metabolites. The active form of vitamin D has been shown to be both anti-proliferative against SCC in cell lines in vitro and in mouse models(McElwain *et al.*, 1997). Conversely it is also known that sun exposure increases the risk of developing lip cancer. UK National Cancer Registration and Analysis Service is provided by Public Health England (http://www.ncin.org.uk/about_ncin/). It aims to collect data on cancer cases in England so that it can be used to support research, public health policy and healthcare planning. The data collected informs on the trends in cancer cases and outcomes in various regions and times throughout England. In England pathology reports of cancer are submitted to the NCRAS National Health Service from NHS and private

pathology laboratories. The information collected is matched with data from the Patient Administration System and Cancer Outcomes and Services Dataset to create a more detailed cancer record (Venables *et al.*, 2019). It would therefore be useful to use these data resources to explore sunshine-vitamin D- oral cancer associations, including by replicating the methodology used in this thesis incorporating ecological studies to explore associations with geographical variation in sunshine.

6.7.2 Medium Term Research Proposals

Repetition of the empirical studies but with more accurate exposure data would be beneficial. The weakness of the studies completed here, as discussed prior, is the large area sunshine hours data. Other studies have successfully used personal UV dosimeters to record UV exposure and this approach could be utilised here (McKenzie *et al.*, 2013). Ideally studies would collect longitudinal daily UV dosimeter data and longitudinal serum vitamin D and oral health data for robust assessment of relationships.

6.7.3 Long Term Research Proposals: Experimental Research

If the short term and medium-term research produced consistent results regarding the association between sunshine hours and dental caries then ideally a randomised controlled trial (RCT), the highest level of evidence when investigating a causative relationship, would be considered. There is insufficient evidence presented here to conduct an RCT currently but should an RCT of general health outcomes and artificial UVB exposure ever be undertaken the possibility of adding a dental health component may be an option.

More practically, traditional experimental approaches such as RCTs could not be easily conducted with natural UVB exposure due to the limitations of randomising participants into high/low sunshine exposure groups. A potential option to consider in experimental research may be a natural experiment approach, for example when people move areas there is a ‘natural’ change in exposure levels. If it is possible to identify appropriate anonymised datasets and data linkage methods which could be used in this way this may be a more appropriate option for this type of environmental exposure research.

6.8 Conclusion

In conclusion the literature reviewed here and the studies undertaken as part of this thesis support the hypothesis that increased exposure to UVB light from natural or artificial sources is associated with a small but consistent decreased risk of dental caries in the primary and secondary dentition in children. Consideration of the Bradford-Hill causation criteria are suggestive of a causal relationship, but subject to a number of limitations. No association was found regarding reduced gingivitis or periodontal disease in adults. Although the work discussed here has contributed to the evidence regarding UVB, vitamin D and the effect on oral health, it is suggested further research is needed in the form of analysis of data from other datasets and long-term, longitudinal studies to investigate if this association is reproducible and consistent. In the absence of other recent, robust ecological studies which address this research question, the novel research conducted as part of this thesis may still be useful to inform public health guidance, policy and interventions regarding sunshine, UVB exposure and oral health.

Appendices

Appendix 1: A Systematic Review of Exposure to UVB and Oral Health

Jane Collingwood, David Moles, Ben Wheeler, Nicholas Osborne

Citation

Jane Collingwood, David Moles, Ben Wheeler, Nicholas Osborne. A systematic review of exposure to UVB and oral health. PROSPERO 2016 CRD42016047655 Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42016047655

Review question

Do populations exposed to higher levels of UVB radiation have better oral health than those who are exposed to lower levels?

Objective 1: What are the effects of sunlight exposure on: i) Caries ii) Gingivitis iii) Periodontal Disease iv) Plaque Deposits

Objective 2: What are the effects of artificial UVB exposure on: i) Caries ii) Gingivitis iii) Periodontal Disease iv) Plaque Deposits

Searches

A preliminary search was done as part of an initial literature review of evidence of vitamin D, UVB exposure and gingivitis, periodontal disease and caries. This literature review used keyword search strategies in Medline to identify relevant papers and previous reviews on this topic. The reference lists of papers and reviews were also searched for other relevant papers to get an idea of the number and type of studies available.

The findings of this were used to develop a full search strategy. The following electronic databases and grey literature sources will be used:

• MEDLINE via EBSCO

• EMBASE via OVID

• Dentistry and Oral Sciences Source via EBSCO

• Cochrane Central Register of Controlled Trials- Wiley

• ERIC via EBSCO

• British Education Index via EBSCO

• Child development and Adolescent studies via EBSCO

• Scopus database via Elsevier

• Proquest dissertation database via Proquest

• Web of Science via Thomson Reuters

• ZETOC Conference Proceedings (1980 to 7 March 2013) via MIMAS

There will be no date restriction on searches; they will be conducted from earliest recorded records on the database to date.

The search strategy for MEDLINE will be adapted for use in the other databases:

Search results will be imported into Endnote and will be screened according to the review's inclusion criteria.

Types of study to be included

All types of trials on humans will be included in the analysis. Longitudinal studies will be included Comparative studies will be included Ecological or observational studies will be excluded Cross sectional studies will be excluded Reviews will not be included in the final analysis but if found using the search strategy will be used as a source for supplementary literature searching- the reference list will be used for backward searching – to check for any other references not found using the search terms Case reports and series will be excluded also. Studies which look at temperature only, Season only, Month only, Latitude only will be excluded.

Condition or domain being studied

Exposure to sunlight or any light containing UVB wavelengths between approx. 295-310nm will cause vitamin D production in the skin. It is not possible to produce vitamin D naturally at certain months of the year in the UK due to the strength of the sun being reduced by atmospheric changes and changes in the zenith angle. Chronic vitamin D deficiency is therefore common in the UK, especially in certain group. Previous studies have shown an association with some oral conditions and low vitamin D levels. It is important to investigate this further as if exposure to UVB does improve oral health this could be a cheap and effective treatment modality to reduce oral disease and to be investigated further.

The oral health conditions being included in this review are:

Dental Caries: Caries is the decay of the hard tissues of the teeth. Teeth are made up of a hard outer highly mineralized layer of enamel formed from hydroxyapatite crystals over a slightly softer dentine layer and a central dental pulp, which is the blood and nerve supply to the dentine (but not enamel which is avascular). Bacteria form plaque on the surface of the teeth and use dietary fermentable carbohydrates, especially sugar, to metabolise and produce acid as a waste produce. This acid lowers the pH of the oral environment and causes dissolution of the enamel. In the early stages of enamel caries teeth can remineralise under favourable conditions and the caries can arrest and reverse. Once the lesion is established into deeper enamel and dentine the caries cannot be reversed and will generally not arrest. Eventually the caries will progress through dentine towards the pulp causing pain and eventually pulp necrosis and abscess.

Caries is a common childhood illness and in the UK a diagnosis of dental caries is the most common cause of admission to hospital for those aged under 19 each year, in 2013-14 this number was approximately 46,500. The WHO recognises the burden of oral disease and there is increasing periodontal disease and untreated caries globally (James *et al,* 2017).

Gingivitis: Gingivitis is inflammation of the gingivae, which can progress to periodontal disease and bone loss in some individuals and is a sign of poor periodontal health and poor plaque control. It was shown definitively by Loe that

gingivitis, the initial lesion of periodontitis, is caused by substances derived from microbial plaque accumulating at the gingival sulcus and margin. Acute necrotising gingivitis will not be included in this review as this is a separate disease of different underlying aetiology and not typical of chronic gingivitis

Periodontal Disease: Periodontal disease (periodontitis) is a chronic inflammation of the gingivae. A build-up of plaque and bacteria can initiate a host response, which causes inflammation and destruction of the teeth and underlying alveolar bone and can cause tooth loss. It is now recognised that periodontal disease is associated with other diseases of chronic inflammation and immunity including diabetes and cardiovascular disease.

Plaque deposits: Plaque is the build-up of bacteria and food debris to form a biofilm on all the oral surfaces. This biofilm is present in both oral health and oral disease, however the quality and quantity of the biofilm may differ for each state. The bacteria ferment dietary carbohydrate and produce acidic by-products which lead to tooth caries, others form noxious by-products and cause gingival inflammation. Therefore, the presence of oral plaque is a pre requisite for dental caries, gingivitis and periodontal disease. Although plaque is not a disease of the oral cavity it will be included in this review as it is the primary risk factor for gingivitis, periodontal disease and caries. Any intervention which reduces plaque deposits will reduce the risk of developing the oral diseases of interest.

Participants/population

Inclusion: Any human population

Exclusion: Animal studies

Intervention(s), exposure(s)

For objective 1:

UVB radiation between approx. 270-315 nm from natural sources

E.g., variation of UVB exposure caused by latitudinal variation, sunlight hours variation, seasonal variation, monthly variation.

For objective 2:

UVB radiation between wavelengths approx.270-315nm from artificial sources

Comparator(s)/control

For objective 1:

Exposure to different amounts of natural UVB e.g., sunlight

For objective 2:

No exposure to artificial UVB

Exposure to different amounts of artificial UVB light

Context

Primary outcome(s)

Primary outcomes: Any measure of caries, gingivitis, plaque or periodontal disease including but not limited to:

Caries

1. (Lifetime) DMFT (Decayed, Missing, Filled Teeth),

2. DMFS (Decayed Missing Filled Surfaces)

3. New carious lesions during a trial period, caries incidence

4. Lifetime Tooth loss

Gingivitis

1. Any Gingival bleeding index

2. Gingival Index (Loe and Silness)

Periodontal Disease

1. Bleeding on probing

2. Pocket depths

3. Alveolar bone loss

4. Loss of attachment

5. Lifetime tooth loss

6. Community periodontal Index (CPITN)

Plaque deposits

1. Plaque scores

2. Silness and Loe PI

3. O'Leary

4. Any other published plaque score

Measured clinically and/ or where appropriate radiographically.

Secondary outcome(s)

Any harmful health outcomes, to the population, individual or teeth, which are reported

Data extraction (selection and coding)

Selection of studies

JC will screen the titles and available abstracts of all reports identified through the electronic search. This will also be done by 2nd and 3rd reviewers.

For all studies appearing to meet the inclusion criteria, or where the abstract does not contain sufficient information to determine if the inclusion criteria are met, the full report will be requested. The full text will similarly be screened by JC and 2nd and 3rd reviewers against the inclusion criteria before final decision taken. Rejected papers will be recorded along with reasons for exclusion.

A bespoke data extraction form will be designed for this review. This will be piloted on 5 papers prior to the start of the review and modified before use if required.

JC will do the data extraction and a second reviewer will check the results. If any discrepancies are found and a consensus cannot be agreed a third reviewer will be asked to arbitrate.

For each study we intend to record where possible

• Year of publication, country of origin and source of any study funding.

• The primary study aims

• Details of the participants including demographic characteristics (socio-economic status (SES), ethnicity), primary/permanent dentition and criteria for inclusion and exclusion.

• Details of the type of intervention environment where it was delivered, who provided the intervention, comparator and co-interventions.

• Details of the outcomes reported, including method of assessment, and time intervals.

• Details of confounding factors considered (potential confounders of relevance to this review include sealant use, exposure to other UVB sources, different latitudes of place of study, exposure to fluoridated water, sugar consumption, SES, ethnicity and the use of other fluoride sources).

• Details on comparability of groups with regard to confounding factors.

• Details on methods used to control for confounding.

• Details on results and effect estimates

• Details of Quality Assessment

Quality Assessment including risk of bias assessment

This will be done by JC as part of the data extraction process and checked by a second reviewer with disagreements settled by discussion or a third reviewer if a consensus cannot be found.

The quality assessment and risk of bias will apply the principles outlined in the CRD handbook using appropriate tools e.g., Cochrane Collaboration Risk of Bias tool for RCTs, Hamilton Quality Assessment Tool for Quantitative studies, rating studies as strong, weak or moderate in the study of components of selection bias, design, confounders, blinding and data collection methods, withdrawals and drop-outs. Results will be tabulated.

Additionally, the quality assessment and risk of bias across all the studies via possible publication bias will be discussed and a funnel plot will be constructed.

Strategy for data synthesis

Results from the systematic review will be presented according to PRISMA recommendations. A flow diagram will provide the number of citations retrieved from electronic searches. The flow diagram will summarize the number of articles retained at each screening stage and provide the reasons for exclusion. Summary statistics and data from all studies will be presented and described in the results as text and tables.

Analysis:

The analysis will include all the papers irrespective of bias however papers will be grouped for analysis according to those addressing objective 1 and those addressing objective 2.

They will be tabulated by objective and listed by study type.

Assessment of heterogeneity

Differences in how the UVB is given artificially, amount of UVB/sunlight exposure, outcome measures and technique of measuring the outcomes are all possible sources of heterogeneity.

Heterogeneity will be evaluated using the I-squared statistic and observing any differences or overlap in the effect of different studies.

If enough homogeneity between the trials is found for either of the objectives separate random effects meta-analyses will be undertaken. If appropriate there will be a narrative synthesis of the data extraction table to compare the strength and direction of associations between exposure to natural and artificial UVB exposures, and more specifically each of the oral diseases of interest.

The narrative synthesis will aim to:

• Develop a theory of how the intervention of UVB exposure may work

• Develop a preliminary synthesis of findings of included studies

• Explore relationships within and between studies

• Assess the robustness of the synthesis

Where possible, the risk ratio or mean difference and a measure of statistical significance (using the 95% confidence interval or P value) of the finding will also be included.

Confounders which could affect oral health will also be considered

Analysis of subgroups or subsets

If meta-analysis is performed: analysis of subgroups or subsets

If possible, the following subgroup analysis will be performed:

1. Age

2. Sex

Sensitivity analysis:

The following sensitivity analysis will be performed:

1. Quality: studies that are rated as weak can be removed from analysis

Contact details for further information

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Organisational affiliation of the review

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Anticipated or actual start date

01 October 2016

Anticipated completion date

01 October 2017

Funding sources/sponsors

NIHR funded ACF post

Conflicts of interest

None known

Language

English

Country

England

Stage of review

Review Ongoing

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

Dose-Response Relationship, Radiation; Humans; Oral Health; Ultraviolet Rays

Date of registration in PROSPERO

13 September 2016

Appendix 2: Final search strategy for Medline. Adapted for use in other Databases.

1. (UV radiation or UVB or Ultraviolet or Irradiation or Broad spectrum light or sunshine or sunbed or sunlight or Solar irradia* or Latitud* or seasonal variation or geographic variation or zodiac).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
2. ultraviolet radiation/ or ultraviolet b radiation/
3. (gingiv* or periodont* or carie* or plaque or enamel hypoplasia or enamel dysplasia or enamel defects or tooth loss or DMFT or DMFS or PUFA or CPITN).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
4. exp periodontal disease/
5. tooth disease/
6. exp dmf index/
7. 1 or 2
8. 3 or 4 or 5 or 6 or 7
9. 8 and 9

Appendix 3: Summary of Data Collection from ADHS 2009 (Adapted from Foundation Report: Adult Dental Health Survey 2009: Technical information. (O'Sullivan *et al*, 2011))

Dental Examination	Code	Criteria
Debris	P	Any visible plaque or supragingival calculus visible to naked eye
	C	Clean- no plaque/ calculus visible to the naked eye
Coronal Condition	M	Missing (and not a bridge pontic)
	A	Adhesive bridge pontic
	B	Conventional bridge pontic or implant pontic
	T	Implant
	F	Amalgam filling
	R	Intracoronal restoration, but not amalgam, including GIC, composite, inlays and onlays
	V	Veneers, retentive wings of adhesive bridges
	K	Full Crown, permanent or temporary, including full coverage bridge abutments
	X	Sealants

Dental Caries	0	Sound
	Y	Failed restoration
	H	Hard, arrested decay
	4	Visual dentine caries (underlying shadow- non cavitated
	5	Distinct cavity with visible dentine
	6	Extensive cavity with visible dentine- teeth so broken down pulpal involvement would have occurred
	9	Not possible to code
Condition of Root Surfaces	F	Amalgam restoration
	R	Filling or restoration, not amalgam
	N	No exposed root surface
	0	Exposed root surface but no evidence of current or past disease
	W	Worn to a depth of 2mm or more, but with no caries or restoration
	H	Hard, arrested decay
	4	Caries on the root surface equivalent to coronal caries 4 or 5
	6	Extensive cavity- deep wide and pulpal involvement
	9	Unscorable

Tooth Wear- Upper and lower Canine to canine only	0	All Surfaces	Sound, wear restricted to enamel, none into dentine
	1	All Surfaces	Loss of enamel just exposing dentine
	2	Buccal and Lingual Surfaces	Loss of enamel exposing dentine for more than an estimated one of the individual surface area (B, L)
		Incisal	Loss of enamel and extensive loss of dentine not exposing secondary dentine or pulp
	3	Buccal and Lingual Surfaces	Complete loss of enamel on a surface, pulp exposure, or exposure of secondary dentine
	8	All	Fractured tooth- clear traumatic loss of tooth substance rather than wear
	9	All	Unscorable
Some examiners performed additional examination procedures, described below			
BEWE: Basic Erosive wear Examination Sextants- worst score in sextant recorded	0	Buccal, Occlusal, Lingual	Sound
	1	BOL	Loss of enamel surface texture

	2	BOL	Distinct defect, hard issue loss less than 50% of the surface area
	3	BOL	Hard tissue loss more than 50% of the surface area
	9	BOL	Unscorable. All crowns and bridge abutments are given this code
Occlusion- functional occlusal contacts (all examiners)	0	Posterior and molar regions	No posterior functional contact
	1	Posterior and molar regions	Posterior functional contact present
Spaces, aesthetics and dentures Each tooth in anterior region between 2nd premolars.	N		No space
	T		Implant retained restoration
	S		Space equal to, or more than
	D		Space restored by a removable prosthesis
	B		Space restored by a fixed bridge
Dentures (recorded separately for upper and lower arch)	1		Partial
	2		Full
	3		Complete overdenture

	4		Implant retained
Denture Base Material	1		Metal
	2		Plastic
Denture Status	0		Intact
	1		In need of repair
PUFA Index (Pulp, Ulceration, Fistula, Abscess)	0	Whole mouth	No problem or pain
	1		Yes, problem or pain
	P		Open pulp in permanent dentition
	U		Obvious ulceration
	F		Fistula in permanent dentition
	A		Abscess in permanent dentition
	0		No lesions evident
	1		A single lesion present
	2		2 or more lesions present

Periodontal Condition: PD, calculus and bleeding in under 55 yrs. PD, Calculus and bleeding plus LOA in over 55 yrs.			
Pocket depth (PD) and Loss of Attachment	0	Record worst score in each sextant	Up to 3.5 mm
	1		4-5.5 mm
	2		6-8.5 mm
	3		9+ mm
	9		Unscorable
Calculus	0		None visible/ detectable
	1		Any supra/sub gingival calculus
	9		Unscorable
Bleeding	0		No bleeding
	1		Bleeding
BPE	0		No bleeding/pocketing detected
	1		Bleeding on probing- no pocketing >3.5mm
	2		Plaque retentive factors- no pocketing >3.5mm
	3		Pockets >3.5mm but < 5.5mm
	4		Pockets > 5.5mm

Appendix 4: Summary of Data Collected in CDHS 2013 (adapted from Child Dental Health Survey 2013: Technical Report. 2015)

Component	Age Groups	Notes	Information recorded- summary
1) Developmental Defects of Enamel (natural light)	12	Upper 4 to 4 teeth only	Type and Extent of defect coded
2) Simplified IOTN aesthetic component	12,15		10 point aesthetic component scale
3) Periodontal Condition 1 3 segments for each of upper and lower jaw. Assessed by three segments- buccal and lingual surfaces included. Middle (canine to canine), Left, Right	5,8,12,15	Gingival Health	0, gingivae appear healthy 1, gingivae are not healthy 9, assessment cannot be made
		Plaque	0, The teeth appear clean

			1, Plaque visible without probing 9, assessment cannot be made
		Calculus	0, No calculus 1, Calculus is present 9, Assessment cannot be made
4) Tooth Condition	5,8,12,15	Dry tooth with cotton wool only	Coded to record: Absence/ presence of tooth O, Sound AV, Visual change in enamel AC, Visual change in enamel with cavitation 2V, Visual caries – non dentine cavitated 2C, Cavitated dentine caries 3, Decay with pulpal involvement 4V, Filled and recurrent decay (no cavitation) 4C, Filled and recurrent decay (cavitation) 5, Filled no dentinal decay

			R, Filled, needs replacing (not carious into dentine) T, Traumatised surface X, Obviously sealed surfaces C, Crown/ advanced restorative procedures
5) PUFA Index (Pulp, Ulceration, Fistula, Abscess)	5,8,12,15	Pain and problems related to dental caries only	P=open pulp in primary or permanent dentition U=obvious ulceration F=fistula in primary or permanent dentition A= abscess in primary or permanent dentition 0, No lesions present 1, A single lesion present 2, 2 or more lesions present
6) Trauma (adult teeth only)	5,8,12,15,	Adult teeth only	0, No trauma 1, Discolouration 2, Fracture involving enamel 3, Fracture involving enamel and dentine

			4, Fracture involving enamel, dentine and pulp 5, Missing due to trauma 6, Acid-etch composite restoration 7, Permanent replacement including crown, denture, bridge pontic 8, temporary restorations 9, Assessment cannot be made
7) Tooth Surface loss/ tooth wear	5,8,12,15	Buccal and Lingual Surfaces Incisal edge not considered Upper BAAB UR6,2,1 UL1,2,6 LR6 and LL6	0, Normal 1, Enamel Only 2, Enamel and Dentine 3, Enamel into Pulp 9, Assessment cannot be made 0, Normal 1, Less than one third of surface involved 2, One third to less than two thirds of surface involved

			3, Two thirds or greater of surface involved 9, Assessment cannot be made
8) Simplified IOTN- Dental Health Component	12,15	Children wearing an orthodontic appliance are excluded from this component	Missing teeth- reason Overjet Crossbite Displacement of contact points Overbite
9) Orthodontic appliances	12,15	When in orthodontic treatment only	0, No appliance 1, Fixed orthodontic appliance 2, Removable orthodontic appliance 3, Other OR 0, Never worn 1, worn fixed appliance in past and no longer worn 2, worn a removable appliance in past and finished wearing it.

			3, worn an appliance in the past and is still wearing it.
10) Anomalies (where present)	5,8,12,15	Cleft lip/palate Other craniofacial anomalies	0, none 1, present Hypodontia 0, none 1, extensive hypodontia with restorative implications.
11) Perio II- Modified BPE	15	Modified BPE UR6,1,UL6 LR6,LL1,6	Standard BPE codes 0,1,2,3,4
12) Asterisk/ Comments		If dentist wishes to record anything else not covered already	

Appendix 5: Summary of Data Collected for The Oral Health Survey of five-year-old children 2014-15 [Adapted from Oral Health Survey of five-year old children 2014-15. National Protocol 11.3. Conventions pg.13 (PHE, 2014)

Code	Clinical assessment	Notes
0	Teeth appear clean	Clinical data recorded for assessment of plaque levels used a modified Silness and Low index,
1	Little plaque visible	
2	Substantial amount of plaque visible	
9	Assessment cannot be made of upper anterior sextant	
Individual Tooth Codes		
0	Sound	a) A tooth is deemed to have erupted when any part of it is visible in the mouth. Unerupted surfaces of an erupted tooth will be regarded as sound. b) The presence of supernumerary teeth will not be recorded. If a tooth and a supernumerary exactly resemble one another then the distal of the two will be regarded as the supernumerary. c) Missing primary incisors are assumed exfoliated d) Caries takes precedence over non-carious defects, e.g., hypoplasia.
1	Arrested	
2	Caries into dentine	
3	Decay with pulpal involvement	
4	Filled and decayed	
5	Filled no decay	
R	Filled needs replacing, no decay	
C	Crown	
T	Trauma	

$	Sealant type unknown	e) Retained roots following extraction or gross breakdown should be recorded as decayed with pulpal involvement. f) Discoloured, non-vital incisors, without caries or fractures should be scored T for trauma on all surfaces. g) Surfaces that are obscured, e.g., banded teeth, should be assumed to be sound “
N	Obvious sealant restorations	
Abscess/Sepsis		
0	No abscess/sinus	
1	Abscess/sinus present	

Appendix 6: Additional Analysis of Chapter 5 Data

Table 5.16 Results of Logistic Regression Models for Presence of any Clinical Decay in more than Five Primary Teeth

N-2771 Age 5,8		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.99	0.97-1.01	0.285	0.99	0.97-1.01	0.331	0.99	0.98-1.01	0.328
Sex	M	-	-	-	1.00	-	-	1.00	-	-
	F				0.89	0.58-1.37	0.604	0.92	0.60-1.42	0.714
Age	5yrs	-	-	-	1.00	-	-	1.00	-	-
	8yrs				1.67	1.18-2.37	0.004	1.29	0.89-1.87	0.174
IMD Quintile										
1 (Most deprived)		-	-	-	1.00	-	-	1.00	-	-
2					0.94	0.63-1.40	0.753	0.99	0.66-1.47	0.950
3					0.61	0.36-1.03	0.063	0.65	0.38-1.10	0.108
4					0.54	0.28-1.07	0.076	0.56	0.28-1.12	0.102
5 (Least deprived)					0.22	0.10-0.50	<0.001	0.22	0.10-0.50	0.001
Current Plaque:	Yes	-	-	-	-	-	-	1.00	-	-
	No							0.29	0.178-0.470	<0.001

Table 5.17 Results of Logistic Regression Models for Presence of Decay into Dentine in more than Three Primary Teeth

N=2771 Age 5,8		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.98	0.97-1.00	0.039	0.98	0.97-1.00	0.035	0.98	0.97-1.00	0.030
Sex M		-	-	-	1.00	-	-	1.00	-	-
F					0.89	0.68-1.17	0.398	0.92	0.68-1.23	0.565
Age 5yrs		-	-	-	1.00	-	-	1.00	-	-
8yrs					1.58	1.13-2.20	0.007	1.23	0.89-1.70	0.202
IMD Quintile										
1 (Most deprived)		-	-	-	1.00	-	-	1.00	-	-
2					0.97	0.63-1.47	0.868	1.01	0.66-1.55	0.964
3					0.61	0.36-1.04	0.069	0.65	0.38-1.11	0.112
4					0.40	0.23-0.68	0.001	0.40	0.23-0.70	0.002
5 (Least deprived)					0.28	0.15-0.50	<0.001	0.27	0.14-0.51	<0.001
Current Plaque:	Yes	-	-	-	-	-	-	1.00	-	-
	No							0.32	0.21-0.49	<0.001

Table 5.18 Results of Logistic Regression Models for Presence of any Clinical Decay Experience in Primary Teeth (Cavitated Lesions Only)

N=2771 Age 5,8	Model 3 Model 2 as before + Adjusted for plaque		
Sunshine Hours as Integers	OR	95% CI	P
Categorical Model	1.00	-	-
149	2.36	1.63-3.41	<0.001
150	1.02	0.67-1.57	0.912
161	1.59	1.16-2.17	0.004
165	1.17	0.70-1.95	0.535
172	1.34	0.72-2.48	0.354
176	1.35	0.86-2.13	0.189
181	1.02	0.65-1.59	0.937
184	0.99	0.57-1.72	0.979

Table 5.19 Results of Logistic Regression Models for Presence of any Clinical Decay Experience in Primary Teeth (Cavitated and Visual Lesions)

N=2771 Age 5,8	Model 3 Model 2 as before + Adjusted for plaque		
Sunshine Hours as Integers	OR	95% CI	P
Categorical Model	1.00	-	-
149	1.62	0.99-2.65	0.055
150	1.01	0.52-2.00	0.966
161	1.07	0.65-1.76	0.795
165	1.13	0.55-2.32	0.727
172	1.09	0.59-2.00	0.774
176	0.97	0.45-2.12	0.941
181	0.88	0.49-1.60	0.680
184	0.85	0.44-1.62	0.607

Table 5.20 Results of Logistic Regression Models for more than Five Permanent Teeth with Clinical Decay

N=3941 Age 8,12,15		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.98	0.96-1.01	0.144	0.98	0.96-1.01	0.136	0.99	0.97-1.01	0.274
Sex	M	-	-	-	1.00	-	-	1.00	-	-
	F				1.40	0.85-2.29	0.183	1.53	0.93-2.50	0.092
Age	8yrs	-	-	-	1.00	-	-	1.00	-	-
	12yrs				5.95	2.38-14.87	<0.001	6.43	2.50-16.56	<0.001
	15yrs				13.11	5.72-30.08	<0.001	15.61	6.56-37.13	<0.001
1	(Most deprived)	1.00	-	-	1.00	-	-	1.00	-	-
2					0.59	0.33-1.06	0.076	0.60	0.33-1.08	0.085
3					0.48	0.22-1.03	0.059	0.51	0.25-1.06	0.072
4					0.41	0.19-0.90	0.027	0.46	0.22-0.98	0.044
5	(Least deprived)				0.23	0.09-0.59	0.003	0.26	0.10-0.63	0.004
Current Plaque:	Yes	1.00	-	-	1.00	-	-	1.00	-	-
	No							0.37	0.22-0.63	<0.001

Table 5.21 Results of Logistic Regression Models for more than Three Permanent Teeth with Decay into Dentine

N=3941 Age 8,12,15		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.98	0.96-1.01	0.192	0.98	0.96-1.01	0.220	0.99	0.96-1.01	0.36
Sex	M	-	-	-	1.00	-	-	1.00	-	-
	F				1.27	0.81-2.01	0.295	1.43	0.91-2.25	0.121
Age	8yrs	-	-	-	1.00	-	-	1.00	-	-
	12yrs				3.79	1.90-7.57	<0.001	4.19	2.05-8.54	<0.001
	15yrs				5.10	2.72-9.57	<0.001	6.28	3.32-11.88	<0.001
1	(Most deprived)	1.00	-	-	1.00	-	-	1.00	-	-
2					0.39	0.22-0.68	0.001	0.39	0.22-0.68	0.001
3					0.43	0.20-0.92	0.031	0.47	0.22-0.98	0.045
4					0.21	0.8-0.55	0.002	0.24	0.10-0.60	0.003
5	(Least deprived)				0.26	0.73-0.90	0.033	0.28	0.08-0.96	0.043
Current Plaque:	Yes	1.00	-	-	-	-	-	1.00	-	-
	No							0.27	0.16-0.45	<0.001

Table 5.22 Results of Logistic Regression Models for Presence of Clinical Decay in Permanent Teeth (Cavitated Lesions Only)

N=2,529 Age 12,15	Model 3 Adjusted for sex, age, index of derivation, plaque, eats sugary food more than four times a day, smoking		
	OR	95% CI	p
Sunshine Hours	0.98	0.97-1.00	0.009

Table 5.23 Results of Logistic Regression Models for Presence of Clinical Decay in Permanent Teeth (Cavitated and Visual Caries)

N=2,529 Age 12,15	Model 3 Adjusted for sex, age, index of derivation, plaque, eats sugary food more than four times a day, smoking		
	OR	95% CI	p
Sunshine Hours	0.97	0.96-0.99	0.001

Appendix 7: Dissemination of Findings: Draft paper 1.

Impact of sunshine exposure on plaque and bleeding on probing in participants of the UK 2009 Adult Dental Health Study: A cross sectional study

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Key Words: Sunshine, UVB, vitamin D, plaque, bleeding on probing

Abstract

Objectives: Vitamin D, which may be associated with periodontal health outcomes, is produced in the skin after sunshine exposure. This paper investigated the possible association between mean sunshine hours, which vary substantially across England, and plaque and presence of bleeding on probing (BOP) in an adult population.

Basic research design: Logistic regression models were used to investigate association between regional mean sunshine hours and presence of plaque or BOP in participants of the Adult Dental Health Survey 2009.

Results: In the fully adjusted model, presence of plaque was independently associated with sunshine hours and a 10% decrease in the presence of plaque was seen per category increase in sunshine hours (OR=0.90, 95% CI 0.87-0.92, p<0.001), treating the 10 regions as categories in ascending order of mean sunshine hours. Thus, this association was observed even when confounding factors were considered.

In the unadjusted model, there was a significant 3% decrease in BOP (OR=0.97, 95% CI 0.95-0.99 p=0.009) per category increase in sunshine hours However, when the model was adjusted for sex, age, smoking and deprivation, the association with BOP only, was attenuated (OR=0.98, 95% CI 0.96-1.01 p=0.125).

Conclusion: This study, supports the view that increased sunshine exposure may decrease the presence of plaque, a risk factor for oral disease. The study is subject to design limitations, but is the first observational population study to fully take into account demographic and confounding factors.

Introduction

Vitamin D is a secosteroid hormone essential for bone metabolism, immuno-modulatory functions, which also has antimicrobial properties (Hewinson, 2012, Prietl, 2013). Although some vitamin D is obtained from the diet, in the UK most of an individual's vitamin D is obtained from exposure to sunshine (Macdonald et al., 2008, Hypponen and Power, 2007). When UVB radiation, at approximately the wavelengths of ~290-315nm, is absorbed by the skin, a photolytic process occurs, involving the cholesterol derivative 7-dehydrocholesterol (7-DHC) (Holick., 1981). This converts 7-DHC to pre-vitamin D3, which is then isomerised to vitamin D3. In the UK vitamin D deficiency, less than 50 nmol/L of the major circulating metabolite 25(OH)D, is thought to be common, with levels being highest in September and lowest in February, such that the majority of the UK population become vitamin D deficient during the winter (Webb et al, 2010). Sunshine hours exposure is positively correlated with vitamin D status, even in individuals who avoid sun exposure and populations resident in areas with higher average sunshine hours have higher levels of vitamin D than those living in areas with lower average sunshine hours (IOM, 2011., Brot et al., 2001, Cherrie et al., 2015).

Vitamin D status may influence gingival health, as determined clinically by outcome measures such as presence of plaque and presence of bleeding on probing, suggestive of inflammation (Dietrich et al., 2005). A previous RCT (Hiremath et al., 2013b), demonstrated a dose dependent improvement in gingival health with Vitamin D supplements, but a Finnish study found no association between lower serum levels of Vitamin D and gingival bleeding (Antonoglou et al., 2015). However, despite the known links between sunshine and blood Vitamin D levels (Brot et al., 2001), no recent studies have analysed the evidence in an attempt to link sunshine exposure to specific markers of oral health, nor have they fully considered confounding factors and demographic data as part of the analysis.

The aim of this study therefore, was to investigate the association between long-term mean environmental UVB exposure (using sunshine hours as a proxy and an indicator of potential vitamin D status) and the presence of plaque and bleeding on probing in an adult population in England, adjusting for potential confounding factors.

Methods

The exposure measure for sunshine hours was obtained from The Medical and Environmental Data Mash-up Infrastructure Project (MEDMI), which utilised Met Office data from 78 meteorological stations around the UK (2017). In order to create long term averages indicative of chronic exposure, the recorded raw daily sunshine hours from all stations were obtained and aggregated into monthly total bright sunshine hours for each month 2010-14, where data were available for every day in the month. Following this, the data were averaged by calendar month and station across the five years, and using ArcGIS 10.5 (ESRI, Redlands, USA) mean monthly data were plotted geographically using station British National Grid references. Inverse distance weighted interpolation was used to estimate monthly mean sunshine for a grid of 5 km square cells across the study area. The twelve monthly grids were then overlaid with the geographical boundary data of the ten English Strategic Health Authorities (SHAs), and an average of the five-year monthly mean hours of sunshine for each SHA for the period 2010-2014 was obtained (example shown in Figure 1). These data were summarised to produce a five-year monthly mean for the whole period for April to

October, the months when Vitamin D production is possible (Table 1). For analyses the SHA areas were treated as ten categories ordered by sunshine hours, or grouped according to high, medium and low estimated exposure for sensitivity analysis.

These data therefore provided an estimate of long-term, population exposure to sunshine. They were linked with secondary data obtained from the results of the 2009 Adult Dental Health Study (ADHS) (Office for National Statistics, 2012). The original data set included 11,380 individuals from across England, Wales and Northern Ireland, however for this study the data analysed were limited to England residents only (Table 2). This restriction was applied since area deprivation is a key potential socio-economic confounder, and is measured differently in each country, meaning that combined analysis adjusting for deprivation is not possible. The original survey consisted of a questionnaire interview, of all adults aged over 16 years within sampled households, and an oral examination of the mouth and teeth of all adults who had at least one natural tooth. If on examination of the buccal surfaces of the upper teeth, or the lingual surfaces of the lower teeth, bleeding on probing (BOP) was positive from any of the pockets in a sextant, the sextant was recorded positive for BOP. For the purposes of analyses in this study, if one or more sextant was recorded as positive for BOP then our study categorised that participant as having gingival bleeding. The presence of visible plaque on any tooth was recorded as positive in the original dataset also giving two binary outcome measures for analysis. The oral examination was only carried out on approximately half of the sample, therefore analyses here using examination-based outcomes are based on this sub-sample only. Primary reasons for non-participation in the examination were a) being edentate; b) no consent given; c) consent given but examination not performed due to lack of dental examiner availability (Office for National Statistics, 2012). The field work for the ADHS was completed between October and December 2009, and January to April 2010 by dentists who had undergone calibration and training.

Sunshine data at SHA level were linked to ADHS survey data based on the SHA of residence for each participant. For data protection reasons, this is the highest geographical resolution identifier provided with the ADHS survey data obtained from the UK Data Service. It was hypothesised that participants living in areas with higher mean sunshine hours would be less likely to have visible plaque, and consequently less likely to have BOP (Theilade et al., 1966). Logistic regression was used to initially compare the odds of the presence of plaque across categories of mean sunshine hours (Sperandei., 2014). Potential confounders were then added to the model, to adjust for age, the socioeconomic metric Index of Multiple Deprivation (IMD) and smoking status. In order to account for the study sampling design, weighting was applied to the regression models with sampling weights for the examination data obtained from the original ADHS dataset. These models were then repeated with the presence of BOP as the outcome. Finally, in the case of BOP, a third full model was run, which also adjusted for plaque, to investigate whether plaque might mediate any protective association between sunshine and BOP. Only individuals with examination participation and with no missing data from any of the covariates were included in each model (complete case analysis) to ensure a consistent analysis sample.

Results

The main analysis sample consisted of 5,601 study participants (Table 2). The vast majority of exclusions from the total study sample were due to the focus on the sub-sample who had an oral examination. The first model considering the association between sunshine and plaque, even after accounting for potential confounders of age, sex, IMD and smoking status, showed increased sunshine hours was associated with a significant 10% reduction in the prevalence of plaque (OR=0.90 95% CI 0.87-0.92 p=<0.001) (Table 3). Secondly, when considering an association with BOP, in the unadjusted model, individuals living in the SHA with the highest sunshine hours had a 31% reduced risk of positive bleeding on probing (OR=0.69 95% CI 0.52-0.91, p=0.008, n = 5601), when compared to the SHA with the lowest sunshine hours, but ORs did not decrease in a linear fashion across categories (Table 4). Overall, in the unadjusted model, each increase in sunshine hour category from 1 to 10 was associated with a 3% decrease in bleeding on probing (OR=0.97 95% CI 0.95-0.99 p=0.009). However, when the model was adjusted for sex, age, smoking and IMD this small association was attenuated (OR=0.98 95% CI 0.96-1.01 p=0.125). Despite there being no remaining protective association, the final model to investigate mediation by plaque was run and resulted in negligible change to the results (OR=1.01, 95% CI 0.99-1.04 p=0.30). Sensitivity analysis was completed using a three-level classification of low, medium and high sunshine hours. Unadjusted results suggested that the odds of reported bleeding on probing was lower in high and medium UVB regions compared to low UVB regions, OR 0.82 (95% CI 0.71-0.94 p=0.004) and 0.88 (CI 0.75-1.03 p=0.122). In the adjusted model this association attenuated but still suggested a protective association, although only in the 'medium' versus 'low' sunshine group comparison with p-value below conventional significance (OR=0.86, 95% CI 0.76-0.99, p=0.035). Including plaque in this model resulted in complete attenuation, as per hypothesis.

Discussion

Key findings

These findings indicate that residing in a SHA with higher sunshine hours is associated with lower odds of presence of plaque, and this is robust to adjustment for confounding. Results also suggest a small decrease in the prevalence of bleeding on probing in the simple model, but this association reduces in the adjusted model, when confounding factors were considered. When the effect of plaque was considered in the full model, the relationship attenuated even further. There is therefore support for the hypothesis that individuals living in areas with increased hours of sunshine are less likely to have visible plaque. The findings here are consistent with the odds of gingivitis being potentially lower in areas with more sunshine, as this is mediated through plaque, but these findings are subject to considerable statistical uncertainty to the extent that the role of chance cannot be ruled out. The findings are also in agreement with a recent systematic review, not considering these cofounding factors as done here, but which also demonstrated weak evidence to support the relationship between Vitamin D and gingivitis (Cagetti et al., 2020). However, in this study, decreased BOP , which decreases with gingival health, did not mirror the pattern observed with plaque, despite plaque being known a causative for gingivitis. BOP is also dependent on the host immune response, and thus further studies would be warranted to provide a mechanistic explanation for dichotomy of findings, which is beyond the scope of this study.

Strengths & limitations

Our study benefits from a large population sample at national scale, with considerable variation in environmental UVB exposure estimated over a relatively long period. The ADHS outcome data is based on consistently applied examination procedures, undertaken by trained and calibrated examiners, and includes individual data on important covariates. It is accepted however that variation between ADHS examiners exists at SHA level, and there are limitations of assessing clinical outcomes in non-clinical environments that can lead to inaccuracies and residual confounding. It is suggested that inaccuracy is less prevalent in the binary outcome measures used here, with plaque and gingival bleeding being present or not present rather than the need for precise measurement, for example with pocket depths.

A positive correlation between sunshine hours and blood Vitamin D levels is generally accepted in the UK (Brot et al., 2001). However, within these current analyses, vitamin D levels were not considered directly, but instead mean annual sunshine hours (April – October) in areas of residence was used as a proxy for UVB exposure of the individual. In England, it is recognised that most people get the majority of their Vitamin D from this endogenous source (Webb et al., 2010), however there is likely a complex relationship between living in an area with higher UVB levels, individual Vitamin D levels and oral health. Since data are based on a cross-sectional survey, how long an individual has resided in the area they were examined also remains unknown, and sunshine hours allocated to them may not be representative of their residential history. There is also a small mismatch in the timing of data (survey 2009, sunshine 2010-14), although the sunshine data can be assumed to be representative of long-term geographical variation, with long-term averages as indicators of chronic exposure.

Since exposure data are based on area-level estimates, these analyses are potential subject to the ecological fallacy (Sedgwick., 2011). Importantly, due to data protection, the survey participants could only be located to large geographical regions. The relative crudity of the geographical exposure area and consequent potential error in exposure estimation is therefore a limitation. The use of an examination-based outcome means that the analysis sample only includes around half of the total ADHS sample (i.e., those that were eligible, agreed to and had the examination). It is possible that this introduces bias, although the use of weighting provided specifically for the examination sample counters this to some degree. While the analysis was based on a large population sample, the relatively small effect sizes mean that the study may still have been underpowered to detect an effect.

Bleeding on probing signifies the presence of active inflammation in the gingival sulcus or pocket, but does not distinguish between gingivitis and periodontitis. Therefore, bleeding on probing can only measure current inflammation of the superficial gingival tissues and is not a measure of past disease. In this study we have adjusted for age, IMD and smoking as confounding factors. However, we have also adjusted for the presence of visible plaque, included as a separate full model, to investigate plaque as a possibly mediator. It has been suggested that vitamin D has host immune moderating properties and may influence the quality or quantity of plaque (Wang et al., 2013). It cannot be determined whether the null results reported here for bleeding on probing were due to study limitations, or a true influence of increasing sunshine exposure altering either the quantity/quality of plaque or the host response. Finally, our findings

may also suggest, that there is not a linear response, rather a number of sunshine hours is needed to reach threshold before an effect is seen. This dose dependent response was seen in previous studies looking at gingivitis and vitamin D supplementation (Hiremath et al., 2013). Nevertheless, this all remains speculative and thus further studies using individual dosimetry readings for UVB exposure and measurement of plasma Vitamin D levels are advised as future work.

Conclusion

From this study, it is shown that increased sunshine exposure is associated with a decrease in the presence of plaque. This is one of the first observational population studies to consider confounding factors in this context, and when these were considered the association between increased sunshine hour and reduced plaque remained. As plaque is a risk factor for poor gingival health this finding could be considered in public health messages regarding sunshine exposure. Furthermore, it can be concluded that whilst residing in an area of higher mean sunshine in the UK is associated with reduced bleeding on probing, in this study the effect was attenuated by confounders such as age, sex and IMD. We advise that further studies are required with more accurate individual measures of sunshine exposure to investigate these relationships further.

Funding:

We acknowledge support for funding this project from the NIHR, as part of a clinical fellowship from the Peninsula Dental School, University of Plymouth. Sunshine data were provided via the MEDMI project: U.K. Medical Research Council (MRC) and the Natural Environment Council (NERC) for the MEDMI Project (MR/K019341/1); thanks to Christophe Sarran at the Met Office for support.

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Table 1: Mean Annual Hours of Sunshine (April - October) 2010-2014 per Strategic Health Authority England

Strategic Health Authority (SHA)	Mean Sunshine Hours	UVB Grouping	N (9,663)
North East	149.14	3 lowest	992
North West	149.87		970
Yorks and Humber	150.78		1,021
West Midlands	161.77	4 middle	876
East Midlands	165.69		1,130
East of England	172.60		1,033
South West	176.23		1,012
South Central	177.17	3 highest	968
London	181.77		762
South East Coast	191.87		899

Table 2: Demographics of the study populations

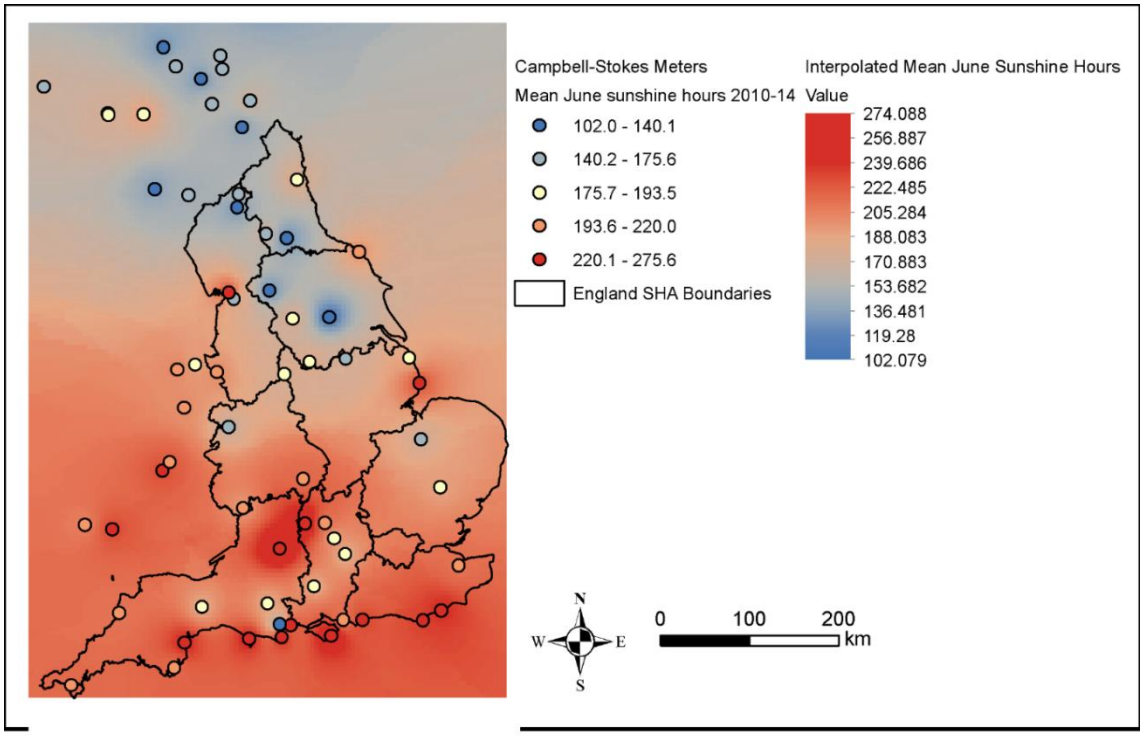
	Full Sample N (%)	England Sample N (%)	Analysis Sample. N (%)
Number of people	11,380 (100)	9,663 (100)	5,601 (100)
Male	5,086 (44.69)	4,314 (44.64)	2,557 (45.65)
Female	6,294 (55.31)	5,349 (55.36)	3,044 (54.35)
Age			
16-34	2,539 (22.31)	2,182 (22.58)	1,346 (24.03)
35-54	4,090 (35.94)	3,464 (35.85)	2,131 (38.05)
55 and over	4,751 (41.75)	4,017 (41.57)	2,124 (37.92)
Current Smoker	2,404 (21.12)	2,019 (20.89)	1,079 (19.26)
Non smoker	8,962 (78.85)	7,633 (79.08)	4,522 (80.74)
English Index of Deprivation	-	9,657 (100)	5,601 (100)
Deciles 1-5 (most deprived)	-	4,361 (45.16)	2,364 (42.21)
Deciles 6-10 (least deprived)	-	5296(54.84)	3,237 (57.79)

Table 3: Association of presence of visible plaque with mean annual sunshine hours/SHA of residence (N=5601)

Sunshine hours per SHA (n)	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE		
	OR	95% CI	p	OR	95% CI	p
149.14 (565)	1.00	-	-	1.00	-	-
149.87 (592)	1.80	1.34-2.41	<0.001	1.93	1.41-2.62	<0.001
150.78 (496)	0.91	0.69-1.21	0.527	0.95	0.71-1.26	0.710
161.77(485)	1.81	1.32-2.47	<0.001	1.87	1.35-2.61	<0.001
165.69 (706)	1.49	1.13-1.96	0.005	1.59	1.19-2.12	0.002
172.60 (646)	0.32	0.25-0.41	<0.001	0.36	0.27-0.47	<0.001
176.23 (658)	0.86	0.67-1.12	0.270	0.92	0.70-1.21	0.556
177.17 (606)	0.63	0.49-0.82	0.001	0.88	0.66-1.17	0.368
181.77 (400)	0.86	0.65-1.16	0.328	0.89	0.66-1.19	0.436
191.87 (447)	0.35	0.27-0.47	<0.001	0.40	0.29-0.54	<0.001
p for trend	0.89	0.86-0.91	<0.001	0.90	0.87-0.92	<0.001
Low	1.00	-	-	-	-	-
Medium	0.66	0.57-0.77	<0.001	0.69	0.59-0.80	<0.001
High	0.50	0.42-0.60	<0.001	0.56	0.47-0.66	<0.001

Table 4: Association of gingival bleeding in any sextant with mean annual sunshine hours/SHA of residence (n=5601)

Sunshine hours / SHA (n)	Model 1			Model 2 Adjusted for sex, age, smoking, IMDE			Model 3 Model 2 + Adjusted for plaque		
	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
149.14 (565)	1.00	-	-	1.00	-	-	1.00	-	-
149.87 (592)	0.65	0.50-0.84	0.001	0.65	0.50-0.84	0.001	0.54	0.41-0.71	<0.001
150.78 (496)	1.02	0.79-1.34	0.837	1.01	0.77-1.33	0.984	1.03	0.77-1.37	0.835
161.77(485)	0.99	0.76-1.30	0.963	1.00	0.76-1.32	0.978	0.86	0.64-1.14	0.291
165.69 (706)	0.94	0.73-1.21	0.629	0.97	0.75-1.25	0.801	0.85	0.66-1.11	0.231
172.60 (646)	0.29	0.23-0.38	<0.001	0.32	0.25-0.41	<0.001	0.40	0.30-0.53	<0.001
176.23 (658)	0.84	0.65-1.07	0.165	0.89	0.69-1.14	0.356	0.90	0.70-1,17	0.444
177.17 (606)	1.12	0.87-1.45	0.382	1.38	1.06-1.82	0.019	1.51	1.14-2.00	0.004
181.77 (400)	0.59	0.45-0.78	<0.001	0.59	0.45-0.78	<0.001	0.58	0.44-0.78	<0.001
191.87 (447)	0.69	0.52-0.91	0.008	0.78	0.59-1.03	0.077	1.03	0.77-1.37	0.849
p for trend	0.97	0.95-0.99	0.009	0.98	0.96-1.01	0.125	1.01	0.99-1.04	0.300
Low (1653)	1.00	-	-	1.00	-	-	1.00	-	-
Medium (2495)	0.82	0.71-1.03	0.004	0.86	0.76-0.99	0.035	0.94	0.81-1.09	0.42
High (1453)	0.88	1.17-1.46	0.1222	0.96	0.81-1.14	0.651	1.14	0.95-1.37	0.15



. Figure 1: Map showing interpolated 5 km grids of mean June sunshine hours 2010-2014 overlaid with SHA boundaries

Appendix 8: Dissemination of Findings: Draft paper 2.

Association of dental caries with sunshine exposure in participants of The Children's Dental Health Survey 2013 and The Oral Health Survey of Five-year-old Children 2014-15

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Key words: Caries, vitamin D, solar irradiation, UVB,

Objective: To investigate the relationships between sunshine hours and prevalence of dental caries in children in England, in the primary dentition.

Basic research design: Oral health outcome measures were taken from the Child Dental Health Survey (CDHS) 2013 (individual data) and National Dental Epidemiology Programme Oral Health Survey of Five-year old Children (NDEP OHS, aggregated area data) 2015. Sunshine hours for relevant geographical regions were obtained from Met Office data via the Medical & Environmental Data Mash-up Infrastructure Project dataset (MEDMI). Regression models were used to investigate the relationships between sunshine hours and measures of experience of caries.

Results: In fully adjusted models using data from the CDHS on the primary dentition, comparing children residing in the highest and lowest sunshine regions, there was a 35% (OR 0.65, CI 0.44-0.94, p=0.024) reduction in the number of children with any decay experience, excluding visual caries. Marginal statistical evidence of a protective trend was observed when any decay experience, including visual caries, was considered (OR 0.67 CI 0.43-1.05 p=0.082). Analysis of the OHS data suggested the difference between the lowest and highest hours of sunshine/local authority was associated with a decrease in mean decayed, missing, filled teeth of -0.50 (Coef -0.50, CI -0.68, -0.33, p<0.001)

Conclusion: A small, but consistent, reduction in cavitated caries experience in primary teeth was associated with living in an area with higher mean sunshine hours.

Introduction

In recent times the importance of vitamin D sufficiency has been revisited frequently, and whilst its importance in maintaining systemic health is established, its role in maintaining oral health, with particular reference to protecting against dental caries in children, remains disputed. Vitamin D is a secosteroid required by the body for calcium and phosphate metabolism. It can be obtained from dietary sources, but more commonly in UK populations it is produced endogenously in the skin following sunlight exposure (Webb *et al.*, 2010). Vitamin D deficiency in the UK, as defined by the UK Scientific Advisory Committee on Nutrition, is the approximate level indicative of increased risk of poor musculoskeletal health with serum concentrations of less than 25 nmol/L (10 ng/mL) [2]. The proportion of UK children with 25(OH)D concentration < 25 nmol/L(10 ng/mL) in the winter (October to late March) has been reported as 31%, in children (4-10 years) and 40% in adolescents (11-18 years), decreasing to 2% and 13% in the summer respectively (SACN, 2016). The occurrence of dental caries in children remains a major health problem throughout the world with the WHO recognising a burden of oral disease and untreated caries globally (Marcenes *et al.*, 2013). In the UK approximately 31% of 5 year olds have untreated tooth decay and 46% of 15 year olds have obvious decay experience (Office for National Statistics, (2015).A diagnosis of dental caries is the most common cause of admission to UK hospitals for those aged under 19 each year and in 2013-14 this number was approximately 46,500 (*RCS, England*, 2015).

Vitamin D supplementation has been associated with decreased incidence of dental caries in children since early in the 20th century. May Mellanby, among others, published extensively on a role for vitamin D rich diets, supplements, or UV light exposure, in the prevention of childhood caries (Mellanby and Pattison, 1928; Mellanby and Pattison, 1932; Mellanby, Pattison and Proud, 1924; McBeath, 1934). In recent years, the relationship between vitamin D and dental caries has been revisited, with one recent systematic review concluding that supplemental vitamin D reduced the risk of developing dental caries in children, pooled relative-rate estimate from 24 trials of 0.53 (95% CI, 0.43–0.65) (Hujoel, 2013). More recently, whilst higher vitamin D status in children has been found to be associated with reduced caries experience in some studies (Schroth *et al.*, 2016; Gyll *et al.*, 2018), others have not found this not to be the case (Herzog *et al.*, 2016).

Numerous mechanisms are proposed via which vitamin D may decrease the prevalence of childhood caries. Firstly, in the pre eruptive development stages of both the primary and permanent dentitions, vitamin D optimises mineral metabolism during enamel formation and maturation (Papagerakis, MacDougall and Berdal, 2002; Onishi *et al.*, 2008), potentially providing protection against future decay. Post eruption, the supersaturation of saliva with calcium and phosphate provides further caries protection, via remineralisation of tooth enamel subject to acids produced by plaque bacteria (Singh *et al.*, 2015). Vitamin D deficiency has also been associated with reduced parotid gland function, reduced saliva production and saliva calcium content (Glijer, Peterfy and Tenenhouse, 1985). Vitamin D sufficiency leads to increased production of salivary antibacterial peptides (AMP) such as cathelicidins, and salivary IgA, (He *et al.*, 2016), which increased protection from the caries associated bacteria *Streptococcus mutans* (Rose *et al.*, 1994).

No recent studies have considered the relationship between sunlight exposure, as a key determinant of vitamin D status, and dental caries in children. This study aimed to investigate the relationships between sunshine hours in area of residence and prevalence of dental caries in children in England, in the primary dentition.

Methods

Participants

Secondary data for this study were obtained from two national oral health surveys. The Child Dental Health Survey (CDHS) 2013 for 5, 8, 12 and 15 year olds in England, Wales and Northern Ireland, and the National Dental Epidemiology Programme for England, Oral Health Survey of Five-year-old Children (NDEP OHS), 2015. In England 71% of primary schools and 40% of secondary schools selected took part in the CDHS survey. Data available from the CDHS included individual level oral health data, and the area of residence was reported at Government Office Region (GOR) level (nine administrative regions of England)(Office for National Statistics, 2015). The National Dental Epidemiology Programme Oral Health Survey of Five-year-old Children (NDEP OHS) 2015 was a cross-sectional epidemiological survey, which aimed to measure the prevalence and severity of dental caries (Pine, Pitts and Nugent, 1997a; Pine, Pitts and Nugent, 1997b; Pitts, Evans and Pine, 1997). Although individual level data were collected, only data aggregated at lower tier local authority level were released. The survey population was defined as all five year old children attending state-funded primary schools within the local authority, and the survey covered 324 out of 326 lower-tier local authorities (LA). The data for both surveys were collected by trained and calibrated clinicians.

Methodology

To estimate sunshine hours, data were obtained by the author from The Medical and Environmental Data Mash-up Infrastructure Project (MEDMI) which compiles Met Office data from meteorological stations. Daily bright sunshine hours, as recorded using a Campbell-Stokes meter (Stanhill, 2003), were available from 78 stations around the UK. The raw daily data from each of the stations were selected and aggregated into monthly total bright sunshine hours per month, where data were available for every day in the month. The data were averaged by calendar month and station across a five year time period, 2010-2014 and then interpolated to create a 5 km grid of estimates of mean monthly hours of sunshine using ArcGIS (ArcGIS, 2016). The gridded data were allocated to geographical boundary data as required for each analysis, GOR in the case of CDHS and LA for NDEP OHS. The five-year monthly mean hours of sunshine for each geographical area of interest over 2010-2014 was then summarised to a five-year monthly mean for the months April to October. Using this method, the monthly mean sunshine hours per GOR ranged from 149.0 to 184.2 over the nine regions, whilst for the LAs it ranged from 133.5 to 208.7 hours over the 324 smaller LA regions (Figure 1).

The outcome measures for oral health from the CDHS 2013 were obtained from the original dataset (Office for National Statistics, 2015). Primary outcome measures were the presence of any clinical decay experience (CDE) for primary teeth; given this focus on primary teeth we considered participants aged 5 and 8 only. The CDHS 2013 technical document defines any CDE as "*evidence of tooth decay in the enamel,*

dentine or pulpal layers of the crown of the tooth. All teeth with cavitated or visual dentine caries, restorations with cavitated or visual dentine caries, teeth with filled decay (otherwise sound), teeth extracted due to caries and teeth with visual or cavitated enamel caries would be included"[27]. Due to the wide ranging definition of decay the analysis was stratified by two outcome measures, CDE excluding visual caries and CDE including visual lesions, with visual caries defined as caries suspected on visual inspection but enamel not yet cavitated. Individuals with missing data were excluded. Logistic regression using survey sampling weights were completed for the crude model (CDE regressed on sunshine hours only), after which adjustment was applied for sex, age, area deprivation (the Index of Multiple Deprivation for England (IMDE) quintile) and presence/absence of plaque. For the NDEP OHS 2015, the aggregate outcome measures used by the authors for analysis were the mean dmft per LA and mean dmft amongst children with dmft >0. In this survey, only decay reaching dentine, or decay with signs of previous decay reaching dentine, were considered a carious lesion. All lesser extents of demineralisation, for example enamel white spots, were classified as sound i.e. not decayed. The participants for this survey were five years old so again only deciduous teeth were considered. These variables were chosen as for both surveys they provided evidence of past and present decay experience.

Results

The unweighted demographics of the CDHS population are described in Table 1. In total 1,526 five-year-olds and 1,369 eight-year-olds participated in an examination. (*PHE,* 2015). In the crude model using individual-level CDHS data, for every one extra hour of sunshine per month (April-October) there was a reduction in the prevalence of CDE in primary teeth of 1% (Odds Ratio, OR 0.99 CI 0.98-1.00 p=0.019). The result persisted with the addition of potential confounders, initially sex, age and index of deprivation, and also plaque in the full model (OR 0.99 CI 0.98-1.00 p=0.024) as shown in table 2. With CDE with visual caries lesions as the outcome, the result was similar but was of marginal statistical significance (Table 3, OR 0.99 CI 0.97-1.00 p=0.083 and 0.99 CI 0.98-1.00 p=0.061 respectively). Scaling these coefficients to compare the highest and lowest sunshine regions (36 hours difference), suggested a moderate protective potential effect of 35% (OR 0.65, CI 0.44-0.94, p=0.024) for any CDE, and similarly 33% when CDE with visual lesions were included (OR 0.67 CI 0.43-1.05 p=0.082).

In total, 111,500 five year old children underwent clinical examinations in the OHS survey, representing 95.7% of the consented sample and 16.5% of the population of this age cohort attending mainstream state schools (Table 2). For the NDEP OHS 2015, the crude linear regression model included only sunshine hours and the oral health outcomes of interest. The second model adjusted for fluoridation status for each LA (PHE, 2014) and area index of deprivation (Smith, 2015). The percentage of children with substantial plaque was added in to the third and final model. All the outcome measures were published as weighted prevalence data, so no sample weighting factors were applied.

In the crude model, considering sunshine hours only, there was a reduction in mean dmft of approximately 0.01 for each one hour increase in sunshine (β -0.009, CI -0.013, -0.007, p<0.001). This relationship attenuated, but persisted, when fluoride and IMD

were added to the model (β -0.007, CI -0.009, -0.004, p<0.001). The final addition of the prevalence of substantial plaque deposits to the model did not alter the relationship, suggesting that this was not acting as a potential confounder or mediator. When the adjusted coefficient was scaled, over the range from the lowest to highest hours of sunshine per LA, 75 hours difference, this represented a potential decrease in mean dmft of 0.50 (β -0.50, CI -0.68, -0.33, p<0.001) (Table 5).

Considering mean dmft amongst those children with dmft>0, the crude model demonstrated an hourly increase in mean sunshine hours associated with a similar reduction in mean dmft of -0.011 (CI -0.016, -0.006, p<0.001). This persisted in the fully adjusted model with a decrease in mean dmft of -0.007 (-0.011, -0.003, p<0.002) per hourly increase.

Discussion

Our findings across both datasets indicate that a moderate decrease in caries prevalence is associated with higher bright sunshine hours. After adjustment for potential confounders, in the CDHS each increase in sunshine hours was associated with a 1% decrease in odds of caries, and in the NDEP OHS a decrease in population mean dmft of 0.01.The reduction in caries, when considered across the full range between maximum and minimum sunshine hours, thus has the potential to be clinically important, with a potential decrease in population mean dmft of 0.5 and CDE prevalence of up to 35% in primary teeth of children from the sunniest areas.

This study therefore suggests that children living in areas with increased sunshine hours, where vitamin D levels would consequently be higher, have a decreased risk of caries in primary teeth. As both sunshine exposure and vitamin D are modifiable risk factors, these findings could inform novel public health interventions.

Each study had its own strengths and limitations. The CDHS provided individual level oral health data, but the areas used to assign sunshine exposure (GOR) were large. Conversely, the NDEP OHS 2015 had greater geographical sensitivity, but presented aggregate oral health outcomes at LA level only. The inclusion of NDEP OHS data with smaller geographical areas allowed for increased sensitivity of the interpolation technique used to allocate sunshine data from meteorological stations to area boundaries, and this was demonstrated in the larger range between the highest and lowest mean sunshine hours compared to GOR-level analysis. Undertaking analysis of two independent datasets with different approaches allowed for cross-checking of the apparent protective trend.

Both studies had limitations typical of this type of environmental exposure design. Standard limitations of ecological studies apply, for example allocating sunshine exposure based on geographical area of residence is a potential source of error, given the important role of individual behaviours and other characteristics in determining sun exposure. The data are cross-sectional, and we assume that estimates based on the 2010-14 sunshine data are a reasonable proxy for exposure prior to development of outcomes. As there is no measure of plasma vitamin D, the results depend on the assumption that reduced sunshine exposure at area level leads to reduced vitamin D status of all individuals in that area. It is also noted that adjustment for confounding in ecological studies can be less accurate due to the data being at population rather than

individual level. Residual confounding will remain and cannot be excluded as an explanation for the protective effect observed.

Finally, there are limitations in the primary data in collection and interpretation. The oral health surveys are conducted in non-clinical environments which can hinder caries diagnosis by visual assessment alone. Furthermore, the definition of what constitutes caries can include wide ranging decay experiences leading to inaccurate assessment and recording of caries present, although this is somewhat addressed through standardised approaches, examiner training and calibration.

When comparing with previous studies, although the use of ecological studies to identify environmental factors influencing oral health is well established, the prime example being water fluoridation (Schluter *et al.*, 2020), the authors are not aware of any other recent publications that have analysed correlations between sunshine exposure and prevalence of decay experience. Similar to our findings, one much older study has reported decreases in the number of carious lesions occurring in sunnier seasons (Erpf, 1938). Others have reported that children develop fewer new carious lesions if they receive topical skin exposure to UVB light (McBeath, 1934; Schoenthal and Brodsky, 1933), or if their classrooms have full spectrum light, which includes vitamin D inducing UVB (Hargreaves and Thompson, 1989; Hathaway, 1995; Mayron *et al.*, 1975). However, these older studies lacked robust methodologies or statistical analysis and their findings cannot be extrapolated to recent generations due to the numerous changes in society and healthcare. This paper is therefore the first ecological study in the 21st century to address these issues, however further work using individual level, longitudinal data, with improved exposure assessment and confirmation of vitamin D as mediator, is required to investigate the proposed associations further.

Conclusion

In conclusion a small, consistent reduction in the prevalence of decay experience (CDE or dmft) in primary teeth of five- and eight-year-olds was associated with living in an area with increased mean sunshine hours, even after consideration of confounding factors and comparable analysis of two different data sets.

Supplemental Material- none

Acknowledgements

Sunshine data were provided via the MEDMI project: U.K. Medical Research Council (MRC) and the Natural Environment Council (NERC) for the MEDMI Project (MR/K019341/1); thanks to Christophe Sarran at the Met Office for their support. We also acknowledge support for funding this project from the NIHR, as part of a clinical fellowship from the Peninsula Dental School, University of Plymouth.
Jane Collingwood was funded by a National Institute for Health Research NIHR ACF in Primary Dental Care for this research project

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Table 1: Demographics of the CDHS population

		England Sample (N %)	Age 5	Age 8
Number of children		5,642 (100)	1,526 (100)	1,369 (100)
Male		2,736 (48.49)	763 (50.00)	661 (48.28)
Female		2,906 (51.51)	763 (50.00)	708 (51.72)
Region	Mean Sunshine Hours 2010-14 April-October			
North East	149.0	692	162	177
North West	150.0	855	198	191
Yorkshire and Humber	150.4	594	169	137
West Midlands	161.6	591	171	154
East Midlands	165.4	488	147	142
East of England	172.5	520	137	126
South West	176.2	583	158	136
London	181.5	685	176	140
South East	184.2	634	208	166
IMDE 1 (most deprived)		2,214 (39.24)	591 (38.72)	495 (36.16)
IMDE 2		1,073 (19.02)	283 (18.55)	286 (20.89)

IMDE 3	756 (13.40)	236 (15.47)		194 (14.17)
IMDE 4	724 (12.83)	184 (12.06)		175 (12.78)
IMDE 5 (least deprived)	649 (11.50)	173 (11.34)		163 (11.91)
Missing data	226 (4.01)	59 (3.87)		56 (4.09)
Smoking				
Current smoker	155 (2.75)	N/A		N/A
Non Smoker	2,544 (45.09)	-		-
Missing data	48 (0.85)	-		-
N/A (age specific question)	2,895 (51.31)	-		-
Plaque				
Plaque visible	3,433 (60.85)	732 (47.97)		1000 (73.04)
No Plaque	2,196 (38.92)	789 (51.70)		365 (26.66)
Missing Data	13 (0.23)	5 (0.33)		4 (0.29)
	England Sample (N %)	Age 5		Age 8
Number of children	5,642 (100)	1,526 (100)		1,369 (100)
Any clinical decay experience in primary teeth (*excl visual caries)	Yes	1,413 (25.04)	563 (36.89)	674 (49.23)

	No	4,229 (74.96)	963 (63.11)	695 (50.77)
Any clinical decay experience in primary teeth (*incl. visual caries)	Yes	1,787 (31.67)	768 (50.33)	827 (60.41)
	No	3,855 (68.33)	758 (49.67)	542 (39.59)

Table 2: Results of logistic regression models of CDHS data: presence of any clinical decay experience in primary teeth (excluding visual caries)

N=5403		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		0.99	0.98-1.00	0.019	0.99	0.98-1.00	0.022	0.99	0.98-1.00	0.024
Sex M		-	-	-	1.00			1.00		
F					0.95	0.79-1.14	0.559	0.97	0.80-1.17	0.757
Age 5yrs		-	-	-	1.00	-	-	1.00	-	-
8yrs					1.75	1.45-2.12	0.00	1.46	1.21-1.75	<0.001
IMD Quintile										
1 (Most deprived)		-	-	-	1.00	-	-	1.00	-	-
2					0.82	0.62-1.07	0.144	0.84	0.64-1.10	0.211
3					0.54	0.38-0.77	0.001	0.56	0.41-0.78	0.001
4					0.42	0.28-0.61	<0.001	0.42	0.29-0.62	<0.001
5 (Least deprived)					0.36	0.25-0.53	<0.001	0.35	0.24-0.53	<0.001
Current Plaque:	Yes	-	-	-	-	-	-	1.00	-	-
	No							0.45	0.36-0.58	<0.001

Table 3: Results of logistic regression models for CDHS data: presence of any clinical decay experience in primary teeth (including visual lesions)

N=5403		Model 1			Model 2 Adjusted for sex, age, IMDE			Model 3 Model 2 + Adjusted for plaque		
		OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Sunshine Hours		1.00 0.99	- 0.97-1.00	- 0.083	1.00 0.99	- 0.98-1.00	- 0.096	1.00 0.99	- 0.98-1.00	- 0.061
Sex M F		-	-	-	1.00 0.88	 0.71-1.10	 0.263	1.00 0.90	- 0.71-1.14	- 0.369
Age 5yrs 8yrs		-	-	-	1.00 1.47	- 1.22-1.78	- <0.001	1.00 1.18	- 0.98-1.42	- 0.056
IMD Quintile 1 (Most deprived) 2 3 4 5 (Least deprived)		 - 	 - 	 - 	 1.00 0.77 0.55 0.48 0.46	 - 0.58-1.04 0.38-0.80 0.32-0.71 0.29--0.71	 - 0.083 0.002 <0.001 0.001	 1.00 0.80 0.57 0.48 0.46	 - 0.60-1.07 0.40-0.80 0.32-0.72 0.28-0.70	 - 0.134 0.002 0.001 0.001
Current Plaque:	Yes No	-	-	-	-	-	-	1.00 0.40	- 0.30-0.53	- <0.001

Table 4: Summary of Demographics of Oral Health Survey of Five-Year Old Children England 2015

Variable	Mean per LA	Std. Dev.	Min	Max	Inter-Quartile Range	Number of LAs
Number of children examined in each LA	344.1	354.8	0	3156	203.5-337.5	324
Percentage with DMFT=0	76.3	8.1	43.9	91.8	71.3-81.9	321
Percentage with DMFT >0	23.7	8.1	8.2	55.7	18.1-28.7	321
Percentage with substantial plaque	1.8	3.4	0	28.4	0-1.7	321
IMD	19.5	8.0	5.0	42.0	12.9-25.3	324
Mean DMFT overall	0.8	0.4	0.1	2.5	0.5-1	321
Mean DMFT in those with decay	3.2	0.6	1.2	5.3	2.8-3.6	321
Mean Monthly Hours of Sunshine April-October 2010-2014	170.2	13.8	133.5	208.7	158.0-179.3	324

Table 5 Linear Regression of Mean dmft and Mean Monthly Hours of Sunshine April-October 2010-2014 : Oral Health Survey of Five-Year Old Children England 2015

	Model 1 Sunshine hours only			Model 2 Model 1 plus Fluoride			Model 3 Model 2 plus IMD			Model 4 Model 3 plus plaque		
	Coef.	P	CI	Coef	P	CI	Coef	P	CI	Coef	P	CI
Mean monthly hours of sunshine Apr-Oct	-0.010	<0.001	-0.012, -0.007	-0.011	<0.001	-0.136, -0.078	-0.007	<0.001	-0.009, -0.004	-0.007	<0.001	-0.009, -0.004
Fluoride				-0.254	0.002	-0.410, -0.097	-0.203	0.002	-0.328, -0.077	-0.182	0.004	-0.306, -0.059
IMD average							0.027	<0.001	0.023, 0.031	0.028	<0.001	0.024, 0.320
Plaque										0.016	<0.001	0.007, 0.025

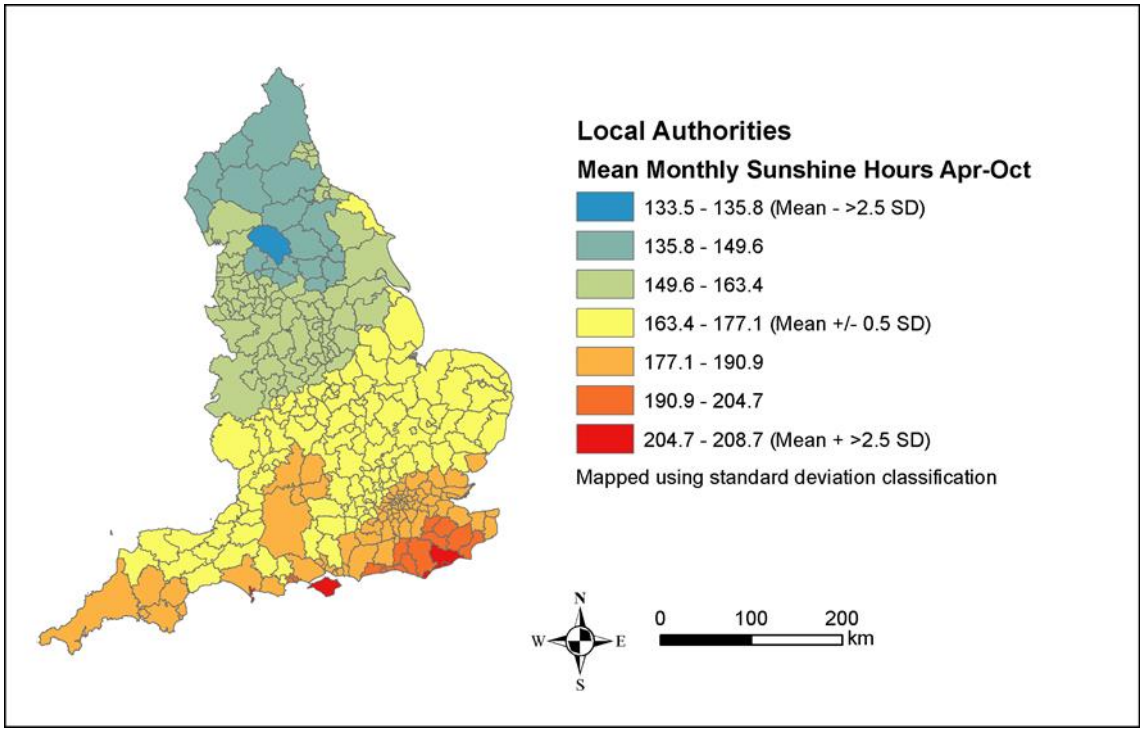

Figure 1: Variation of mean monthly sunshine hours per LA from Apr-Oct averaged between 2010 - 2014

Glossary

1-alpha-hydroxylase: Enzyme in the liver which converts 25(OH) D to 1, 25 (OH) 2D

25-hydroxylase-CYP2R1: The enzyme in the liver which converts vitamin D to 25 (OH) D

Calcitriol: another name for 1, 25 (OH) 2D

Cathelicidins: A group of antimicrobial peptides stored in the secretory granules of neutrophils and macrophages, released extracellularly upon leukocyte activation.

Caries: Tooth decay

Cholecalciferol: Vitamin D3, the form derived from cholesterol in plants

CYP27B1: the enzyme which regulates the level of biologically active vitamin D

Primary Teeth/ Dentition: The first set of teeth to erupt in children, which exfoliate naturally and are replaced by the permanent dentition.

Defensins: Antimicrobial proteins with a role in the innate immune system

Ergocalciferol: Vitamin D2, the form derived from ergosterol in plants

Half-life: Time it takes for half the initial amount to be metabolised.

Latitude: Often written as degrees of latitude. $X^{O\cdot}$ Distance from the equator.

Melanin: Melanin is a dark skin pigment occurring in both humans and animals which may help protect the skin from UV rays.

Metabolite: Intermediate molecule formed along the metabolism pathway

Permanent or Secondary Dentition: The second set of teeth to erupt, providing the permanent lifelong dentition which does not exfoliate

Pleiotropic: having more than one effect

Previtamin D: The precursor to the formation of vitamin D

Rickets: Rickets is a bone condition in children caused by lack of vitamin D and calcium. It can lead to softening of the bones and bone deformities.

Serum: the clear yellowish fluid that remains from blood plasma after clotting factors (such as fibrinogen and prothrombin) have been removed by clot formation.

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