An investigation into the effects of vitamin D supplementation during pregnancy and infancy, on early childhood dental health in New Zealand

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Abstract

Study Purpose: To investigate the effects of early-life vitamin D supplementation on the dental health of New Zealand (NZ) children.

Background: NZ has a high caries burden, where dental disparities exist from a very young age. Children of Māori and Pacific ethnicity have a higher risk of developing early childhood caries (ECC) compared to NZ European children, as do children living in socioeconomically deprived households. Vitamin D deficiency (VDD) has been linked with ECC, particularly if it is present during tooth developmental periods. VDD is prevalent in pregnant NZ women and young Māori and Pacific children.

Aim: To assess the effects of pregnancy and infancy vitamin D supplementation on ECC and developmental dental defects.

Hypothesis: Pregnancy and infancy vitamin D supplementation protects the primary dentition against ECC and enamel developmental defects.

Objectives: To compare the dental health of preschool children, who received one of two vitamin D supplementation doses or placebo, during pregnancy and infancy.

Methodology: Participants were recruited from the Pregnancy and Infancy Vitamin D (PIVID) study, where 260 pregnant women and their infants were randomly assigned to one of three treatment groups. Vitamin D or placebo supplementation was given to pregnant women from 28 weeks gestation until delivery, and their infants from birth to age six months. The three supplementation groups comprised of: higher dose vitamin D, lower dose vitamin D (mothers: 2000/1000 UI, children: 800/400 IU per day, respectively), and placebo (Grant et al., 2014). The study sample comprised of approximately 30% Māori and 50% Pacific children.

Children from the PIVID study were dentally examined at ages two, three and four. Data were collected on dental outcomes, demographics, and maternal and infant dental risk factors.

Descriptive statistics and regression models were used to compare the effects of vitamin D supplementation on childhood dental health outcomes. These were: the presence of

all 20 primary teeth, decayed missing and filled teeth (dmft), enamel developmental defects, dental plaque present and plaque and gingival indices.

Results: This observational study followed a cohort of children previously enrolled in the PIVID randomised control trial (RCT). This study was not powered to be able to show statistical significance for clinically important differences in oral health outcomes between study groups. Children in the higher dose vitamin D group had odds of 3.16 (95% CI 0.90-14.78, p = 0.096) of having all 20 primary teeth present at age two, compared to placebo, and odds of 0.39 (95% CI 0.13-1.11, p = 0.081) of having decayed teeth at three years old.

Children in the higher dose vitamin D group had increased odds of dental plaque present on teeth, at ages two (OR = 7.29, 95% CI 1.87-48.52, p = 0.012) and three (OR = 4.20, 95% CI 1.27-19.79, p = 0.032), compared with placebo.

Additional risk factors associated with increased odds for ECC were irregular dental visits, ethnicity, and a higher intake of sugar snacks.

Conclusion: In NZ, where ECC and vitamin D deficiency are prevalent, vitamin D supplementation in pregnancy and early infancy may be beneficial in protecting the primary dentition against ECC.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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"Acknowledging the good that you already have in your life, is the foundation for all abundance"

-Eckhart Tolle

Abbreviations

25(OH)D	25-hydroxyvitamin D
AAP	American Academy of Pediatrics
AAPD	American Academy of Pediatric Dentistry
ADA	American Dental Association
AMA	American Medical Association
AMPs	Antimicrobial peptides
ANUG	Acute necrotising ulcerative gingivitis
AUTEC	Auckland University of Technology Ethics Committee
BPE	Basic Periodontal Examination
CAMBRA	Caries management by risk assessment
CPP-ACP	Casein phosphopeptide-amorphous calcium phosphate
CPTIN	Community of Periodontal Index of Treatment Needs
CRA	Caries risk assessment
DNA	Deoxyribonucleic acid
DDE	Developmental defects of enamel
dmft	Decayed, missing, and filled teeth
deft	Decayed, extracted, filled teeth
dmfts	Decayed, missing, and filled tooth surfaces
ECC	Early childhood caries
FDA	Food and drug administration
Ftt	Failure to thrive
FiCTION	Filling children's teeth indicated or not
EHP	Enamel hypoplasia
G	Grams
GA	General anaesthesia
GIC	Glass ionomer cement
GBD	Global burden of disease
GP	General practitioner
HDEC	Health and Disability Ethics Committee
ID	Identification

IQs	Intelligence quotients
IU	International units
L	Litre
LED	Light emitting diode
LL-37	Antimicrobial peptide from the Cathelicidin family
MFP	Sodium monofluorophosphate
MOH	Ministry of Health
MOMs	Maternal Organics Monitoring study
NaF	Sodium fluoride
Ng/mL	Nanograms per ml
NHMRC	National Health and Medical Research Council
Nmol/L	Nanomoles per Litre
NZ	New Zealand
NZACRes	New Zealand Association of Clinical Research
OH	Oral health
OHIP	Oral health impact profile
OHT’s	Oral health therapists
PMI's	Primary maxillary incisors
PIVID	Pregnancy and Infancy Vitamin D
Ppm	Parts per million
PTH	Parathyroid hormone
RCT's	Randomised control trials
QOL	Quality of life
S. mutans	*Streptococcus mutans*
S-ECC	Severe early childhood caries
SES	Socioeconomic status
SPF	Sun protective factor
SSC	Stainless-steel crown
SSCs	Stainless-steel crowns
UK	United Kingdom
US	United States
UV	Ultraviolet

UVR	Ultraviolet radiation
VDD	Vitamin D deficiency
VDRR	Vitamin D-resistant rickets
VDRs	Vitamin D receptors
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
WHO	World Health Organization
WSLs	White spot lesions
µg/day	Micrograms per day

Chapter 1 Introduction

Childhood dental caries remains the most widespread non-communicable chronic disease in New Zealand (NZ) and worldwide (Misra et al., 2007; Oral Health Collaborators, 2017), Twetman, 2018). According to the Global Burden of Disease (GBD) study it was reported that 573 million children were affected by untreated dental caries in their primary teeth (Righolt, 2018; Kassebaum et al., 2017). Despite the decline in dental caries over the past few decades, they still occur in 60-90% of children worldwide, aged between two and 11 years (Boyce et al., 2010; Marinho et al., 2013; Bernabe et al., 2020). As per the GBD study in 2010, untreated dental caries in primary teeth affected 9% of the world's child population and it was the tenth most prevalent of all health conditions (Marcenes, 2013). In 2015, globally there was a peak in untreated dental caries in primary teeth of children aged between one and four years old, with its prevalence being 7.8% by 2017, with 532 million children being affected (Kassebaum, 2017).

Before discussing the development of dental caries from a clinical and scientific perspective, a summary will be given on why childhood dental caries remains an issue in NZ and internationally.

1.1 A need for improved oral health in NZ children

Childhood dental caries is an important health issue in NZ that affects children of all ages (Bach & Manton, 2014). Despite dental caries being preventable, they are highly prevalent in early childhood. According to the Ministry of Health (MOH) 2010 Oral Health Survey, 49% of five to 11-year-old children in NZ experienced dental caries in their primary teeth (MOH, 2010). Early childhood caries (ECC) is more prevalent in Māori and Pacific children compared with children of other ethnic groups. This places them at higher risk of future dental caries. Furthermore, 40% of children aged two to four do not access dental services in NZ, with the proportion being larger for Māori and Pacific children (Curzon & Preston, 2004; Moffat et al., 2017).

Selwitz (2007) states that dental caries is "one of the most common preventable childhood diseases". Despite this, 7% of hospitalisations in NZ, from 2000 to 2009, were for children below 14 years old with preventable dental conditions (Craig, 2012;

Bach & Manton, 2014). In 2018-2019, 15% of hospital emergency admissions were for children aged below 14 years old, including those requiring dental care (Ministry of Health, 2019a). Further to this, a 20-year review of potentially preventable dental hospital admissions between 1990 and 2009, revealed that the highest rate of admissions were consistently for preschool children aged between three and four (Whyman, 2014). A NZ population-based study reported that potentially avoidable non-dental hospitalisations for children under six years old, such as those related to respiratory illnesses, were significantly associated with children having ECC (prior to adjusting for demographic variables) (Aung, 2019). In another recent nationwide study on ambulatory sensitive hospital admissions for preventable conditions in NZ, it was shown that up to 30% of all admissions for children below five years old, including dental conditions, were potentially avoidable. The study noted that oral health could be improved by water fluoridation, better housing, and improved access to healthcare services in NZ (Hobbs, 2019).

In the most recent NZ Oral Health (OH) Survey, 39% of children and adolescents aged between five and 17 years old, required invasive dental treatment, such as filling work or removal of teeth due to dental caries (MOH, 2010). Unfortunately, without effective management of this disease, its occurrence in childhood predicts its continuation into adulthood (Broadbent et al., 2006). Therefore, caries prevention strategies need to be introduced earlier, to limit and reduce the likelihood of children acquiring dental caries in their primary and permanent teeth.

1.2 Definition and development of dental caries

The word 'caries' is derived from a Latin word meaning rotten or decayed (Krol, 2003). Dental caries occur by a process of demineralisation; defined as the loss, deprivation or removal of minerals or mineral salts from the body, for instance, the loss of calcium from bones or teeth (Kidd, 2005). Tooth enamel is broken-down by acids, which are produced from the metabolism of sugars, by *Streptococci mutans (S. mutans)* and other acid producing bacteria (Fejerskov & Kidd, 2003; Kidd & Fejerskov, 2004).

Early childhood caries (ECC) is the term used for childhood dental caries that are developed on children's teeth in reference to the child's age (being below six years old) and influenced by specific child, family and community influences on oral health

(WHO, 2019a). ECC will be further discussed in the context of this study in chapter two, section 2.5.

The range and severity of ECC varies with genetic predisposition, socio-economic status and cultural differences within populations (Subramaniam & Prashanth, 2012). Higher rates of ECC have been recognized in ethnic minority groups, and infants with poor feeding habits or diets with high and/or frequent sugar consumption, which as a result can cause malnourishment in children (Mobley, 2009, Anil, 2017). Therefore, children from some ethnic groups or those from lower socioeconomic backgrounds may have higher rates of decayed, missing and filled teeth (dmft) than others.

1.2.1 Symptoms, management, treatment, and prevention strategies for ECC

The symptoms and consequences of childhood dental caries may include: pain, difficulty eating, nutritional deficiencies, disturbed sleep, cellulitis, fever and/or tooth necrosis (Çolak et al., 2013; Schroth et al., 2014). ECC can affect children's nutritional and growth status by affecting eating ability and therefore causing pain and distress, as well as influencing mental and emotional wellbeing (Sheiham, 2006). Non-physical effects can include poor social interaction and upset or anxious behaviour due to the physical and psychosocial impacts of the disease (Nunn et al., 2000).

The management of childhood dental caries has implications for not only children, their parents, caregivers, health services, providers, and the economy. Management of painful ECC often requires time away from school and other activities for children (Schroth et al., 2014) and it affects school performance and developmental progress (Gaynor & Thomson, 2012). Parents and caregivers may need to take time off work or other duties when their children require dental treatment (Petersen, 2003). Further, the financial costs of delivering acute and extensive dental services are significantly higher for health service providers, especially when general anaesthesia (GA) is required to provide restorative dental treatment to children who are younger, apprehensive, have special needs or are medically compromised (Krol, 2003).

Dental caries may require invasive restorative treatment such as fillings, pulpotomies, pulpectomies or the extraction of teeth (Fejerskov & Kidd, 2008). However, there are other non-conventional restorations for children who may not be able to cope with

those procedures. Examples include the Hall technique, which involves placing stainless steel crowns on teeth or atraumatic restorative treatment (ART) fillings (Rosenblatt, 2008; Carvalho et al., 2009). The restorations are undertaken by manual excavation of dental caries by hand instrumentation. Local anaesthesia is required to minimize pain or discomfort for dental procedures (Kidd, 2005). Regular recall appointments are necessary to examine existing dental fillings or temporary restorations, new dental caries/lesions, as well as monitor dietary and oral hygiene habits, so that once children are treated, they can live comfortably with their re-established dental health.

Preventative strategies for dental caries include reducing the quantity and frequency of intake of dietary sugars, good oral hygiene habits put into practice, the use of fluoride dentifrices or other modes of application, and protective fissure sealants or fissure protections, which protect teeth from bacterial invasion (American Academy of Pediatric Dentistry (AAPD), 2012).

1.3 A novel means of reducing ECC

This thesis investigated a novel approach to preventing dental caries in early childhood. Much of the focus to date has been on caries prevention and treatment strategies post tooth eruption. However, by this stage, not only have teeth erupted into the mouth but children have also acquired microflora. In earlier dental studies, where interventions have been undertaken to reduce the prevalence of ECC, the efforts have focused on improving oral hygiene, introducing earlier access of dental services for prevention strategies, oral health education, fluoride exposure, nutrition and reducing transmission of microflora within families (Health Promotion Agency, 2015). Infancy and early childhood are critical periods for tooth development, and it is where ECC is initiated. This PhD study investigated the effects of early life vitamin D supplementation on oral health and whether it was effective in preventing childhood dental caries. In the study within which this thesis was developed, study mothers and their infants were given two different doses of vitamin D supplementation during pregnancy and aged up to age six months old respectively, thus during periods of active tooth development.

It was first reported in the 1920s that a diet containing adequate vitamin D could prevent dental caries (Mellanby et al., 1924). Vitamin D is a prohormone that is recognised to influence the formation of teeth by stimulating tooth calcification in utero (Watson, 2013). A lack of vitamin D during pregnancy is known to increase the risk of dental diseases and defective tooth enamel (Holick, 2007). Since tooth development begins in utero, this happens to be an important phase to consider when trying to prevent childhood dental caries.

Vitamin D is essential for tooth formation, where it facilitates the absorption of calcium and phosphorous required for the disposition of bones and teeth (Grant & Holick, 2005). Vitamin D acts on ameloblasts and odontoblasts during tooth development, the cells responsible for forming the first two layers of teeth (enamel and dentine), as well as oral bone (Murguía-Peniche, 2013). A deficiency of vitamin D during these phases is associated with rickets, enamel defects, periodontal disease and an increased risk for childhood dental caries (Schroth, Lavelle, et al., 2005; Holick, 2007). It is therefore essential for tooth development that the foetus and young infant have adequate amounts of vitamin D in the embryonic stages and initial stages of life during tooth development.

Vitamin D is obtainable in three different ways. Firstly, vitamin D_3 or cholecalciferol is primarily a fat-soluble vitamin produced in the skin from exposure to direct sunlight (ultraviolet [UV] B radiation). Skin exposure to sunlight is the principal mode in which human beings acquire adequate amounts of vitamin D. In most countries, including NZ, more than 90% of vitamin D is derived from direct sunlight exposure (Lips, 2006). Secondly, vitamin D can be consumed through the intake of certain foods or drinks, however, very few dietary sources naturally contain vitamin D. Some examples of vitamin D inclusive foods are: oily fish, egg yolk, and animal liver or fortified milk (Holick, 2012). There are also a few plants that produce vitamin D (in the form of vitamin D_2 or ergocalciferol), one example is a shiitake mushroom (Holick, 2007). Thirdly, vitamin D can be obtained from vitamin D supplements or via vitamin D fortified food or drink intake (Watson, 2013).

Vitamin D status is lower in NZ compared to many other developed countries (MOH & Cancer Society of NZ, 2012). Serum 25-hydroxyvitamin D (25(OH)D) is the biomarker

used to define vitamin D status. The average serum 25(OH)D concentration in adults in NZ is 20 nmol/L lower than in the United States (US) (MOH & Cancer Society of NZ, 2012). The reasons for this include our sun avoidance public health policy, the low vitamin content of the NZ diet and the limited use of vitamin D fortification and supplementation relative to North American and European countries (Moore et al., 2004).

Pregnant and lactating women are vulnerable to vitamin D deficiency (VDD) in NZ and internationally (Grant et al., 2009). In NZ, the prevalence of VDD is higher in pregnant NZ women and in young children (Ekeroma et al., 2014). The US Institute of Medicine define VDD as a serum 25(OH)D concentration <50 nmol/L. based upon this definition, VDD is present in 57% of new born children in NZ (Camargo et al., 2010).

Almost one fifth (19%) of NZ new born babies have a serum 25(OH)D concentration below <25 nmol/L, which is a concentration low enough to place the child at risk of rickets (Camargo et al., 2010). Such severe VDD during early childhood remains prevalent in NZ. It was reported that 10% of NZ infants aged six-to-23-months-old had serum 25(OH)D concentrations below <27.5 nmol/L. Further, Māori and Pacific children have poorer vitamin D status at birth and in early childhood, compared with NZ European children (Grant et al., 2009).

Over the past four decades, VDD has been reported to be associated with an increased risk for certain cancers (Grant & Garland, 2002), multiple sclerosis (Hayes et al., 1997) and diabetes mellitus (Hypponen et al., 2001 Jarveli & Virtanen, 2001). Other potential benefits of vitamin D in disease prevention and preservation of health have also been reported (Grant & Holick, 2005). The Auckland NZ based Pregnancy and Infancy Vitamin D (PIVID) study, showed that vitamin D supplementation during pregnancy and infancy prevents aeroallergen sensitisation and primary general practitioner (GP) visits for acute respiratory infections, in children as measured when they were 18 months old (Grant et al., 2015; Grant et al., 2016).

Deficient vitamin D status of pregnant women can affect the calcification of their children’s teeth prior to birth, thereby putting the children at higher risk of acquiring dental defects post-birth and increasing the risk of ECC (Schroth et al., 2014).

Vitamin D is known to stimulate the calcification of tooth tissues (Mellanby & Pattison, 1928; Schroth et al., 2014). Therefore, the rationale behind this PhD study is that vitamin D supplements taken during tooth development periods may provide dental benefits.

The overarching hypothesis for this PhD study is that vitamin D prevents childhood dental caries and developmental dental defects.

1.4 PhD study aim

The aim of this PhD study was to determine if vitamin D supplementation during pregnancy and early infancy has a beneficial effect on dental health in early childhood.

The primary aim was to investigate, through this longitudinal, randomised placebo-controlled trial, the effects of higher and lower dose pre-natal and early-life vitamin D supplementation, on early childhood dental health compared with placebo. It is well known that other factors influence oral health status. These include diet, the amount of sugar intake, oral hygiene habits, oral microbiome profile from the child's dental plaque, access to dental services and maternal oral health. All the above factors contribute to the risk of dental caries and were all considered in conjunction with vitamin D supplementation.

The specific research objective of this study was to assess the effects of early life vitamin D supplementation on early childhood dental health.

1.5 Thesis hypothesis

Perinatal and early childhood vitamin D supplementation protects the primary dentition against developmental dental defects and childhood dental caries.

The specific research questions of this study are:

1.5.1 Primary research question:

What were the effects of vitamin D supplementation during pregnancy and infancy on early childhood dental health?

1.5.2 Secondary research questions were:

A. What were the effects of other potential risk factors on dental caries in this study?
B. Were the mother's oral health statuses in all three study groups equal?

In this quantitative prospective longitudinal study, the three following methods were used to assess the dental health of the children enrolled in the PIVID study, who were aged between two and four years:

1. A clinical dental examination of each child.
2. Self-reported study questionnaires completed by mothers on their child's oral hygiene practices, dietary habits, and visits to the dentist.
3. A clinically validated self-reporting oral health impact profile (OHIP) questionnaire completed by mothers on their own oral health habits and use of dental services for their personal dental health.

1.6 Significance of this oral health study

The NZ Ministry of Health (2010) Oral Health survey in 2010, reported that NZ is a relatively high-caries population, and that dental caries remain one of the most prevalent chronic diseases in adults and children (MOH, 2010). Disparities in dental health become apparent from early life between higher and lower socioeconomic groups and are detectable from two years of age. Moreover, Māori and Pacific children and children living in socioeconomically deprived households, have poorer oral health than the rest of the NZ population.

The study population in which this hypothesis was tested was an ethnically diverse group of individuals, who had generally come from a more socially deprived region of Auckland, NZ. In this study, it is considered that the possibility that poor vitamin D status before and after birth may be a factor in increasing the odds of childhood dental caries and developmental dental defects in children.

NZ provides the ideal setting to conduct research into the dental health effects of vitamin D, as there are limited sources of vitamin D (such as supplementation and food fortification) other than sunlight. NZ public policy with respect to sunlight, promotes sun avoidance due to high rates of skin cancer. Individuals' sunlight exposure shows

wide seasonal variation, and the population is ethnically diverse with a wide range of skin pigmentation.

A systematic review and meta-analysis of 24 clinical trials with 2,827 children, reported an overall risk reduction by almost half (47%), where vitamin D supplementation was given to prevent dental caries (Hujoel, 2013). The mean age of the children enrolled in these studies, was approximately 10 years old however, and all the clinical trials of vitamin D supplementation (as opposed to UV light) predated modern clinical trial designs (Hujoel, 2013). An observational study by (Schroth et al., 2014), reported a positive relationship between vitamin D statuses of pregnant women during the second and third trimesters, with the dental health of their infants at the age of 12 months.

This is the first robustly designed study, to prospectively assess the effects of pre-natal and early life vitamin D supplementation on early childhood dental caries and developmental dental defects in NZ children.

1.7 Structure of thesis

Chapter two presents a literature review pertaining to ECC, reviewing the protective and disease-causing risk factors required for ECC and its developmental stages. Chapter three presents a literature review that describes the relationship between vitamin D status and childhood dental health. Chapter four describes the study design and methodology of this PhD research. The study results are reported in chapter five, followed by a discussion of the study strengths and limitations in chapter six, in which the wider implications of this research will be discussed as well. Chapter seven presents the study conclusions drawn from the study findings and placed in the context of the literature reviews.

Chapter 2 Literature review of childhood dental caries

2.1 Preamble

The literature review for this study topic is presented over two chapters. The purpose of these two chapters is to review the existing evidence from published literature on ECC and the role of vitamin D in the prevention of childhood dental caries.

Childhood dental caries is an important and preventable disease. In this chapter an explanation of the definition of childhood dental caries will be given, with a description of the clinical development of dental caries, including the disease aetiology and pathophysiology. The types of dental caries that occur in childhood will be outlined, with a description of their presentation and risk factors. The prevalence of dental caries in NZ children is then summarised and comparisons made to the global prevalence and severity of childhood dental caries.

Chapter three provides a description of vitamin D and its association with childhood dental caries. A review of the available evidence in relation to the association between vitamin D and childhood dental caries is presented.

2.2 Introduction

Initially, the background on ECC is explored, with a focus on its underlying causes, the diverse types of childhood dental caries and the potential associated risk factors. This facilitates an understanding of current intervention strategies that seek to prevent ECC. Current prevention and management options for childhood dental caries will then be reviewed. The prevalence of childhood dental caries globally will be hereafter outlined, followed by a summary on the knowledge of the current dental caries burden experienced by NZ children.

This literature review of childhood dental caries includes information from primary sources and reviewed articles, published in peer review literature and from textbooks. Published literature relevant to this chapter was identified using search databases, for instance: “Medline”, “PubMed”, “EBSCO”, “Web of Science”, “Science Direct” and “Google Scholar”. The reference lists of some selected articles were also reviewed, and

relevant cited articles were studied. The key words used to search the literature on childhood dental caries are listed next in **Table *1***.

Table 1

Key words for the literature search on ECC

Key words	Variations
Childhood dental caries	Childhood dental decay
Early childhood dental caries	ECC, baby bottle or nursing caries
Dental caries in preschoolers	Dental decay in preschoolers
Childhood oral health	Child dental health
Caries in deciduous teeth	Caries in primary teeth
Dental caries in NZ children	Dental decay/disease in NZ children

2.3 Defining dental caries

Dental caries is defined as “the localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates” (Selwitz, 2007; Newman et al., 2014). To simplify, dental caries occurs because of specific bacteria that are present in dental plaque. These bacteria metabolise fermentable carbohydrates, such as sugars (for instance, monosaccharides: glucose, fructose, and galactose as well as disaccharides: sucrose, maltose, and lactose) (Moynihan, 2016). The metabolism of these sugars produces weak acids and generates extra-cellular polysaccharides. The acids produced reduce the pH of plaque to below five, which initiates demineralisation of tooth tissues (Kidd, 2005). A carious lesion develops if the remineralisation process does not balance the demineralisation process in a timely manner. The tooth tissue is then placed at risk of cavitation from progression of the carious lesion, which can lead to the internal tooth structure becoming undermined. Tooth necrosis or death can then potentially occur. This destructive process only persists if demineralisation is not reversed by remineralisation in the preliminary stages. Dental caries is a non-communicable disease and ECC can be acquired by bacterial transmission from mother to child, even prior to tooth eruption (Çolak et al., 2013; WHO, 2019a). The distinct types of transmission are discussed later in section 2.6.1.4 (the microbiology of dental caries).

2.4 Etiology and pathophysiology of dental caries

The process of dental caries is dynamic with many contributing factors. These factors will firstly be outlined, followed by an explanation of the disease progression in its succession sequence.

The following five prime factors play an essential role in the initiation and progression of dental caries:

- A susceptible host (tooth surface)
- Microorganisms (bacteria; primarily *Mutans Streptococci* and *Lactobacilli*)
- Substrate (sugars)
- Time, and
- Saliva (Davenport, 1990; Gussy et al., 2006; Selwitz, 2007; Basavaraj et al., 2011), see Figure 1.

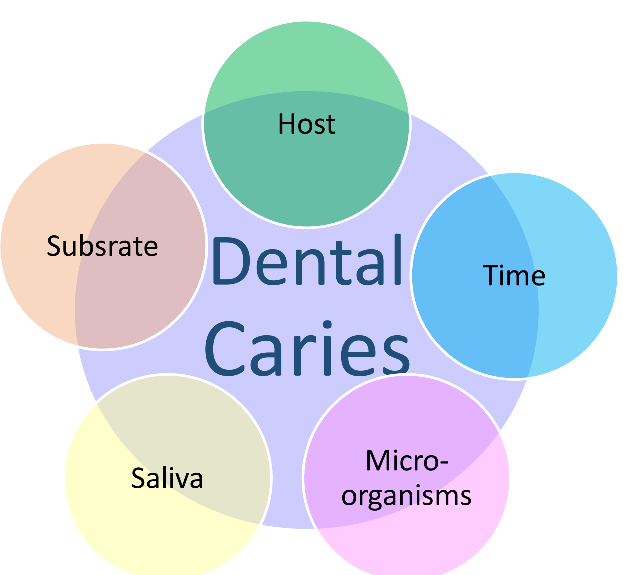


Figure 1

Venn diagram for dental caries, illustrating the five prime factors jointly required for the initiation and development of dental caries.

2.4.1 The initiation and process of dental caries

The initiation of dental caries begins with the attachment of biofilm and dental plaque to a susceptible tooth surface. Biofilm contains endogenous bacteria required for the

initiation of dental caries, primarily *S. mutans* and *Streptococcus sobrinus* and *Lactobacilli* (Caufield & Griffen, 2000). Bacterial micro-colonies have a shield of protection from host defences and disturbances, such as antimicrobial agents. They are surrounded by deoxyribonucleic acid (DNA), polysaccharides and proteins secreted by the bacteria (Fejerskov & Kidd, 2003). After initial attachment, the microorganisms in dental plaque metabolise sugars from consumed fermentable carbohydrates and produce extra-cellular polysaccharides and weak acids. The acids reduce the oral pH to below five in one to three minutes. This creates a favourable environment for demineralisation, where calcium, phosphate and carbonate ions can readily diffuse out of tooth tissues (Featherstone, 2004; Xuedong, 2015). Over time, the organic acids cause dissolution of the tooth structure and as a result a carious sub-surface lesion develops (Kidd, 2005). If demineralisation is not quickly reversed by remineralisation, the lesion will further progress to cavitation (Kawashita et al., 2011).

Dental cavities develop on tooth surfaces where plaque accumulation is most abundant (Holick, 2008). Depending on where dental plaque accumulates, cavities may develop on either crown and/or root surfaces of teeth. The fissures and pits of teeth represent particularly risk areas, as they increase the retention of food and plaque and are difficult to clean. Other high risk areas include interproximal contact points between teeth and gingival margins (Selwitz, 2007). Early carious lesions may remain limited to enamel if remineralisation occurs. This occurs by restoring the oral pH balance and creating a favourable environment for calcium, phosphate and carbonate ions to diffuse back into tooth tissues (Schafer & Adair, 2000). If remineralisation is absent, lesions may progress further into decay and potentially pulp death (Schroth, Smith, et al., 2005; Oliveira et al., 2006). Therefore, the development and progression of carious lesions is dependent on continuous regression and progression cycles (Fejerskov, 1997; Cury & Tenuta, 2009).

2.4.2 The progression of dental caries

Dental caries is an on-going process. The extent of carious lesions depends on the events which have taken place during initiation and over their period of development (Fejerskov, 1997; Kidd, 2005). Dietary habits that contribute to progression of dental caries include the amount and frequency of ingesting free sugars, those naturally

contained in food, and others which are added to food and drinks (Tinanoff et al., 2019). Consequently, the intake of sugar is a primary risk factor for dental caries (Moynihan, 2016).

Initially, white spot lesions (WSLs) will occur on smooth tooth surfaces, due to prolonged plaque causing demineralisation of the enamel. WSLs appear as white and chalky spots on teeth due to the decalcification of enamel, and its porosity becomes compromised (Deveci et al., 2018). The carious lesions that remain non-cavitated could stay limited to enamel and have the potential to repair entirely if remineralisation occurs. However, if carious lesions progress into forming cavities on the enamel surface, they may extend into the dentine and pulp of teeth (Davies, 1998; Fejerskov & Kidd, 2003; Amaechi et al., 2013; Angus & Richard, 2013). Dental caries cannot be reversed after cavities occur and extend into the dentine layer of tooth, reaching the critical point of no return when perforation of the enamel has occurred. At this point, it becomes impossible to entirely repair the tooth by remineralisation, however, the lesion can be arrested. A diagnosis can be made by clinical signs and radiographs, these techniques indicate non-cavitated and cavitated lesions, as per their development stages (Axelsson, 2000; Çolak et al., 2013). It is recommended that infants and children have regular dental examinations to identify carious lesions early, in order to prevent, monitor and treat or attempt to halt their progression accordingly (WHO, 2019a).

2.5 Early childhood caries (ECC)

Dental caries that present in children aged 6 years and below, are termed ‘early childhood caries’ (ECC); ‘ECC’ describes the pattern of dental caries that is specifically seen in young infants or children (WHO, 2019a).

In the past, different terms have been used to describe the phenotype of ECC and childhood caries that relate to specific feeding practices. ECC has been commonly referred to as “baby-bottle caries” or “nursing caries”, having been believed to be due sugar sweetened drinks in the baby’s bottle and overnight bottle and/or breastfeeding for prolonged periods. Other terms that have been used to describe ECC include:

- “nursing bottle syndrome”
- “rampant caries”

- "mottle bottle syndrome"
- "early childhood dental decay"
- "comforter caries"
- "maxillary anterior caries" and
- "breast milk tooth decay"

These terms for ECC often refer to and reflect the distinct appearance that it exhibits (Tinanoff et al., 2019).

It was first proposed at a workshop, sponsored by the US Centers for Disease Control and Prevention in 1994, that ECC be the new common descriptive term for this childhood dental disease. This was to incorporate the multiple causative factors involved in its occurrence and so as to not attribute ECC primarily to feeding practices (Featherstone, 1999; Lam & Chu, 2012). This terminology and definition for ECC was then further re-established at a workshop by the National Institute for Dental and Craniofacial Research in 1997 and 1999, and then by the American Academy of Pediatric Dentistry (AAPD) in 2005. The most current universal definition of ECC is: the presence of one or more decayed (non-cavitated or cavitated lesions), missing (because of caries), or filled tooth surfaces in any primary tooth in a child aged 71 months or younger (AAPD, 2011; WHO, 2019a).

ECC has distinct characteristics that are usually associated with feeding practices, dietary intake, and children's tooth eruption patterns (Mathur et al., 2011; Tinanoff et al., 2019; WHO, 2019a). Therefore, the classification of ECC relates to the disease characteristics and age of the children. The classification of ECC was initially delivered at a workshop entitled "diagnosing and reporting early childhood caries for research purposes". The goals of this workshop were to simplify and standardise diagnostic methods, definitions and clinical classifications used for ECC (Drury et al., 1999). This classification system is used by the AAPD (2011) whereby "ECC is the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of six years." Similarly, ECC has been defined as "the presence of one or more decayed, missing (due to caries) or filled teeth in a child under the age of 72 months" in NZ by Bach and Manton (2014). The most up to date classification includes the WHO's definition in their recent toolkit

to prevent childhood dental caries ("Ending childhood dental caries"), where ECC has been characterised by the presence of having one or more teeth affected by carious lesions including WSLs, tooth loss due to caries and filled teeth in a child below age six (WHO, 2019a). This standardised classification was used for ECC in this PhD study (refer to **Table *2***).

Table 2

Classification of ECC in this study

ECC was characterised by:	Age of child
Definition: The presence of one or more teeth affected by dental carious lesions or WSLs, or missing or filled teeth	Zero to six years old

The classification for recognising and recording ECC has been standardised in most places all over the world (Drury et al., 1999; Wyne, 1999; WHO, 2019a; Machiulskiene et al., 2020). However, some variances do exist in classifications and terms for ECC (Çolak et al., 2013). These alternative classification systems include those defining ECC as the presence of caries on any primary maxillary incisor tooth, and some having no references to age. For example, one study defined ECC simply as the presence of a cavitated carious lesion on any tooth (Cariño et al., 2003). The reasoning behind more limited definitions may be to make the diagnosis for ECC simpler, particularly in situations where the oral examinations may be more restricted, for instance with only visual and tactile detection. Therefore, most often ECC has been classified in children aged six and under as one or more decayed, missing or filled teeth (dmft) due to dental caries.

2.5.1 Severe early childhood caries (S-ECC)

Severe early childhood caries (S-ECC) is the term used to define the more advanced presentation of ECC. S-ECC describes rampant dental caries, when children below three years old and present with smooth surface dental caries on any primary teeth. In children aged between three to five years old, S-ECC comprises of:

- One or more maxillary anterior teeth that are either missing, filled, or cavitated from dental caries, or
- Four or more decayed, missing or filled tooth surfaces (dmfts) on primary teeth (AAPD, 2011; Çolak et al., 2013).

2.5.2 The presentation of ECC

ECC presents in various stages, with each stage corresponding with the advancement of the disease. The stages of ECC are determined by the extent of caries progression and the number of teeth affected.

2.5.3 The appearance of ECC

ECC usually begin along the gingival margins of teeth, where dental plaque frequently accrues (Selwitz, 2007). The first clinical signs of ECC present as WSLs, these are dull in colour, with a chalky appearance. Non-cavitated WSLs are usually hard in texture where the enamel is still intact. If the progression of dental caries continues, WSLs will become cavitated and extend into the dentine. The lesions will also change in colour, progressing from white to yellow and sometimes advance from brown to black (Zafar et al., 2009; Caufield et al., 2012). The texture of the lesions will also alter progressing from initially firm to soft. The distinct clinical appearance of ECC makes it obvious to parents or caregivers, who can detect its presence, as well as practitioners who can treat and prevent its progression appropriately.

2.5.4 An outline of the three stages of ECC

The stage of ECC depends on the number of teeth involved and the distinct causes behind its manifestation. The three stages of ECC have been outlined below.

2.5.4.1 **ECC type one:** is in the mild to moderate range, occurring in children aged two to five years, who present with ECC on their incisor and/or molar teeth. Type one ECC is usually caused by the intake amount and frequency of sugar containing food and poor oral hygiene practices.

2.5.4.2 **ECC type two:** is in the moderate to severe range and can affect children as young as six months old, after their first teeth erupt. Type two ECC, generally presents on the labial and palatal surfaces of maxillary incisor teeth, but it can also present on

molars. It generally does not affect the lower incisor teeth. The primary causes of type two ECC are linked with poor feeding habits, such as prolonged bottle and/or breast-feeding, as well as poor oral hygiene practices, which adds to its severity.

2.5.4.3 **ECC Type three:** is in the severe range and occurs in children aged between three and five years. Type three ECC occurs when type two has advanced, with more lesions on most teeth and most if not, all teeth affected. It occurs due to poor dietary habits, unfavourable feeding practices, neglected oral hygiene and a lack of arresting earlier lesions; otherwise known as untreated dental caries (Zafar et al., 2009).

2.5.5 Anatomical location sites of ECC related lesions

ECC typically follows tooth eruption patterns (Zafar et al., 2009), where the central primary maxillary incisors (PMIs) are the first teeth to be affected, followed by other PMIs and other anterior teeth before lower canines. The primary maxillary and mandibular molars are usually the last to be affected by ECC and are not always affected (Curzon & Preston, 2004). The lower incisors are rarely affected as the tongue and lower lip provide protection over them during feeding, along with the sublingual salivary glands in the floor of the mouth which provide continuous saliva flow around the incisors to clear sugar substances and plaque (Poureslami & Van Amerongen, 2009).

The carious lesions for ECC are observed on labial and/or lingual surfaces of primary teeth. However, they can begin in one area and spread over the entire tooth (Davies, 1998). The spread of caries can also extend to more than one primary tooth and cause detriment to other teeth over time (Berkowitz, 2003). In children over two years who have teeth erupted for 18 months or more, extensive deterioration of the primary dentition can be expected from ECC (Çolak et al., 2013). For this reason, children's teeth should be monitored as soon as the first tooth erupts to observe ECC occurrence, so it can be prevented and/or lesions can be arrested.

2.6 Potential risk factors for ECC

A risk factor is something that influences the manifestation of a disease and to which a proportion of the disease can be attributed. There are many risk factors contribute to dental caries. In this section, these internal and external risk factors will be reviewed to

demonstrate their influence on ECC, and how they may be modified to reduce the occurrence of this destructive disease (Basavaraj et al., 2011).

The development of ECC requires causative factors to be present, for instance the presence of dental plaque (microflora) and exposure to sugar (substrate) (Çolak et al., 2013). In addition to these, there are other mitigating factors of ECC, these include genetic, immunological, behavioural, environmental, and nutritional factors which influence its existence and extent and are modifiable (Ling et al., 2019; Moynihan et al., 2019; WHO, 2019a). Known risk factors which increase the prevalence of ECC include improper feeding practices, frequent sugar consumption, family SES, and previous dental visits (Anil & Anand, 2017; Tinanoff et al., 2019). The main risk factors in relation to ECC will be discussed next, which were considered in this study as co-factors which influence dental caries. The host risk factors that occur within the mouth will be described first, followed by external risk factors (both are outlined in **Figure *2***).

The risk factors for early childhood caries (ECC)

Host risk factors

- Tooth structure
- Developmental defects
- Salivary composition and flow
- Oral microbiome composition
- Dental plaque
- Dietary sugars and pH levels
- Micronutrient deficiencies

External risk factors

- Diet/Feeding practices
- Fluoride
- Low birth weight
- Socioeconomic status
- Ethnicity
- Poor oral hygiene habits
- Previous dental diease
- Time
- Irregular dental visits

Figure 2

The host and external risk factors for ECC

2.6.1 Host risk factors

The host risk factors in relation to dental caries will be discussed briefly in the following sections, most of these were not measured in detail due to being outside the scope of this study.

2.6.1.1 Tooth development and structure

Tooth development or odontogenesis begins in the womb, from the sixth week in utero. Dental lamina first starts to form from primary epithelial bands, from which 20 pimary tooth buds will originate from. Tooth buds for the permanent dentition also develop lingual to the primary tooth buds, this excludes the permanent molar teeth. In the the eighth embryonic week, the bud stage follows, where primary incisor teeth, canines and molars start to develop. In the ninth and tenth embryonic week, known as the cap stage, the epithelial buds continue to proliferate and spread laterally. This is

where histodifferentiation (separation of tissues) and morphodifferentitation (tooth germs formation) begins. During the elenth and twelveth embryonic weeks, the bell stage starts to take place, where the enamel organ forms a bell shape to be the crown. The epithelial cells then start to differentiate into inner and outer epithelium, stellate recticulum, and stratum intermedium cells, these later support enamel production. Dentinogenesis (dentine formation) takes place before amelogenesis (tooth enamel formation), dentine formation starts for primary teeth in the eigthtenth embryonic week, and at 32 weeks, for permanent first molar (Nanci, 2017; Rathee, 2020).

Inadequacy in maternal vitamin D or calcium status during pregnancy is known to be associated with insufficient tooth tissue formation in children (Botelho et al., 2020). As vitamin D, is required to assist calcium and phosphorous absorption into tooth structure during pregnancy. Studies have shown low maternal vitamin D statuses and insufficient intake from diet and skin exposure to sunlight, are linked with enamel developmental defects which can then increase risk of ECC. It is important for women to consider vitamin D supplementation during pregnancy, for sound tooth development in their children (Wagner et al., 2012). This will be further discussed in the next chapter.

The composition of tooth structure will be briefly discussed prior to enamel developmental defects. Integrity of tooth structure is essential for protection against dental caries. Defective tooth enamel formation predisposes teeth to dental caries, as they are more likely to have inferior quality and crevices that can harbour dental plaque. Distinct qualities of tooth structure that predispose teeth to dental caries include enamel defects, tooth morphology and/or orthodontic status (Carvalho et al., 2006). Irregular tooth structure or anatomy, their quality and teeth positioning is able to make primary teeth more prone to dental caries (Ozdemir, 2013).

Before explaining how the tooth structure is a risk factor for dental caries, the composition of a tooth will be outlined (see **Figure *3***). Each layer of tooth will be individually explained in a systematic manner, which will be in the order in which they are usually affected by childhood dental caries.

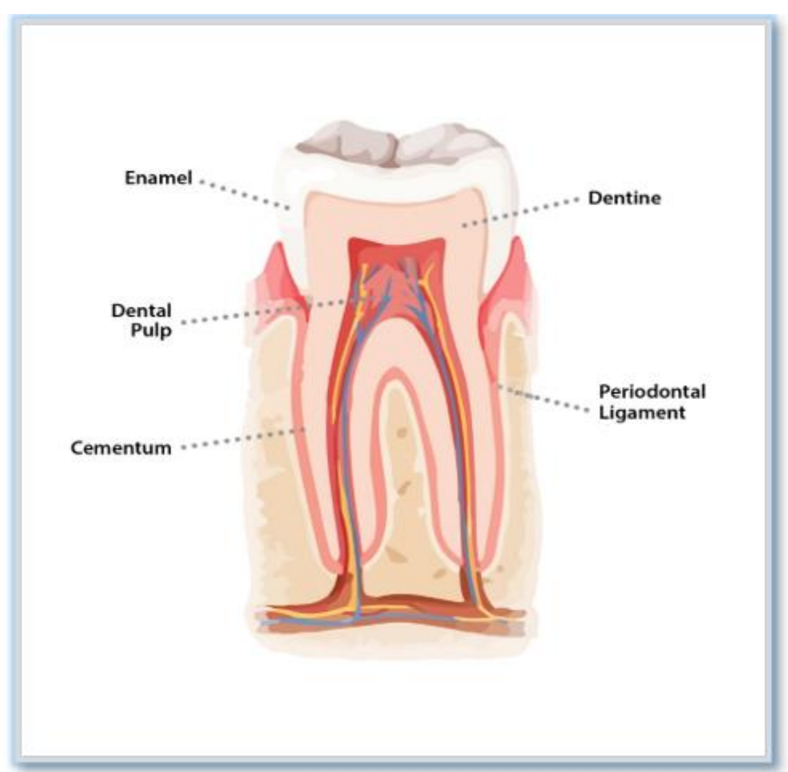


Figure 3

Tooth structure composition

Enamel is the first mineralised layer of tooth tissue, which covers the crown of the tooth. It is the hardest substance in the body, with 96% of its composition, by weight, being inorganic hydroxyapatite crystals. These crystals contain calcium and phosphate ions and 4% of its organic material being water by weight. Enamel is the first dental hard tissue surface of the tooth, affected in the dental caries development process. Tooth enamel cannot be remodelled, therefore any disease which may affect tooth enamel formation will leave a permanent which cannot be repaired (Gil-Bona, 2020).

Dentine is the next layer of hard tissue and is the most abundant component of tooth structure. Dentine comprises of 70% hydroxyapatite by weight, 20% consisting of collagen fibres and the remaining 10% is water by weight. If dental caries extends into the dentine, the breakdown of this tooth tissue by dental caries is more rapid than enamel since dentine is not as hard as enamel.

Dental pulp is the soft vascular connective tissue below dentine, situated in the pulp chamber in the centre of the tooth. The dental pulp spreads from inside the crown portion of a tooth into the root canals. The canals are much smaller in primary teeth. The substances within the dental pulp consist of blood vessels, nerves, and collagen

fibres, where macrophages or defence cells that will respond to inflammation are contained.

Cementum covers dentine on the root surfaces of the tooth. It is 65% hydroxyapatite by weight, 23% collagen and 12% water. It is softer than dentine and therefore more permeable. In children, this surface is usually unexposed and therefore at minimal risk of dental caries.

The periodontal ligament is the connective tissue, which attaches to the cementum of the roots of the tooth. It is made up of mainly collagenous fibres and is served by a rich blood supply (Milgrom et al., 2000). The periodontal ligament acts as an anchor for connecting the tooth to bone.

Compromised tooth structure is a prime risk factor for dental caries. Enamel, once broken down, cannot regenerate, whereas the underlying pulp can produce secondary dentin. Enamel is the first layer of tooth which is affected by dental caries. When dental plaque interacts with sugars, it produces lactic acid which lowers the oral pH of saliva (from 6.0-7.4 to below 5.5) initiating enamel demineralisation (Fejerskov & Kidd, 2008). Calcium and phosphate ions diffuse out of the enamel matrix during demineralisation, making the tooth susceptible and porous if remineralisation does not occur.

Bacteria can cause damage to other tooth tissues if progression continues. It does so by successively infecting dentine tubules. The dental pulp will eventually be affected if infective activity does not stop. This results in inflammation of the pulp or pulpitis, and, finally, ischemia of the dental pulp and death of the tooth (Walsh, 2007).

Predisposing factors for ECC include:

- Developmental dental defects such as enamel hypoplasia (EHP)
- Tooth morphology, fissures, and groove patterns and
- Orthodontic conditions such as crowding or malocclusion (Schafer & Adair, 2000).

Thus, each of the structure (anatomy), quality and positioning of tooth enamel can make the primary teeth more prone to plaque accumulation which leads to ECC.

2.6.1.2 Developmental dental defects

Developmental dental defects are irregularities seen within the hard tissues of tooth structure, namely the enamel or dentine. They occur when formation of enamel and/or dentine is faulty. These irregularities occur due to disturbances in the matrix and during the apposition and mineralisation of dental hard tissues in odontogenesis (Walsh, 2000). The disturbances can occur in the antenatal and/or postnatal periods. Conditions known to cause these disturbances include maternal sickness during pregnancy, viruses affecting the unborn or new born child or excess fluoride exposure during tooth development (Milgrom et al., 2000). Developmental defects of enamel (DDE) have also been linked with pre-term births, low birth weight and malnutrition (Carvalho et al., 2011). “Like ECC, enamel hypoplasia has also been found to be more prevalent among children in populations with lower socioeconomic status” (Poureslami & Van Amerongen, 2009).

Developmental dental defects comprise of two main categories, they can be either quantitative or qualitative and found on the enamel, dentine or both (Lagerlöf & Oliveby, 1994).

Qualitative dental defects occur because of disruptive forces during the calcification and maturation of dental hard tissues. EHP disturbances in primary teeth for instance, are from stresses that impact on ameloblasts and odontoblasts during matrix secretion (during tooth formation), resulting in either hypo-plastic or hypo-mineralised (qualitative) defects in enamel or dentine (Caufield et al., 2012). Qualitative defects in enamel that are known as hypo-calcification or hypo-mineralisation give rise to *enamel opacities* which appear as “opaque white spots” (Milgrom et al., 2000). The appearance of enamel is more translucent and distorted where enamel opacities present, they are commonly seen on upper maxillary incisors and may stain (Milgrom et al., 2000).

EHP is a quantitative defect where enamel is thinner because of hypo-mineralisation (Drummond & Kilpatrick, 2015). Hypoplastic teeth can be quite white in colour, or yellow to brown, with pitted hard tissues, making it difficult to distinguish enamel opacities from ECC.

Quantitative dental defects are when there is a defect that involves an alteration in the depth or thickness of the dental tissues, such as EHP with a reduced thickness of the enamel layer of tooth (Vargas-Ferreira & Ardenghi, 2011).

VDD in pregnancy, neonatal tetany and maternal smoking are conditions linked with increased risks for EHP, as are pre-term gestation and being of low birth weight (Reed et al., 2016). The above conditions are associated with EHP and are mainly due to deficiency in minerals, such as calcium and phosphorous, which are essential for the adequate formation of hard dental tissues (Seow, 2014).

When children's teeth are affected by dental defects, such as EHP, they are more at risk of ECC. This is because teeth have pits and grooves, which acquire and retain more plaque. If dental plaque is not removed frequently, by appropriate oral hygiene measures, it will quickly multiply, increasing the bacteria available for caries to initiate (Thie et al., 2002; Oliveira et al., 2006; Carvalho et al., 2011). Posterior teeth affected by EHP are more at risk of developing dental caries, as their pits and fissures on chewing surfaces accumulate more plaque than smooth surfaces.

Studies show a strong correlation between EHP and the incidence of ECC (Milgrom & Chi, 2011). In a study by Lagerlöf and Oliveby (1994), the probability of caries for children who had EHP on their posterior teeth was three times greater than those without EHP. Another study described the ECC probability as 15 times higher for children with dental defects compared to those without defects (Oliveira et al., 2006). Hence, young children with dental defects are at increased risk for ECC and S-ECC, therefore their dental defects must have close monitoring for the initiation of caries, particularly if oral hygiene is poor.

2.6.1.3 Saliva

Saliva is secreted from major and minor salivary glands to lubricate the oral hard and soft tissues, dilute and clear substances from the oral cavity and to provide antimicrobial effects (Widmer, 2010). The submandibular glands secrete a total of 60% of saliva, the parotid glands secrete 20%, the sublingual glands secrete 5% and the minor glands secrete 15% (Marsh, 2010). Saliva secretion varies between individuals and it impacts on "oral clearance" for instance, the amount of sucrose or glucose that

may be cleared from the oral cavity (Tanzer et al., 2001). Limited saliva secretion is a risk factor for ECC, especially where sugar containing beverages are given to young children and are consumed through a bottle for long periods of time or overnight (Caufield et al., 1993). A decrease in salivary flow dries out the oral cavity, and creates a more favourable environment for cariogenic bacteria to multiply (Hurlbutt & Young, 2014).

Several childhood illnesses also cause alterations in salivary flow. Children with respiratory diseases for example, have altered salivary flow as well as breathing difficulties, this puts them at higher risk for dental caries (Harris et al., 2004). Every attempt should be made to lubricate the oral tissues of children who are unwell, to promote salivation. Recommended ways are by giving them water frequently, tasty food to eat, and/or chewing gum to stimulate their saliva secretion.

2.6.1.4 Microbiology of dental caries

According to Berkowitz (2006) approximately 700 microorganisms can be found in the human mouth. *S. mutans* are the cariogenic bacteria predominantly involved in the breakdown of fermentable carbohydrates into sucrose, fructose and glucose, to produce acid which dissipates tooth tissues (Li & Caufield, 1995; Caufield & Griffen, 2000). Infants and toddlers can easily be infected with *S. mutans* as it is transmittable horizontally and vertically (Doméjean et al., 2010; Damle et al., 2016). Horizontal transmission can be from one child to another and vertical transmission is from mother to child (Baca et al., 2012). The transmissions can occur in a variety of ways, but generally mothers tend to inoculate their pre-dentate infants with *S. mutans* by dispersing it through saliva (Marrs et al., 2011). Poureslami and Van Amerongen (2009) have affirmed that, “mothers with dense salivary reservoirs of *S. mutans* are at higher risk for infecting their infants very early in life”. Children who acquire *S. mutans* in the first two years of life have more dental caries than those who do not. This period of acquisition is known to be the ‘window of infectivity’ (Li et al., 2005a; AAPD, 2020b). It is generally the period when teeth are erupting, anywhere between the age of six and 36 months (Caufield et al., 1993; Alaluusua & Malmivirta, 1994). To reduce the risk of ECC, it is important to minimise transmission of cariogenic bacteria, as well as reduce the presence of cariogenic bacteria within plaque itself.

Cariogenic bacteria can be transferred by sharing utensils when eating, parents or caregivers sucking on pacifiers to clean them, or by kissing children (Marsh, 1994). Dense salivary reservoirs in mothers, poor maternal oral hygiene, high sugar diets and frequent snacking all increase the probability of vertical transmission (Berkowitz, 2006).

Neonatal factors associated with the risk of vertical transmission have also been identified. Studies have reported, vaginal delivery of babies, gives direct exposure to vaginal and rectal microbes compared with cesarian delivery, which was associated with exposure to maternal skin and hospital environment microbes first (Kaan et al., 2021). Infants born by caesarean section delivery have an increased risk of acquiring *S. mutans* early, due to the aseptic delivery conditions, which result in less exposure to several bacteria. In comparison vaginally born children have great exposure to the mother's microbiota by method of delivery and subsequent colonisation is believed to make the child less prone to *S. mutans* acquisition (Touger-Decker & Van Loveren, 2003; Li et al., 2005b). However, some studies have also identified that despite the type of birth delivery (vaginal or cesarian) there were no differences found in the colonization of *S. Mutans* (Ubeja & Bhat, 2016; Loureiro et al., 2019).

2.6.1.5 Dental plaque

The early accumulation of dental plaque and its presence on primary teeth is strongly linked with dental caries (Scardina & Messina, 2012). This is due to the cariogenic bacteria within plaque that interact with sugars to produce the acids that are necessary for dental caries development (Zafar et al., 2009). Marsh (1994) established the ecological plaque hypothesis whereby dental caries can only occur when there is an increase in the ratio of acid-producing bacteria within plaque. This shift in the microbiology of dental plaque can be from any local factors in the oral cavity which may favour this, such as the absence of tooth brushing, lowered salivary flow or frequently ingesting fermentable carbohydrates (Ramos-Gomez, Crystal, Ng, Tinanoff, et al., 2010). An increase or decrease in any of the local factors by behaviour modification can change the bacterial plaque ratio on teeth (Julien, 1994). This will in turn influence the rate of acids being produced and dental caries being formed as well as gingival tissues being affected.

2.6.1.6 Intake of dietary sugars

ECC requires sugar rich foods to be ingested for its development. This is so that the bacteria within dental plaque can metabolise sugars to produce acids, and subsequently demineralise teeth (Kidd, 2005). The digestion process of carbohydrates in the mouth begins with salivary amylase (α-amylase, produced by the salivary glands) which binds to oral bacteria to initiate the hydrolysis of polysaccharides. Salivary amylase converts them into smaller molecules (simple sugars such as glucose), by producing maltose (a disaccharide) and dextrins (glucose polymers) from starch. In the short time that salivary amylase interacts with starches in the mouth, it begins up to 30% of the hydrolysis process (Grand et al., 2004).

Whilst not all fermentable carbohydrates may be metabolised, simple sugars (monosaccharides) such as glucose and fructose, can be readily available for bacteria within dental plaque to digest. Some examples of foods with simple sugars include fruit, corn syrup, or honey. Inversely, complex carbohydrates (polysaccharides) such as glycogen, are not accessible for plaque bacteria to metabolise. Foods which contain these include vegetables, whole wheat or rice and pasta (Touger-Decker & Van Loveren, 2003).

The common dietary sugars glucose, fructose and sucrose will go through glycolysis, for bacteria to produce into acids, and if there is a greater exposure to sugar from diet, lactic acid production also occurs, creating a more acidic environment for dental caries to occur. A highly cariogenic carbohydrate is sucrose β2,1-linked disaccharide (made up of glucose and fructose) when it undergoes metabolism by *S. Mutans* (Lemos et al., 2019).

Once carbohydrates such as glucose and sucrose are metabolised by plaque bacteria, the acids produced will work to cause demineralisation of tooth surfaces (Kidd, 2005). Tinanoff et al. (2002) state, “a dynamic relation exists between sugars and oral health”. This pertains to lowering the pH of the oral environment to below critical (5.5) at the tooth surface by dietary intake, whereby salivary amylase hydrolyses carbohydrates (starches), for cariogenic bacteria (*S. mutans)* to metabolise and produce acids, and lowering the pH values to below 5.5 within dental plaque, for the demineralisation of tooth enamel to occur (Paes Leme et al., 2006).

Research by Stephan in the 1940s established that exposure to sugars can cause the pH of saliva and plaque to drop below five quite rapidly, with this acidic environment causing enamel demineralisation. The resting pH of plaque then is gradually restored to 6.5-7 over 30 to 60 minutes (Milgrom et al., 2000), as depicted in the Stephan curve generated from these studies (see **Figure *4***).

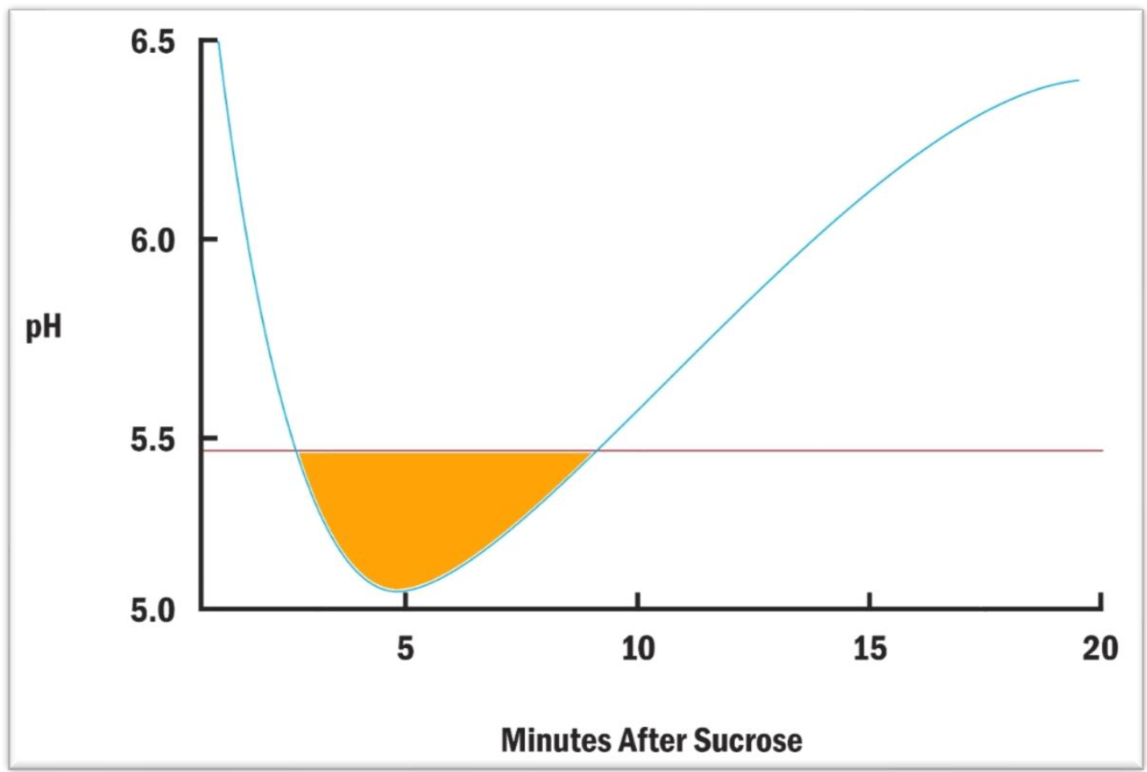


Figure 4

The Stephan curve

Figure 4 Illustrates that the plaque pH curve will drop a lot and quickly after the consumption of fermentable carbohydrates. It will drop below what we call a critical pH, where the demineralisation of the tooth enamel minerals will occur.

The Stephan curve illustrates how one minute following sugar intake the oral pH drops from 6.5 to 5.5, which is the zone where demineralisation can take place. It is not until 30 minutes following sugar intake the oral pH starts to rise again.

The neutralisation of dental plaque takes place with the dispersal of acid out of plaque. Dietary factors pose a large threat to dental caries occurring for this reason, as the plaque pH will remain low if there is a higher and frequent intake of sugar, which favours the demineralisation of teeth.

The incidence of dental caries (dmft rates) has not been identified to decrease when total sugar intake decreases within populations, but rather appears more dependent upon the frequency of sugar intake (Hallett & O'Rourke, 2003; Touger-Decker & Van Loveren, 2003). Therefore, how much and how frequent sugar foods and drinks are consumed the higher the risk for dental caries (Mobley et al., 2009) since pH levels will remain below the critical mark of 5.5 for extended time and favour continual demineralisation of teeth.

The impact of sugar intake on the formation of dental caries also depends on the quality of plaque present on teeth. For instance, a higher amount of cariogenic plaque has a greater effect when exposed to sugar, compared to lower cariogenic plaque, which will not have as much effect when exposed to sugar (Bowden et al., 1975). The frequency and amount of sugar ingestion is the basis for the ecological plaque hypothesis and oral hygiene habits will therefore influence the rate of caries progression (Marsh, 1994). Other host factors, which can affect the caries risk associated with sugar intake, are impaired salivary flow, reduced buffering ability of saliva (Efka, 2020), pre-disposing systemic or localised diseases, medications (Foster & Fitzgerald, 2005), and lowered plaque pH (Loesche, 1986). Dietary texture, consistency, amount, and stickiness, the amount of sucrose available, the timing and frequency of sugar intake all influence the development of carious lesions (Gustafsson et al., 1954; Sanz et al., 2013). This is due to these elements contributing to the length of time food or drink may be present in the mouth, their acidogenity, and how many surfaces of teeth it covers and how quickly it may be cleared (Bibby et al., 1986; Touger-Decker & Van Loveren, 2003; Nirmala et al., 2016). Sanz et al. (2013) state that foods which have 15 to 20% of sugars available, particularly sucrose, can be the most cariogenic quality, especially if they are consumed between meals. Consuming of sugar sweetened foods in the form of candy for children, as well as prolonged breastfeeding has also been significantly associated with a higher risk of dental caries (Grindefjord et al., 1996; Schluter et al., 2007; Nakayama & Mori, 2015). In addition, frequently snacking on sugar sweetened food or drinks allow sugars to be persistently in contact with cariogenic bacteria on tooth surfaces, thus, making teeth more prone to dental caries by lowering the plaque pH for extended time (Loesche, 1986; Paes Leme et al., 2006). Dental caries have been termed a ‘dieto-bacterial disease’ for this reason (Hallett &

O'Rourke, 2006). Sugar containing fluid, although cleared by salivary flow, can be imposed onto tooth surfaces for prolonged periods. This will be discussed further in the next section on feeding practices (in 2.6.2.1).

2.6.1.7 Micronutrient deficiencies

Nutritional deficiencies during tooth development are reported to pose a risk for dental caries. This is due to nutritional deficiencies being linked with EHP (discussed in section 2.6.1.2) and shrinkage of the salivary glands, which leads to lowered salivary flow (mentioned in section 2.6.1.3). Protein-energy malnutrition and deficiency of vitamins A and D are reported to contribute to dental caries (Scardina & Messina, 2012). Maternal malnutrition during pregnancy can impact on the child's tooth formation and development of other oral structures (Jumani, 2016). Vitamin D is essential for building strong tooth structure to withstand the effects of ECC. As the role of vitamin D in the prevention of ECC is the central focus of this thesis, its role will be covered in the next chapter.

2.6.2 External risk factors

Potential external risk factors are those which occur outside the oral cavity and impact on the dental disease. In the context of ECC, demographic risk factors such as socioeconomic factors, ethnicity, individual behavioural factors like oral hygiene and dental history are all external risk factors that may impact upon ECC and will be reviewed next.

2.6.2.1 Feeding practices in early childhood

Feeding practices which favour frequent sugar exposure on teeth for extended lengths of time are a direct factor in the aetiology of ECC for infants and toddlers (Erickson & Mazhari, 1999). Children who drink sugar sweetened beverages from a bottle during the day, or bottle and/or breastmilk feeding for prolonged periods, are at increased risk of ECC, particularly from age 12 to 24 months and onwards (Hallett & O'Rourke, 2003; Gussy et al., 2006; Leong et al., 2013). Sugar sweetened drinks like fruit juices are highly cariogenic, especially when given to children through sipping cups or baby bottles at bedtime, usually to soothe them. These practices produce an acidic environment in the mouth over extended periods and promote demineralisation of teeth (Çolak et al., 2013). A diet high in sugar causes a change in the plaque content as

more neutral species cannot survive in the acidic environment, leaving bacteria with aciduric and acidogenic properties to thrive.

Cows' milk has not been shown to be cariogenic despite containing lactose (Peres et al., 2002). Further, published literature has also showed that human breast milk also non-cariogenic (Erickson & Mazhari, 1999; Trongsilsat et al., 2020). In studies, where human breast milk has been indicated as being cariogenic, there is uncertainty, as confounding variables were not controlled for (Bowen et al., 1997; Prabhakar et al., 2010). It is emphasised that feeding practices, like breastfeeding, are not solely responsible for ECC in infants and that breastfeeding up to 12 months of age has not been indicated to raise caries risk (Richards, 2016; van Meijeren-van Lunteren et al., 2021). However, prolonged breastfeeding practices (>12 months to >24 months of age) and overnight breastfeeding practices, particularly in combination with the intake of other fermentable carbohydrates, are highly cariogenic and do increase caries risk (Tinanoff & Palmer, 2000; Peres et al., 2017; WHO, 2019a). The risk of ECC is further heightened by increasing feeding frequency, feeding on demand and/or sharing of food and utensils with infants and children whilst eating (Zero et al., 2009; Damle et al., 2016).

Data from meta-analyses have affirmed that dental caries is associated with the total consumption of sugars as well the frequency of sugar intake (Moynihan, 2016; van Loveren, 2019). Frequently ingesting sugar rich snacks throughout the day, contributes to the overall intake of sugar, therefore the frequency of snacks for a child has been used as proxy for sugar consumption in this study.

Dental caries have been termed a "dieto-bacterial disease" (Hallett & O'Rourke, 2006), as the repeated intake of food and drinks allow sugars to be persistently in contact with cariogenic bacteria on tooth surfaces, thus, making teeth more prone to ECC (Gussy et al., 2006; Schroth et al., 2007). The caries risk of sugar intake can potentially be modified by mitigating factors such as the exposure to fluoride, this topic will be discussed next (Moynihan et al., 2019).

2.6.2.2 Fluoride exposure

Fluoride can prevent dental decay through both systemic and topical routes. Its effects are primarily by topical exposure on teeth, for instance by using a fluoridated toothpaste (Aine et al., 2000). Fluoride can be systemically ingested through fluoridated water, fluoride supplements, tablets, or fluoridated chewing gum and/or lozenges. The addition of fluoride into enamel during tooth development makes teeth stronger and provides enamel with more resistance to acid dissolution and dental caries (Gussy et al., 2006). Fluoride can be added to non-fluoridated water supplies to protect teeth against dental caries, and the literature indicates that in the 20th century fluoridated water resulted in a 60% reduction in the incidence of dental caries (Boyce et al., 2010). The systemic benefits of fluoride are only attainable with ingestion over a prolonged period (Boyce et al., 2010).

Excessive consumption of fluoride puts teeth at risk of fluorosis or mottling, which appears as opacities on the teeth (Carey, 2014). Fluorosis has a number of clinical presentations and may appear as brown specks on teeth, where the enamel has become cosmetically unattractive (Gussy et al., 2006). Enamel defects may also predispose teeth to fluorosis, which can later put children at risk of ECC (Milgrom et al., 2000).

Fluoride can be used in several different ways topically to prevent ECC. One example of this is shown by how fluoride is discharged from dental plaque on teeth when acids are produced by the bacterial metabolism of fermentable carbohydrates. This discharged fluoride counteracts the lowered pH environment by combining with fluoride ions in saliva; it is then readily absorbed into tooth tissues with calcium and phosphate and thus strengthens the hydroxyapatite layer (Weinstein, 1998). The newly formed tooth structure with fluoride is sturdier. Fluoride contained in dental plaque and saliva also works by inhibiting enamel demineralisation, as its concentrated presence can reverse the progression of early carious lesions by enhancing remineralisation (Aine et al., 2000). This explains why children with low salivary flow or no exposure to fluoridated water, are more at risk of dental caries compared to children who have a higher salivary flow and drink fluoridated water.

2.6.2.3 Low birth weight infants or pre-term gestation

Pre-term or with low birth weight infants are more likely to have dental defects on their primary teeth, for example EHP (Seow et al., 2005). A high prevalence of dental defects in pre-term and low birth weight infants may be due to mineral deficiencies during tooth formation. Illnesses during the neonatal period may cause enamel defects in the primary dentition (Seale & Casamassimo, 2003). As already described in section 2.6.1.2, developmental dental defects increase the susceptibility of primary teeth to develop ECC.

An inadequate diet in pregnancy can predispose children to nutritional deficiencies during tooth development in utero, which in turn, increases the likelihood of EHP in the primary dentition (Poureslami & Van Amerongen, 2009).

2.6.2.4 Socioeconomic status

Socioeconomic status (SES) has a strong association with ECC (Harrison, 2003). Lower SES is associated with early exposure to cariogenic bacteria (Li et al., 2005a; Berkowitz, 2006), a higher intake of sugar, environmental surroundings, poor oral hygiene practices and/or a lack of fluoride (Aine et al., 2000). Differences in tooth enamel calcification are evident in children from lower SES backgrounds, where their teeth are commonly affected by EHP or other developmental dental defects (Schroth, 2010), placing them at higher risk for ECC. The causes of developmental dental defects and their association with ECC has been described in section 2.6.1.2.

Children from lower SES households, frequently have poorer access to dental services leading to an increased risk of untreated dental disease (Ireland, 2006; Anderson et al., 2009). Parents' and caregivers' lack of knowledge about dental services and fear from past experiences, are known to influence parental decision making when accessing dental services for their children (Harrison, 2003). Children from lower SES backgrounds are therefore more likely to present later, with their presentation prompted by symptoms like pain, rather than attending for a preventive dental visit. Regrettably, if the child’s first dental visit is painful and requires a procedure, this can result in future reluctance to access dental care (Harrison, 2003).

Children affected by ECC who come from socioeconomically deprived households, also more frequently come from ethnic minority groups, have single mothers/parents with lower educational backgrounds (Harrison, 2003; Chu, 2006). The children of parents with a lower level of education are reported to have poorer oral health compared with those from higher educational backgrounds (Mobley et al., 2009). Children of parents who have a university level of education, tend to have lower dmft rates, compared with children whose parents have had elementary level of education (Ismail, 2003). Similarly, single mothers with lower educational backgrounds and dental unawareness are reported to exhibit behaviours that predispose their children to ECC, compared to more educated mothers with higher dental intelligence quotients (IQs), and those who belong to two-parent families (Ismail, 2003).

Behaviours that predispose young children to ECC include infants that are bottle fed overnight and/or parents or caregivers sharing utensils with their children, or between children during feeding times. The reasons for these behaviours may be fatigue, with single parents managing solo child rearing, having other priorities in life or simply being dentally unaware that these behaviours can have adverse effects on their child's oral health. In a study of 2,515 children aged four to five years old in Australia, it was found that children who had a higher risk of ECC or S-ECC if they were:

- Fourth or higher in order of birth
- Belonged to single parent families
- Had mothers who were below 25 years old
- Were of non-European ethnicity
- Had prolonged bottle-feeding habits, or
- Were bottle fed with sugar sweetened beverages (Hallett & O'Rourke, 2003, 2006).

Possible reasons for single mothers to have children with poorer oral health outcomes include inadequate living conditions, unemployment, financial hardship, and mental strain. Further, mothers from low SES are more likely to neglect their own oral health as well as that of their child (Reisine & Douglass, 1998). Oral hygiene for children is not usually as high a priority for parents in lower SES groups compared to parents in high SES groups. The reasons behind this include different priorities and needs amongst the

two groups. The use of fluoride toothpaste or exposure to fluoridated water is also less likely to occur in low SES groups which adds to the risk of ECC (Harris et al., 2004).

In the US, children who come from families with an income below federal poverty levels have almost double the incidence of dental caries in primary teeth (55%), compared with children who come from affluent families (31%) (Schroth, 2010). Specker et al. (1992) states “dental caries can never be 100% preventable at the individual and much less at the societal level because of its complex nature”. Within the population group of this PhD study there were many complex factors considered, for the above stated reasons, that ECC it is not entirely preventable for individuals and prevention of ECC is more challenging at a community or societal level. This is particularly true, where feeding practices are unfavourable, in low SES and single parent families, where there is no fluoridation, and in low birthweight or pre-term infants who may have micro-nutrient deficiencies.

2.6.2.5 Ethnic minority groups

Children from ethnic minority groups have higher rates of dental caries (Keirse & Plutzer, 2010), are more likely to live in more socio-economically deprived households, and have parents with low educational backgrounds. They are also exposed to detrimental behaviours such as poor feeding habits or the absence of oral hygiene practices that increase the risk of ECC (Harris et al., 2004; Hallett & O'Rourke, 2006), as discussed in section 2.6.2.6 next.

2.6.2.6 Poor oral hygiene measures

Dental plaque, if not removed from teeth, fosters the growth of cariogenic bacteria, specifically *S. mutans*. Not having established dental hygiene habits at a young age have been shown to increase the risk of ECC in children (Ölmez et al., 2003; Corrêa-Faria et al., 2013). Poor oral hygiene, is therefore, a risk factor for the development of ECC in children (Seow, 1998). Poor oral hygiene habits include not brushing teeth regularly (once or twice daily), ineffective plaque removal and/or not using a fluoride dentifrice (toothpaste) (Fejerskov, 2004; Wagner & Heinrich-Weltzien, 2017). Prioritizing good oral health habits for children is often more difficult for parents and caregivers living in low SES families, and who come from lower educational backgrounds, therefore, oral hygiene education to parents is equally important (Chu,

2006; Tang et al., 2014; Boustedt et al., 2020). A study in Australia reported that ECC occurred more frequently in children for whom routine tooth brushing was delayed until 12 months of age (Hallett & O'Rourke, 2003). Additionally, a systematic review indicated that children who do not brush their teeth at night are additionally at higher risk of ECC (Harris et al., 2004). In a recent UK study, some of the difficulties parents experienced for brushing their children's teeth were national guidelines on tooth brushing, children's behaviour, stressful lifestyles, and unable to carry out supervised brushing on a daily basis (Marshman et al., 2016).

2.6.2.7 Previous history of dental caries

"Children experiencing caries as infants or toddlers have a much greater probability of subsequent caries in both their primary and permanent dentitions" (Keirse & Plutzer, 2010). Previous experience of dental caries in primary teeth during preschool years is a known risk factor for future caries (Seow, 2012; Leong et al., 2013). In particular, primary incisor teeth being affected by childhood dental caries, are a strong predictor for dental caries in the permanent teeth, and carious, extracted and restored teeth in primary and/or permanent dentitions (Seow, 1998; Tinanoff et al., 2002). Tinanoff et al. (2002) state, "with children under the age of five, a history of dental caries should automatically classify a child as high risk for future decay". To avoid future dental caries, it becomes imperative to prevent ECC early in young children.

In infants and toddlers, the presence of WSLs is a good indicator for future cavities (Tinanoff & Reisine, 2009). Therefore, early incipient lesions in young children are monitored, so that future dental caries can be prevented. Children with siblings, parents, or caregivers, who have had S-ECC, are also at increased risk for acquiring ECC (Krol, 2003; Kawashita et al., 2011).

2.6.2.8 Parental dental history

The occurrence of dental caries in parents is a strong risk indicator for whether their children will have dental caries (Vanobbergen et al., 2001). There is a strong correlation between maternal dental caries and periodontal diseases and ECC in their children (Fédération Dentaire Internationale, 1992; Ramos-Gomez et al., 2002; Silk et al., 2008). Therefore, maternal dental history is a risk indicator to be considered, when determining a child's risk for developing ECC (Marrs et al., 2011).

The mother's sugar intake and frequency, presence of *S. mutans* and dental caries are all risk factors for her child developing dental caries (Ramos-Gomez et al., 2002; Thorild et al., 2002). Thus, expectant mothers are advised not to neglect their own dental health during and after pregnancy, as studies report a correlation between the oral health of a mother and her child. Studies illustrate that children incorporate their parents' tooth brushing habits into their own pattern of oral hygiene, therefore this transmitted behaviour should be introduced as soon as primary teeth erupt (Silk et al., 2008).

2.7 Prevention of ECC

Effective prevention measures can reduce the occurrence of ECC in young children. Studies have shown that preventative strategies during pregnancy and infancy reduce the occurrence and progression of ECC (Rosenblatt & Zarzar, 2004; Marrs et al., 2011). S-ECC has further been reported as largely preventable, by addressing the various risk factors which cause it to develop and progress (Farias et al., 2005). "Evidence increasingly suggests that to be successful in preventing oral disease, dentists and other health care professionals must begin preventive interventions in infancy" (Alm et al., 2007). Therefore, childhood dental caries is described as preventable with specific approaches during tooth development in utero and after birth. Mothers and caregivers in this way can minimise the transmission of pathogenic bacteria to infants before and after teeth inoculation, as young children have limited defence mechanisms against this disease at an early age.

2.7.1 Education & knowledge

The ideal preventative approach involves health professionals providing parents, caregivers and the general public in the community, with the knowledge about ECC risk factors and how to reduce or eradicate them where possible (Chu, 2006). Parents and caregivers are the primary caretakers of their child's mouth, for they determine the child's dietary intake, behaviours, and oral hygiene practices. Mothers however, are predominantly the caregivers who determine the family's behaviours in relation to health and therefore oral hygiene habits (Farias et al., 2005). Guidelines have been developed to give parents advice on feeding habits and home care for good child oral hygiene, to prevent ECC in infants and toddlers (Al-Shalan et al., 1997). Measures

aimed at reducing *S. mutans* in the mother's mouth have been recommended during and after pregnancy to limit bacterial transmission from mother to child, to thereby prevent ECC. These include improving the mother's dental hygiene, encouraging the mother to use products with fluoride or chlorhexidine and/or chewing xylitol gum (Marrs et al., 2011).

Promoting good dietary habits by educating parents on low sugary intake and limiting when feeding infants is an approach which has the potential to reduce ECC (Ismail, 1998). The provision of oral health education, particularly in high caries risk groups, has the potential to turn around current attitudes and encourage healthier behaviours to improve oral health. Tooth brushing is one such endeavour in health promotion programmes that has proven to be successful (Curnow et al., 2002).

2.7.2 Preventing bacterial transmission

Discouraging the transmission of cariogenic bacteria from parents to their children when children's teeth are erupting is known as "primary-primary prevention" (Çolak et al., 2013). *S. mutans* is inherited by children from their mothers (Peneva, 2007). It is particularly important to minimise vertical or horizontal transmission of oral bacteria to the child during the crucial early years of a child's life. This can be achieved by mothers not sharing utensils while feeding children, not sucking on their child's pacifiers to clean them, and improving maternal oral health habits to reduce the presence of *S. mutans* (Kawashita et al., 2011; Çolak et al., 2013).

2.7.3 Chewing xylitol gum

Xylitol is a low calorie naturally produced alternative for sugar. It has been incorporated into chewing gum to limit bacteria on teeth and therefore prevent dental caries by its anti-cariogenic qualities (Peneva, 2007). Studies over 25 years showed that pregnant women and mothers with new born infants who chewed xylitol gum on a regular basis, have lower oral *S. mutans* levels. Children aged from zero to two years old, whose mothers chewed xylitol gum have lower rates of dental caries than infants in a control group whose mothers did not chew xylitol gum frequently. This was attributed to the decreased risk of vertical bacterial transmission for mothers who used xylitol chewing gum (Marrs et al., 2011).

Xylitol used in other edible food items also has the potential to stop existing carious lesions from progressing (Peneva, 2007). Over a two-year period, when xylitol had been used in diets in place of sucrose to prevent dental caries, there was an 85% reduction in dental caries in adults (Scheinin, 1976; Scheinin et al., 1976; Scheinin & Makinen, 1977). Xylitol can inhibit the growth of *S. mutans* (Knuuttila & Mäkinen, 1975; Scardina & Messina, 2012). In children xylitol can additionally reduce the risk for ECC by its potential anti-caries benefits following the eruption of primary teeth. A study showed, mothers and infants (from 6 months old) who swabbed xylitol onto their teeth, had a significantly lower incidence of dental caries, compared with control participants (Mäkinen et al., 2013). Similarly, there are dental benefits for children who chew xylitol gum in the aim of preventing dental caries, by reducing the presence of *S. mutans* and lowering dental plaque scores (Caufield & Griffen, 2000).

2.7.4 Exercising good oral hygiene

ECC affects mainly the smooth surfaces of teeth, such as the labial surfaces of maxillary incisor teeth that are not usually plaque retentive. In young children however, plaque accumulates on these surfaces, as children may not be very compliant with tooth brushing (Martignon et al., 2012). Good oral hygiene is linked with reducing ECC risk, as, when the bacterial biofilm is brushed off regularly, the bacteria required for caries formation are removed. In this way early lesions may be prevented and formed carious lesions may be reversed or arrested (Davies, 1998; Selwitz, 2007).

Parents and caregivers are recommended to spend two minutes twice daily brushing their child's teeth with a fluoride toothpaste to effectively remove plaque from tooth surfaces and promote remineralisation (Tinanoff et al., 2002; Gussy et al., 2006). Children do not have the necessary manual motor skills or dexterity to brush their own teeth effectively (Douglass et al., 2004), until they are approximately five years old (Smith et al., 2002),

2.7.5 Fluoride for the prevention of ECC

Fluoride in the oral cavity promotes remineralisation to teeth, by redisposition of the minerals lost from tooth tissues. Fluoride found in enamel strengthens the structure of the hydroxyapatite crystalline layer, by forming fluorohydroxyapatite (FAP). Fluoride

additionally obstructs a step in glucose metabolism and slows down the acid production of bacterial dental plaque (Edgar et al., 2004). It can be applied topically, for example, by fluoridated toothpaste or ingested systemically, for example, in the form of drinking water. Its protective benefits against dental caries have been well proven (Lam & Chu, 2012; Horst et al., 2018). Fluoridation in water supplies is one of the most cost effective methods for preventing dental caries (Plutzer & Keirse, 2011). Fluoridated water should therefore be utilized for young children and can be given in a cup or bottle. There is however some debate as to whether water should be fluoridated or not in some parts of the world and some regional water supplies still lack the addition of fluoride (Ozdemir, 2013), which gives rise to inequalities in the general public for caries prevention. However, fluoride can alternatively be added to drinking water by the addition of dissolvable tablets or by buying bottled water containing fluoride.

In children, the benefits of fluoride can be gained by topical application of fluoridated toothpaste when tooth brushing. There is a proven link between fluoridated toothpaste use and the decline in rate of dental caries. The fluoride compounds used in toothpaste most commonly have been sodium fluoride (NaF), acidulated phosphate fluoride, sodium monofluorophosphate, stannous fluoride and amine fluoride. Concentrations of these compounds vary for fluoridated toothpastes and can be anywhere from 500 to 1500 parts per million (ppm) for the public. In high-risk adult patients, a more concentrated formula of 5,000 ppm fluoride toothpaste is available as a prescription medicine. Children are in the US are generally recommended to use a pea-sized amount (0.25 g/5mm) of toothpaste with a minimum concentration of fluoride, to avoid toxicity and fluorosis if ingested (Ramos-Gomez et al., 2007). In NZ, the MOH guidelines are for children over 18 months of age to have their teeth brushed daily with a mild fluoridated toothpaste (of 400-550ppm) with 0.4–0.55 mg/g of fluoride until five years old (MOH, 2009).

Fluoride varnishes or gels can also be applied to primary teeth to optimize the benefits of fluoride in caries prevention for children. In these forms, the concentration of fluoride is higher than in toothpaste, but the method is safe and effective, therefore it may be applied every six months or more frequently depending on the child’s caries risk category.

2.7.6 Tooth Mousse for remineralisation

Tooth Mousse is a formula of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), that is applied topically to promote remineralisation as it integrates calcium and phosphate on tooth surfaces, as well as working against the bacteria in dental plaque. Studies have reported the effectiveness of Tooth Mousse in remineralising carious lesions (Ferrazzano et al., 2011; Patil et al., 2013; Garry et al., 2017). Tooth Mousse is user friendly as it can simply be applied by a child or parent/caregiver by smearing it over teeth using a finger and leaving it on (Kawashita et al., 2011). Tooth Mousse is beneficial for children to use as a preventative measure for ECC, which can be used specifically for patients at risk of ECC and those who are already affected, to stop the progression of ECC and S-ECC (Kargul et al., 2017).

2.7.7 Improving diet & feeding practices

The aetiology of dental caries is closely linked with exposure of teeth to sugar over time. In this setting the metabolism of sugars by bacteria produces acids, these persist from 20 to 40 minutes after ingestion and create an environment that favours demineralisation (Marrs et al., 2011). The current WHO recommendation is to reduce the overall consumption of free sugars in the diet, and reduce overall free sugars to below five or 10% of the overall energy intake, in the aim of reducing dental caries. This would be taking below 25-50 grams of sugar per day if overall the daily energy intake is 2000 kilocalorie) (WHO, 2015; Moynihan, 2016; van Loveren, 2019). Infrequent snacking on fermentable carbohydrates is also advised to limit prolonged sugar exposure on primary teeth to prevent ECC (Çolak et al., 2013). Other recommendations include discontinuing overnight breastfeeding, not putting babies to sleep with a bottle containing a sweetened beverage and minimising sugar exposure, for instance over shorter periods, to limit the progression of dental caries (Hallett & O'Rourke, 2003; Health Promotion Agency, 2015; WHO, 2019a).

Dietary habits can contribute to the prevention of ECC, for example, when sugar consumption is kept low, and by reducing the frequency of sugar intake (Moynihan, 2002). Foods that have a positive effect on prevention of dental caries (non-cariogenic foods) include whole grain breads, peanuts and chewing gum, all of which require more chewing and as a result will produce more saliva (Moynihan & Petersen, 2004).

Eating cheese is also beneficial as it has a cariostatic effect, it does this by stimulating salivary flow upon chewing, after which the alkaline properties of saliva then help by its buffering capacity to neutralise the acids in dental plaque (Herod, 1991; Kashket & DePaola, 2002; Moynihan & Petersen, 2004). Foods that favour the prevention of dental caries are those that are rich in fibre and low in sugar, so the pH does not drop for an extended period where demineralisation of teeth can occur (Moynihan & Petersen, 2004). Despite this, a young child should receive a well-rounded diet. According to the National food guide, this would entail one third of the diet to be: adequate amounts of breads, cereals, and starchy vegetables, one third of fruit and vegetables, and one third of seafood/poultry and dairy. The key message being limit the intake of sugar containing foods and eating more fruits and vegetables to prevent caries (Moynihan, 2002, 2016).

2.7.8 Regular dental visits

Routine dental visits for children, are the optimal way of identifying early carious lesions and of preventing any new lesions from developing (Ramos-Gomez, Crystal, Ng, Crall, et al., 2010). "The American Dental Association, American Academy of Paediatric Dentistry, and the American Association of Public Health Dentistry currently recommend all children have their first preventive dental visit by 12 months of age" as mentioned by (Ramos-Gomez et al., 2007). The interaction between dental professional, parents/caregivers and families of the child at the dental office helps to build relationships that form positive foundations for future dental health maintenance (Bhaskar et al., 2014). Parents and caregivers of children who have a high risk of acquiring ECC can promote good oral health habits at home, and gain information on how ECC presents to counteract the progression of any dental caries on their child's teeth (Poureslami & Van Amerongen, 2009).

Providers of primary health care who usually see young children before their dental visits, are in an ideal position to give early anticipatory guidance to parents and caregivers, on how to prevent ECC (Gussy et al., 2006). However, more research is needed to provide knowledge on the effectiveness of non-dental providers educating the public on the aetiology of ECC, its risk factors, preventative strategies and referring patients to dental services (Al-Ghutaimel et al., 2014).

2.7.9 Vitamin D supplementation during tooth development

The amelogenesis of primary teeth begins in the third trimester during pregnancy, begining with the primary incisors and first permanent molar teeth (Kumar, 2015). Vitamin D supplementation could possibly positively impact the mineralization of primary teeth during their development, as developmental dental defects originate during amelogenesis stages. This occurs due to disturbances in the formation of the enamel matrix, as discussed in section 2.6.1.2.

There are currently no measures to prenatally prevent developmental enamel defects, which later predispose teeth to ECC. Further, studies have already reported positive assosciations between prenatal vitamin D supplementation and fewer developmental enamel defects in children's primary teeth. It may therefore, be advantageous to implement prebirth vitamin D supplementation for the fetus, to reduce the risk of developmental dental defects as well as ECC. This will be further discussed in section 2.12 in relation to particular societies such as NZ where deficiencies in vitamin D in pregnancy and at birth are common.

The fetus would benefit from a preventative approach for enamel dental defects, as well as dental caries, for which currently no prenatal intervention exists.

2.8 Host resistance

2.8.1 Salivary benefits

Salivary glands release the viscous fluid in the oral cavity that is known as saliva. Saliva consists of 99.5% water and 0.5% of salivary proteins which contain bicarbonate and phosphate ions (Ireland, 2006). Saliva executes its defence functions in the following four ways:

1. Cleansing ability
2. Buffering of acids
3. Antibacterial properties
4. Remineralisation capacity (Kidd, 2005; Stookey, 2008).

The cleansing ability of saliva refers to its rinsing action, as is clears bacteria in the mouth by removing plaque biofilm and the oral clearance of ingested sugary

substances. This decreases the interaction time available for sugar to contribute to the formation of dental caries, by removing sucrose from the oral cavity efficiently.

The main function of saliva is to buffer and provide bicarbonate phosphate and smaller proteins to neutralise acids after carbohydrate intake. Salivary bicarbonate favours remineralisation against dental caries, by its buffering ability to neutralise dental plaque pH everytime it is lowered due from bacterial acid production after the intake of fermentable carbohydrates (Dodds et al., 2015). Several electrolytes including salivary bicarbonate and immunoglobins act as mitigating factor for dental caries, by buffering the biofilm acids, and providing an antibacterial action on microorganisms (Humphrey & Williamson, 2001). As salivary bicarbonate neutralises saliva, it reverses the demineralisation process by raising the pH with its buffering action (Featherstone, 2000).

Saliva also provides a chemical protection for dental caries, as it contains calcium, phosphate and fluoride as well as providing those minerals for remineralisation when it occurs. The fluoride found in saliva strengthens structure of the enamel's hydroxyapatite crystalline layer (essentially calcium and phosphate ions), by forming fluorohydroxyapatite (FAP) by fluoride ions becoming charged by other applied fluoride, such as toothpaste and mouth rinse (Curnow et al., 2002). Fluoride additionally obstructs a step in carbohydrate metabolism and slows down the production of bacterial dental plaque (Edgar et al., 2004).

Saliva has the capacity to promote remineralisation by providing the minerals, calcium, and phosphate, which have been lost back into the tooth structure by diffusion. Saliva contains inorganic ions made up of calcium and phosphorous molecules, to help promote remineralisation of ECC lesions, limiting, and preventing dental caries. The antibacterial properties of saliva additionally include, comprising of immunoglobins to fight the bacterial invasion, thus saliva plays an important part in the prevention of dental caries (Bird & Robinson, 2018).

2.9 The management of ECC

The management of ECC is briefly outlined in this section, as detailed information on managing of ECC is outside the scope of this thesis.

2.9.1 Rationale for restoring and treating primary teeth

Retaining and preserving primary teeth can help children having regular growth and development patterns. Primary teeth not only allow children to eat, speak and smile, but prematurely losing primary teeth can incur space loss for permanent teeth. Space loss can cause orthodontic problems such as malocclusions. It is difficult to masticate food without teeth, therefore children with ECC could develop malnourishment due to weight-loss from not eating sufficiently (Khanh et al., 2015; Folayan et al., 2020). Lower body weight has been found to be associated with mouth pain, in a Vietnamese study investigating links between ECC, mouthpain and nutritional status in children aged one to six (Khanh et al., 2015). Further, a study in the Phillipines also showed evidence of ECC being associated with malnutrition in 11 to 13 year old children, from under nourished populations (Benzian et al., 2011). Missing teeth could lead to low self-esteem, as well as alter speech in children (Kaur et al., 2017). Maximum retention of primary teeth is ideal so that teeth can exfoliate naturally, and so children can have regular growth and development patterns (Ercan et al., 2007).

2.9.2 Caries risk assessment (CRA)

Caries risk is a measure that begins with “caries risk assessment” (CRA), to help determine the likelihood of the disease occurring in a child’s mouth over a given period. CRA enables dental professionals to identify risk factors and ways to modify them, in relation to each child’s individual oral health needs. Parents/caregivers can use this information to apply prevention strategies that are personalised to every child. The advice given is based on the theory offered by Featherstone et al, at the California consensus conference in 2002, whereby caries regression is based on the equilibrium maintained between the pathological factors responsible and preventative factors available (Ramos-Gomez et al., 2007).

Healthcare professionals can perform dental screenings for children and establish their caries risk. This will then enable them to give parents and caregivers appropriate anticipatory guidance and refer patients to dental professionals in a timely manner (Lynch & Milgrom, 2003). CRA determines whether a child is in the low, moderate, or high caries risk category. Dental visits for children must be as early as possible for this to be done (Ramos-Gomez et al., 2007), most countries have suggested between 6-12

months for the child's first dental visit which aligns with the NZ recommendations as well, to attend as soon as the first tooth erupts (Health Promotion Agency, 2015). The CRA involves a parent/caregiver interview to identify risk factors for dental caries. The risk factors comprise of feeding practices with prolonged sugar exposure on teeth, the frequent intake of fermentable carbohydrates, poor oral hygiene practices, bacterial transmission, low socioeconomic status, familial history of dental caries and irregular visits to dental professionals. The interview is followed by a clinical examination of the child where the dmft scores are recorded. The abundance of plaque on teeth and any other factors, like low salivary flow, which add to caries risk are also recognised and noted. A clinical examination should be aided with radiographs where possible. If the child's risk status is high, bacterial cultures may be required for both mother and child, to determine the need for antibacterial therapy (Ramos-Gomez et al., 2007).

"Caries management by risk assessment" (CAMBRA) builds upon the CRA, whereby early identification, prevention and treatment strategies are applied to stop further progression of existing dental caries. CAMBRA stems from the same philosophy that "caries protective factors are biologic or therapeutic measures that can be used to prevent or arrest the pathologic challenges posed by the caries risk factors" (Thorild et al., 2002). Different risk assessment forms exist for both the CRA and CAMBRA. Therefore, it is important to get the appropriate form according to age of the child, for the management of ECC. For instance, the form for birth to five years would be sufficient for infants and young children in the pre-school age group. After the risk level for a child is established, parents and caregivers should be educated on ECC and ways it can be prevented (anticipatory guidance) or limited, treatment requirements and referral of children to dental professionals where necessary. It has been said that to manage ECC the disease process must be addressed along with the cavitated lesions, which require treatment. To do this effectively not only dental providers but also other health providers must also be involved in CRA and CAMBRA to assess, monitor and refer patients to decrease the prevalence of ECC.

2.9.3 Treatment planning

Once the child's level of risk for ECC has been established and parents/caregivers have been given anticipatory guidance on the relevant prevention measures, a treatment

plan must then be devised per the child's needs and age. Firstly, WSLs would be identified and addressed to avoid cavities from occurring. The progression of these can be reversed by home oral hygiene care, routine fluoride varnish applications, and good dietary practices with infrequent exposure to fermentable carbohydrates.

2.9.4 Preventative treatments

Preventative dental treatments can be applied prior to restorative treatments where possible and the appropriate oral hygiene education and instructions must be given to parent/caregivers for children. Tooth brushing, and the importance of fluoride toothpaste should be explained, and fluoride varnishes or gels should be applied to the child's teeth in the clinic. Tooth mousse can be suggested as a home care aid to prevention of dental caries or to arrest existing lesions. If parental feeding practices are detrimental to the child's dental health, they must be improved too.

Fissure sealants or protections are coatings on the child's teeth, which can be applied on the chewing surfaces of posterior teeth or where plaque retentive fissures may be present to prevent ECC. These can be beneficial as not only do they seal off the fissures or grooves from gathering plaque, but they can release fluoride and promote remineralisation too.

Following the CRA and addressing the important risk factors, the clinical exam should be supplemented with a radiographic exam where possible. More invasive treatment may involve removing the infected caries from enamel and dentine of primary teeth to preserve the remaining tooth tissues. The possible restorative approaches are outlined below.

2.9.5 Atraumatic restorative treatment technique (ART)

The ART technique uses a minimal intervention method to restore a tooth using a alternative approach, where the child does not require local anaesthesia or drilling into the tooth with an electrical handpiece. A process of manual excavation using hand instruments to the greatest extent possible will remove the caries. The material for filling the tooth is usually a glass-ionomer cement, which bonds to tooth tissues and releases fluoride to strengthen the tooth structure (Sidhu, 2011). Studies have found the restorations using this technique are successful and the fillings retain successfully,

even when compared with amalgam fillings. This is a cost effective and well-received form of dental treatment for ECC, especially in socioeconomic-deprived populations. Reasons for this include: it is not the most expensive treatment to execute in terms of time required to treat, lower material costs, instrumentation requirements are minimum and no pain management is required (Mouradian et al., 2000).

This is an ideal treatment approach for the child and dental practitioner when children may be apprehensive about dental treatment, and do not like the sound of the drill, are uncooperative for local anaesthesia, or do not have access to dental professionals. This treatment can be provided with very little equipment and therefore does not require a dental clinic to be provided. The contraindications for using this technique would be where there is difficulty accessing the caries, extensive caries extending into dental pulp or teeth have abscessed (Frencken et al., 1994; Waggoner & Nelson, 2019).

2.9.6 Dental fillings (restorations)

Restorations or fillings as they are commonly placed worldwide to restore teeth affected by dental caries (Ercan et al., 2007). The surgical removal of dental caries is indicated when the carious lesions have extended into both enamel and dentine and there is the possibility of infection spreading further. Local anaesthetic is given to children for pain management before filling work where possible and if the child is obliging. Primary teeth, although similar in anatomy to permanent teeth, differ in proportions. These factors must be considered when restoring primary teeth. The main differences are that the enamel of primary teeth is thinner and whiter in appearance, and the dentine layer is thinner than that of a permanent tooth, so caries can quickly penetrate from dentine to the dental pulp, requiring tooth structure to be conserved as much as possible prior to placing a filling.

Post the removal of caries using electrical drills and manual hand instruments, the filling material depending on the size of the cavity, cooperation of the child and materials available, is then chosen. If the parent/caregiver agrees with the recommended materials by the dental practitioner and gives consent to place the filling, the material of choice will be placed into the prepared cavity to replace the lost enamel and dentine hard tissues. The distinct types of filling materials that are primarily used in restoring primary teeth are described next.

2.9.6.1 Intermediate restorative material (IRM)

Intermediate restorative material (IRM) is composed of zinc oxide powder mixed with eugenol liquid. IRM material was originally derived as a temporary filling, but manufacturers suggest it may last for up to one year (Devika & Jayalakshmil, 2016). Since eugenol is derived from clove oil the filling material can smell a bit strong, however it is an easy to mix and quick-setting filling, limiting the time of the child in a dental chair. The properties of IRM include low solubility, strength, good resistance to abrasion and its ability to bond to tooth structure. Dental practitioners who may be referring children for further restorative work may use IRM as a temporising filling material. IRM may also be used as a base for fillings, as well as procedures such as pulpotomies and pulpectomies (Weiner, 2011). Its user-friendly handling characteristics make it ideal to use for a small child, where moisture control may not be ideal, or the child may be unable to sit for extended periods, as IRM requires minimal preparation and application time.

2.9.6.2 Glass ionomer cement (GIC)

Glass ionomer cement (GIC) fillings are the most widely used filling materials in modern day dentistry for the restoration of primary molars. They consist of glass particles usually fluoroaliminosilicate which is in powder form and the other component is a polyacrylic acid (Croll & Nicholson, 2001). Both combine when mixed and slowly the mixture starts to set (Cho & Cheng, 1999). The advantages for restoring primary teeth with GIC include: its chemical bonding properties, it is moisture friendly, fast setting, able to release fluoride and thus enhance remineralisation and it is an aesthetically pleasing option (being tooth coloured).

2.9.6.3 Composite

Composite is a long-lasting resin-based material. It is classified depending on the size of the fillers in them, as this determines their polymerization depth, physical strength and shrinkage properties and polish-ability. Composite is used for filling cavities of different sizes in both anterior and posterior teeth, it is utilised where aesthetics may be more important as it comes in many enamel and dentine shades. Composite fillings require good moisture control and a bonding agent; therefore, they may take a bit longer to place, however they are known to have a good retention rate in children’s

teeth (Pinto et al., 2014). Composite fillings are used more in combination with GIC material for primary teeth, however as the placement of this filling material is more technically challenging, it requires patient cooperation and a longer setting time, using a light emitting diode (LED) curing light. Its benefits are better aesthetic outcomes with respect to colour, greater strength and durability and it contains fluoride. In today's world, modern day resin composite fillings are commonly used in pediatric dentistry. This is in line with the phasing out of amalgam fillings in relation to the Minimata Convention on Mercury, in the efforts to reduce mercury pollution globally by the United Nations Environment Programme (2017). Composite is not the ideal material of choice for high caries risk patients, due to its shrinkage and contamination capabilities (Donly & García-Godoy, 2015). However, composite fillings have demonstrated longevity with being retained in primary teeth that were affected by ECC, where children received dental treatment under GA (Bücher et al., 2014).

2.9.6.4 Amalgam

Dental amalgam is made of a metal alloy, incorporating mercury with copper, silver, tin, and zinc. It is one of the oldest and most durable materials used in dentistry and known for its application properties and longevity. Over time the use of amalgam has decreased with the Minamata Convention, due to its metallic colour not being aesthetically pleasing, with the safety concerns of the public and requirements of a cavity preparation to retain the filling material mechanically. The WHO endorses the Minamata Convention's phasing down or mercury fillings overtime and discontinuing the use of dental amalgam in dentistry (WHO, 2018). The worldwide shift in reducing amalgam fillings has been developed in keeping with current minimal intervention dentistry and protecting individuals and the environment from released mercury and its compounds (Fisher, et al., 2018). Contrary to this, other published literature mentions that with non-amalgam restorations (such as composite) being twice as like to develop secondary caries in adjacent teeth, perhaps the phase down of amalgam fillings may be premature (Boushell et al., 2019). Amalgam is also the only filling material to establish a better interfacial seal overtime, due to reduced microleakage between filled tooth surfaces and adjacent teeth, because of the corrosion products that fill the spaces (Boushell et al., 2019).

In addition to the conventional dental materials discussed for restoring teeth with ECC, data from a recently published trial undertaken in the UK, is of importance to note (Homer et al., 2020). In the Filling children's teeth: indicated or not (FiCTION) trial, one of three methods were applied to treat children teeth affected by caries, the three being:

1. Conventional restorations with best practice and prevention
2. Biological carious lesion management with best practice and prevention, and
3. Best practice prevention only (Homer et al., 2020).

This RCT trial enrolled 1144 children, with available dental data for 1058 children, who were on average six years old. It was found that on average the costs for managing ECC were expensive over a 36 month period, and often follow up infection and pain could be avoided with initial treatment being successful (Homer et al., 2020).

It was summarised that conventional filling treatment was found to be the most expensive in the FiCTION trial, followed by biological approaches and the least expensive option being preventative care only. This was determined by the costs of operative procedures, staff time and referrals required, such as some children requiring GA. Conventional fillings were most costly, however, less effective than biological approaches, when compared with incidence and pain or infection post treatment. Biological and preventative treatment was also costly but most effective, compared with preventative care only (Homer et al., 2020).

The next procedure to be described is more invasive than the traditional fillings that require the only dentine and enamel to be drilled or excavated due to caries, as it involved more removal of tooth structure as well as dental pulp due to carious activity, this will be explained next.

2.9.6.5 Pulpotomies

Pulpotomies involve the removal of the entire dental pulp in the pulp chamber or removal of the infected pulp from the chamber and the roots, known as a partial pulpectomy. Pulpectomies are necessary when infection has spread to the pulp chamber and is giving the child acute pain, which indicates necrosis of the pulp occurring. Dental pulp is removed by drilling and excavation and the pulp chamber is

then filled, usually with IRM material. The ideal finishing restoration for both pulpotomies and pulpectomies is a stainless-steel crown (SSC) placed over the top to secure the filling, as much of the natural tooth structure will have been removed (Rodd et al., 2006).

2.9.6.6 Stainless steel crowns (SSCs) and the Hall technique

The Hall technique is where pre-formed stainless-steel crowns (SSCs) are placed over teeth with a cementing filling material, without any anaesthesia and/or drilling (Ludwig et al., 2014). It is an inexpensive option for dental professionals where no further appointment time is required and the SSCs often last until teeth are naturally ready to exfoliate. Minimal time is required in the dental chair with this non-invasive technique, it serves as ideal for high-risk patients who may be unable to experience more extensive treatment and require the caries progress to be halted (Altoukhi & El-Housseiny, 2020).

The Hall technique is based on isolating bacteria within carious lesions, denying them air and nutrients, this method arrests carious lesions from progressing further (Innes et al., 2007). ECC and S-ECC are treated by the Hall technique for retaining teeth and providing function in the mouth for chewing, speaking, and smiling. Parents/caregivers may appreciate the silver appearance of the crown; however, the advantage remains being able to keep the tooth in the dentition. Tooth coloured veneers or crowns are another option although they are often costlier and will be at a private cost to parents. A literature review and meta-analysis finds that the success rates of SSCs are higher than multi-surface amalgam fillings and they are routinely used after pulpotomies on primary teeth, with some preparation of the tooth (Randall et al., 2000). In a recent study where proximal surfaces were treated in 120 children's teeth, the results showed that clinical and radiographically there was a significantly higher success rate for the Hall technique after 18 months, compared with conventional composite and amalgam fillings (Katbeh & Khasan, 2021).

The ease of placing SSCs using the Hall technique without preparation of the tooth or anaesthesia is an efficient way to treat children's primary teeth that may otherwise require extractions. To date, this is currently the most successful, least invasive, and cost-effective treatment option available for young children with dental caries in their

primary teeth (Innes et al., 2007; Welbury, 2017; Elamin et al., 2019{Katbeh, 2021 #1880; Altoukhi & El-Housseiny, 2020; Katbeh & Khasan, 2021).

2.9.7 Tooth extractions

The removal of teeth by extraction is the eventual and unfortunate management for ECC and S-ECC when advanced (Berkowitz, 2003). It requires local anaesthesia before the primary tooth is removed from the socket where the tooth root is anchored into the bone via the periodontal ligaments. This experience can be very traumatic for children and they may require general anaesthesia in a hospital setting for these procedures, due to the complex nature of the procedure (Poureslami & Van Amerongen, 2009). Following the removal of a tooth the space left can make eating difficult and impact on speech later. Space loss for the permanent tooth is why a space maintenance appliance is indicated in many cases, however, it is not always possible for parents to financially or physically to have the means to take their child to an orthodontist for these appliances. The result may be malocclusion or crowding of teeth when permanent teeth start to erupt, due to space loss.

2.9.8 Regular dental visits

Regular dental visits remain an integral part of caring for children's teeth. A child's risk status and recall intervals for dental examinations are determined by their CRA. Most high-risk patients should be seen every six months ideally, moderate risk patients every 12 months and minimal risk patients at least every 18 months by a dental professional. Regular dental visits facilitate maintenance, for identifying any new lesions, detecting any current treatment needs and modification of any oral hygiene practices at home as required. Parents/caregivers also benefit by bringing their children in for regular dental visits to establish how the child's dental health is at any given time, and how it may be improved or worsen if risk prone behaviours persist. Recall intervals for dental visits are important to adhere to if regular maintenance is to be kept up, otherwise if ECC is detected too late, teeth may be deemed non-restorable and if infection has spread significantly the outcomes can have adverse effects on the general health of children (described in section 2.11 next).

2.10 The consequences of ECC

The consequences of ECC can extend beyond the primary dentition and impact on the general health of a child (Finucane, 2012), as well as their learning and future dental wellbeing (Plutzer & Keirse, 2011). ECC can have both short and long-term effects.

2.10.1 Pain and toothache

Dental pain is often the first consequence children register due to ECC (AAPD, 2009). In some studies, over 50% of children are reported to encounter pain because of dental caries (Adeniyi & Odusanya, 2017; Innes & Robertson, 2018). Pain is more evident in children with unrestored carious primary teeth, or where carious molars are involved, and teeth have several surfaces affected by caries or pulp exposures. Thus, toothache is a common episode for children, and it is observed more in children from lower socioeconomic households with ECC and S-ECC (Ramos-Gomez et al., 2002). Subsequent effects of dental pain that can be experienced, may be the inability to eat, sleeplessness, unable to play or socialise due to discomfort, speech problems and loss of concentration (Finucane, 2012).

2.10.2 ECC affects quality of life

The quality of life (QOL) for children is significantly affected by ECC (Malden et al., 2008; Krisdapong et al., 2012). Young children may not be able to clearly express or verbalise what kind of dental pain or discomfort they may be experiencing, as cognitive thoughts and speech of a child are still limited in early life. Parents and caregivers may therefore fail to observe signs of discomfort or aching from ECC and the pain being experienced by children can be missed. The presence of aching can however be assessed by other factors, such as witnessing chewing difficulty in children, reduced food consumption and in some cases malnutrition. Children can also have reduced sleep from the soreness experienced in teeth with this affecting their concentration and behaviour issues during the day.

Oral health related QOL is poorer in children with ECC especially when treatment under general anaesthesia is required (Gaynor & Thomson, 2012). The treatment of teeth affected by ECC will lead to improvement in eating and better sleeping and

attention patterns. Following dental treatment for ECC under general anaesthesia (GA), parents have observed that the QOL of children does improve.

2.10.3 ECC restricts growth and development

ECC can not only restrict mouth movement due to the pain experienced, but also limit the overall growth and development of children. The term ‘failure to thrive’ is associated with children not being able to grow and develop normally, where weight is 80% below the healthy weight margin as per the age and height of the child. Children affected by ECC have lower height and weight when compared with control (caries free) children (Mohammadi et al., 2009; Murguía-Peniche, 2013).

2.10.4 Social development

Parents have reported poorer academic performances in school for children who are experiencing ECC and more so when the child’s general health is poor as well (Edgar et al., 2004). A child’s cognitive capability and devotion to learning can be significantly affected by ECC.

2.10.5 Space loss for permanent teeth

Severely decayed teeth that may need to be removed if deemed non-restorable, can result in space being left where the primary tooth had been (Ireland, 2006). The space left behind is concerning as the primary tooth was providing a footing for the permanent tooth to erupt into and it may be some time before a permanent tooth is to erupt into the gap. Other primary teeth may close this gap in the meantime resulting in space loss. Space loss for permanent teeth is unfortunately an outcome that occurs after the removal of severely decayed primary teeth for children who have had ECC. This contributes to immediate or future orthodontic problems for paediatric patients.

The most common effects from space loss include teeth in cross bite, crowding of teeth, midline deviations, impacted teeth and ectopic eruption patterns which lead to malocclusion (Zou et al., 2018). These occur because of teeth drifting. Studies reveal molars migrate mesially to the areas of removed molars and canines move in a distal direction, the eruption of permanent teeth is commonly altered owing to these occurrences (Finucane, 2012). To prevent space loss, the application of orthodontic

appliances such as space maintainers can help prevent the drifting of teeth so that arch length is not compromised when permanent teeth erupt (Setia et al., 2013). The negatives of space maintainers are that children may find them uncomfortable on soft tissues, they may not be worn correctly or regularly (if in the form of a removable appliance) and they will accumulate more plaque, therefore increasing the risk of dental caries and periodontal diseases (Arikan et al., 2015).

2.10.6 Systemic consequences

Systemic consequences occur because of infection, which may be uncontrolled, or from advanced dental caries with failed treatment outcomes. Extensive ECC or S-ECC when untreated or where treatment has been unsuccessful, can have adverse outcomes, such as increasing the likelihood of dental sepsis and/or cellulitis occurring (Finucane, 2012; Bach & Manton, 2014). Sepsis is the suppuration of pus from infection and if the infection does not remain localised, to a single tooth, it may spread internally to other soft tissues and/or tooth structures. Sepsis is associated with poor efforts in restoring primary teeth affected by ECC, or untreated dental caries (Pine et al., 2006). Often the reasons behind sepsis could be children were uncooperative in the dental chair and all caries were therefore not removed, or poor moisture control may have compromised restorative treatment, given the child's anxiety in the dental setting, inadequate time to treat, and/or the limited availability of materials. Dental sepsis can present and advance into cellulitis, where if progression continues the life-threatening condition Ludwig's angina can develop. Ludwig's angina occurs when the airway is restricted by the infection spreading along the oral cavity floor, and Finucane (2012) states "the condition is potentially fatal, with a mortality rate of 8-10%". The detrimental effects of ECC can be substantial, what is more potentially preventable disease that begins as a WSL, can result in loss of life for a child if it goes untreated.

2.11 Periodontal diseases in childhood

2.11.1 Gingivitis and periodontitis

Gingivitis is the inflammation of gingiva (or soft tissues) and without clinical attachment loss it is reversible. Plaque induced gingivitis occurs in children, but it can revert to health with good oral hygiene methods (Trombelli et al., 2018). Periodontitis is the progressive form of periodontal disease, which follows gingivitis. It is where

bacterial biofilm on teeth and gingival tissues causes an inflammatory disease response, which may lead to degeneration of connective tissues, the periodontal ligament and alveolar bone. Destruction of the tissues by periodontitis is not reversible. Over time, periodontal disease can result in eventual tooth loss . The periodontal ligament attaches the cementum layer of the tooth root to the surrounding alveolar bone; this is where pocketing occurs in the soft tissues in periodontitis from bacteria that separates the roots of teeth from the gingiva and surrounding structures. Initially, periodontal disease is symptomless and remains undetected, while gingivitis is the most painful stage. Periodontitis may cause discomfort or pain at a later stage and impact on chewing ability. The tooth will experience more instability overtime if untreated until it eventually comes out of the socket (Pihlstrom et al., 2005). Destructive forms of periodontal diseases are more prevalent in adults than children, however, gingivitis presents in children of all ages and in varying forms (Califano, 2005; American Academy of Periodontology, 2008).

2.11.1.1 Aetiology of gingival diseases

Disease causing microorganisms, in combination with genetic, environmental and maternal factors all determine the occurrence and progression of gingivitis and periodontitis in childhood (Pihlstrom et al., 2005; Gasner & Schure, 2021). The initiation of gingivitis begins with bacterial biofilm or dental plaque forming soft deposits and sticking to tooth surfaces. The involved bacterial microorganisms are both gram positive and gram negative, which interact with the gingival tissues and generate a response from the immune system, which is usually gingival inflammation.

Plaque induced gingivitis is the most common form of gingivitis in children, and can be seen by swelling, redness and bleeding upon probing of the gingiva (Oh et al., 2002). Gingival and connective tissue damage that follows is from enzymes being released and tissues being destroyed, from the infiltration of neutrophils and lymphocytes as well as plaque biofilm producing collagenase and endotoxins (Watson, 2013). Gingivitis does not always lead to periodontitis and it is only progressive in susceptible patients. The aetiology for gingival diseases is linked with microbial colonisation, infectivity and inflammatory host responses (Page, 1986; Loesche, 1996; AAPD, 2020a). Chronic gingivitis in children is identified by collagen lost near the junctional epithelium and

infiltration of lymphocytes, with the addition of polymorphonuclear leukocytes, plasma, mast cells and monocytes.

Childhood gingivitis has been characterized as containing more T-lymphocytes than in adult gingivitis and fewer B-lymphocytes and plasma cells in the infiltrate (Page, 1986; Rathee & Jain, 2021). In addition, the junctional epithelium is thicker for children with primary teeth, and this contributes to children being less likely to develop periodontitis (Pari et al., 2014). Experimental models of gingivitis in children have showed higher levels of sub-gingival micro-organisms *Actinomyces, Capnocytophaga, Leptotrichia,* and *Selenomonas*, which usually do not present in adult gingivitis (Newman et al., 2006; Al-Ghutaimel et al., 2014). Gingival diseases in children usually range from mild to moderate and occasionally to severe (Hemalatha et al., 2017). Clinical and radiographic assessment helps to determine the extent of the disease.

2.11.1.2 Classifications of childhood gingival and periodontal diseases

Since the 1930s it has been established that young children are susceptible to gingival and periodontal diseases. Gingival diseases can be acute or chronic and are quite similar in children and adolescents. Clinical observations to identify gingivitis include redness, oedema of gingival margins, bleeding on probing and enlarged or bulbous interdental papillae. In children, T-lymphocytes have been typically observed in gingivitis and B-plasma cells in adolescent gingivitis (Newman et al., 2021). Specific bacteria, as already discussed have been associated with gingivitis in children and adolescents compared with adults; however, the exact microbiology remains to be entirely determined.

The seven main types of gingival diseases in childhood are presented in **Table *3***, with their brief descriptions (Al-Ghutaimel et al., 2014; AAPD,2020a).

Table 3

Classifications for childhood gingival diseases

Classification terms	Brief description
Eruption gingivitis	Gingival inflammation is present from aggravation of tooth eruption.
Pubertal gingivitis	Hormonal changes during puberty cause the on set of this, the gingiva proliferates and vasodilates due to inflammation caused by the hormones.
Mouth-breathing related gingivitis	Mouth breathing in effect dries out the gingival tissues and increases the likelihood of gingivitis from increased plaque production.
Drug induced gingival overgrowth	Gingivitis due to medications causing gingival overgrowth.
Malnutrition associated gingivitis	Vitamin C deficiency can cause scurvy and therefore gingivitis, due to the lowered collagen production.
Acute necrotizing ulcerative gingivitis	Compromised host defences, inadequate oral hygiene, stress, and HIV infection all make patients prone to acute necrotizing ulcerative gingivitis.
Primary herpetic gingivostomatitis	Caused by infection with herpes simplex virus type 1

Periodontal diseases only pertaining to children had various titles given by the American Academy of Periodontology in 1986 and were re-classified in 1989. These consisted of early onset periodontitis, juvenile: either localized or generalized; pre-pubertal and rapidly progressive. Otherwise periodontitis may be from systemic disease, necrotizing ulcerative periodontitis, and refractory type (Dowd, 1999; AAPD, 2020a), refer to **Table *4***.

Table 4

Childhood gingival and periodontal disease classifications

Classification terms	Brief description
Early on-set periodontitis. Pre-pubertal, juvenile, or rapidly progressing periodontitis, could be localised or generalized and generally presents in younger adults.	Periodontal disease in people under < 35 years old, where the bacterial microflora differs from adult periodontitis. Rapid rates of disease progression occur. It is associated with more gram-negative anaerobic bacteria below the gingiva.
Periodontitis from systemic disease	Periodontal disease associated with systemic disease such as diabetes or leukemia.
Acute necrotizing ulcerative gingivitis (ANUG)	This condition is whereby viral infections, fevers, malnutrition, emotion related stress, systemic disease and sleep deprivation can pre-dispose children to ANUG.
Refractory type	Periodontal disease not improving, even with appropriate treatment and good oral hygiene measures. It can be linked with host responses.

Different workshops over time have re-classified the categories for periodontal diseases. The most recent periodontal classification by Caton et al. (2018) reclassified periodontal conditions by three major categories of: i) periodontal health, gingival diseases and conditions, ii) periodontitis and iii) other conditions which affect the periondontium. This is shown in **Figure *5.*** The new system incorporated staging and grading for periodontitis, which can be attributed with the severity of the disease over time, it also further, looks at peri-implant diseases and conditions, however, this is not relevant in children so it is not discussed here.

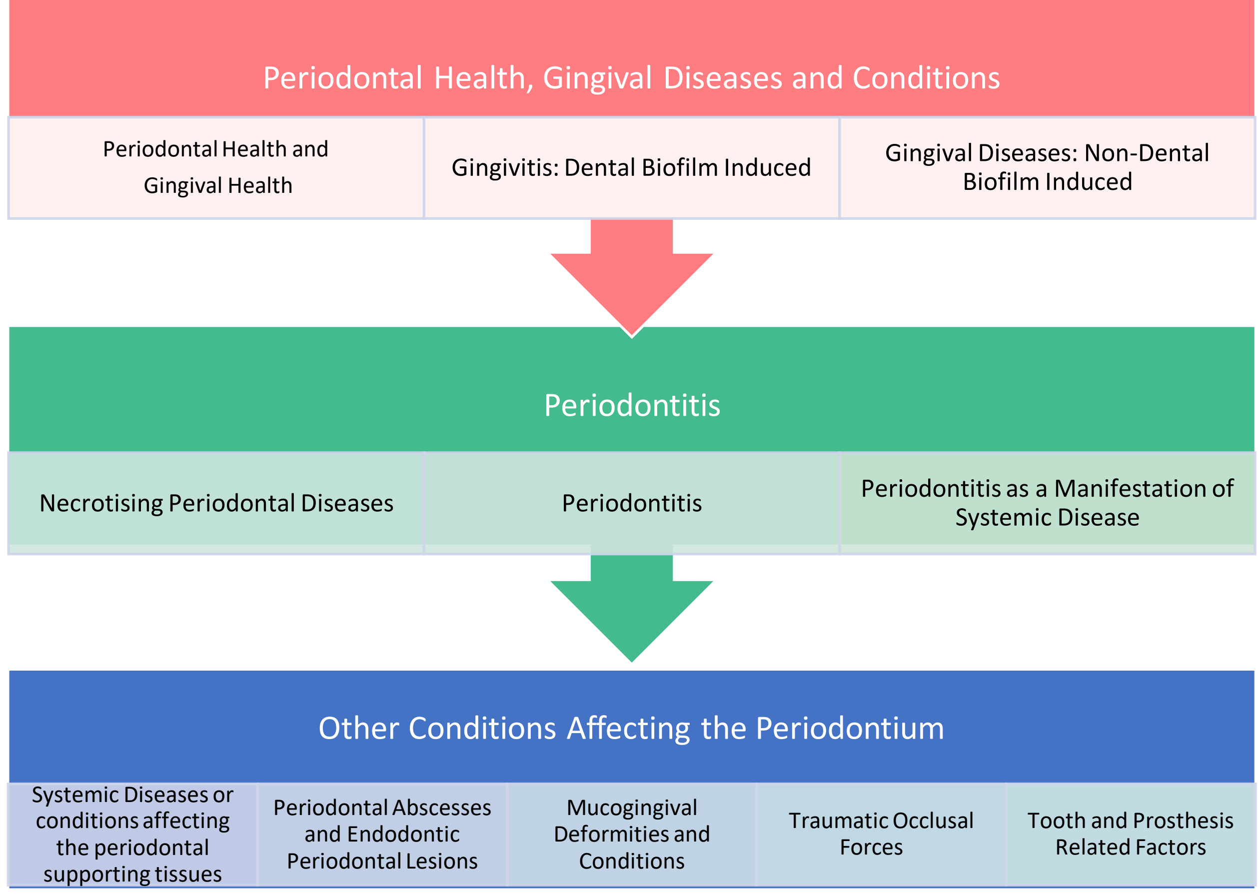


Figure 5

The 2017 classification for periodontitis

Adapted from Caton et al. (2018)

2.11.1.3 Plaque and gingival index scores

Plaque index and gingival index scores were used to measure gingival inflammation when assessing the soft tissues of the study children. Plaque and gingival indices by Löe and Silness (1963) were used in this study, to establish the presence of periodontal disease in these study children.

The Oral Hygiene Index, developed in 1960, measures the presence of calculus and debris on permanent teeth, therefore it was not appropriate for this study and could not be utilized for small children with primary dentitions (Greene, 1967).

The modified gingival index developed in 1986 was another method that could have been used in this study, but it allowed only visual examination and labial, lingual, and

interdental papilla surfaces to be examined (Lobene et al., 1986). Simply by observing the gingival areas these surfaces could have proved more difficult to examine and decipher for gingival disease, in this age group of children, as often bleeding or signs of inflammation (especially mild) are more obvious when probed or air-dried.

There was no calculus index used in this study, as it was unlikely many children would have hard calculus deposits within such a short time following tooth eruption period. It was also not an objective measure to assess presence of calculus on teeth. A periodontal assessment that could be undertaken using the Community of Periodontal Index of Treatment Needs (CPTIN) or Basic Periodontal Examination (BPE) was not used in this study unlike other studies (Preshaw, 2015), as it was not required. The two methods evaluate treatment requirements for periodontal disease, where probing depths over 3 millimetres (mm) would have been required. It was not the aim or intention of this study to take measurements of the teeth and gingival margins to determine the health of the periodontium, therefore the simplest of methods were used to assess periodontal health in the study children.

The relative strengths and weaknesses of the plaque and gingival index scores include: that plaque and gingival indices can be used to quantitatively record and measure gingival inflammation, to show what was clinically observed. The suitability of the plaque index method is that it could be used in primary dentitions. The strengths of this method are the validity and reliability of these indices, as well as being able to be used by a variety of different practitioners and still find the same results for plaque and gingival index scores. The plaque and gingival index scores reveal baseline data of the oral soft tissue conditions, and whether oral hygiene measures are being carried out effectively or not and if at all. The plaque index score allows examination of four tooth surfaces closest to the gingiva and assesses how much plaque is present, this ranged from no plaque (score=0) to an abundance of plaque (score=3) (Löe, 1967).

Gingival index scores are used to describe the severity of gingivitis, ranging from absence of or no inflammation (score=0) to severe inflammation or gingivitis (score=3). This method of scoring also examines four tooth surfaces, required air to blow dry the oral tissues and teeth surfaces, and a blunt periodontal probe was used to assess gingiva (Löe, 1967).

In chapter four section 4.8, the plaque and gingival index scores will be referred to again.

2.11.1.4 Risk factors for gingivitis in childhood

Local and systemic risk factors of gingivitis in childhood are important, as they may be modified, or their presence may indicate the need for extra attention and care to prevent and monitor gingival diseases in individuals. The presence of plaque is of utmost significance and is usually a risk factor when oral hygiene practices are poor or infrequent. Tooth eruption and exfoliation periods also increase the likelihood of gingivitis, since children and/or parents may not brush teeth and gingiva properly or avoid certain areas due to discomfort, as well as food impaction areas presenting when teeth have been lost or are erupting (Newman et al., 2006; Pari et al., 2014). Hormonal changes during puberty also increase the probability of abundantly accumulated plaque on tooth and gingival surfaces. Children who are immuno-compromised, malnourished, suffering from fevers such as malaria, measles, or chicken pox are more prone to getting acute and necrotic gingivitis (AAPD, 2020a).

Mouth breathing also increases the risk of gingivitis as the oral mucosa and therefore plaque dries out as an after effect, which therefore promotes retention of plaque with resulting bacterial multiplication (Jacobson, 1973). Similarly, medications can cause dry-mouth and impaired salivary flow, thereby increasing the susceptibility for inflammation of the gingival tissues (Olczak-Kowalczyk et al., 2019; AAPD, 2020a).

Orthodontic appliances, which include fixed and/or removable appliances in children, also favour plaque retention, contributing to higher incidence of gingivitis and caries (Arikan et al., 2015). Gingivitis in childhood is more prevalent in children from low socio-economic backgrounds, and those living rural or remote areas (Assaf et al., 2006).

2.11.1.5 Prevention of gingivitis in children

The prevention of childhood gingival disease is essential due to the nature of the disease and the high incidence of periodontitis, which may follow. Childhood gingivitis increases susceptibility to periodontal diseases occurring in adulthood, thus, making it a priority to prevent in childhood (Yengopal et al., 2009). The prevention of gingivitis

begins with oral hygiene practices, which foster the frequent removal of bacterial plaque and debris on teeth and gingiva. Young children are mostly reliant on parental aid for effective plaque removal due to age, limited understanding of oral hygiene importance and their limited manual dexterity skills. The removal of plaque in children can therefore be affected by parents/caregivers not assisting or adequately supervising tooth brushing, and/or irregular practices (Assaf et al., 2006). As already described periodontal bacteria may be horizontally or vertically transmitted to children, therefore every attempt should be made to not pass this bacterium on from another person to a child and to limit the disease pathogens in parents or individuals who may be affected (Kagihara et al., 2009).

2.11.1.6 Management of gingivitis and periodontitis in children

To treat gingival and periodontal diseases, the presence of its signs and symptoms needs to be documented first, by thorough intra-oral clinical examination. The examination may require periodontal probing to record pocketing depths, assessment of the gingiva colour, gingival contour, swelling, inflammation, recession and recording the presence of suppuration. Radiographs may be taken if necessary and oral hygiene practices and risk factors must be considered (Lopez et al., 2005). Moynihan and Petersen (2004) state “the main overriding factor in the aetiology of periodontal disease is the presence of plaque, and prevention measures focus on oral hygiene”. Gingivitis and periodontitis in children are usually managed by mechanical debridement of plaque and calculus that is present around teeth and gingival areas. Systemic antibiotics may be considered in conjunction with periodontal therapy when the condition is advanced (Oh et al., 2002). Medically unfit children are predisposed to more plaque accumulation and will therefore be more at risk of gingivitis and periodontal disease (AAPD, 2020a).

Antifungal and anti-bacterial therapeutic oral hygiene agents are strongly recommended for the care of gingival and periodontal tissues of children affected, along with a home care routine, which includes tooth brushing and flossing. The clinical treatment by a dental professional usually includes removal of plaque and calculus by scaling and periodontal debridement. Local and systemic methods can be employed to manage gingivitis and periodontitis in children (American Academy of

Periodontology, 2008). Overall, the prevention of disease and treatment is aimed at minimizing bacterial biofilm and plaque, limiting caries risk factors, and stopping periodontal disease progression, to ultimately save teeth affected by gingival and periodontal disease (Chapple et al., 2015). “Several studies have demonstrated that, under optimal conditions, the careful and regular removal of dental plaque can prevent the occurrence and progression of early periodontal disease” (Lopez et al., 2005). The management of gingivitis and periodontitis is therefore a combination of adequate home oral hygiene care and regular dental visits.

2.11.1.7 Epidemiology of gingival and periodontal diseases in childhood

In recent times, there has been an improvement in childhood gingival and periodontal diseases, however poor outcomes still exist in children with insufficient oral hygiene. Chronic-plaque induced gingivitis is most commonly observed in children, and it varies depending on behaviour, socioeconomic factors and access to dental services. Yengopal et al. (2009) state, “epidemiologic studies indicate that gingivitis of varying severity is nearly universal in children and adolescents”. Children can present with any form of periodontitis, but its advanced forms are rarely seen in infants (Oh et al., 2002). Severe periodontal disease in childhood is usually related to the presence of other systemic disease (Cho & Cheng, 1999; AAPD, 2020a).

2.12 The incidence of childhood dental caries worldwide

Despite the reductions in the incidence and severity of dental caries in developed countries, it remains a prevalent health issue for children worldwide (Bagramian et al., 2009; Kassebaum et al., 2017). ECC is at endemic proportions in non-industrialized countries and within some ethnic minority groups in developed countries (Chen et al., 2019). ECC is five times more frequent than asthma in the US (Ramos-Gomez et al., 2007) affecting 60-90% of children worldwide (WHO, 2020).

Over a third of children aged between two and nine years old in the US who come from poor backgrounds, live with untreated dental caries on one or more teeth (Selwitz, 2007). Immigrant children in The Netherlands, United Kingdom (UK) and Sweden have been reported to experience a three times higher prevalence of ECC compared to non-immigrant children within the same countries (Hallett & O'Rourke,

2003). Reasons for these disparities include limited access to dental services, cost of dental care and lack of knowledge about available dental provisions.

The prevalence of dental caries among five-year-old children in the Africa and the Middle East is reported to be >50%, with the incidence of ECC having been between 36-85% for three-year-old children in Far East Asia for some time now (Cariño et al., 2003; Chen et al., 2019). In Canada, the incidence of ECC is reported to be between 46-65% for children aged one to three years (Newman et al., 2006). Further, ECC affects 50-80% of more at risk Canadian children, such as those from poorer SES backgrounds, from ethnic minority groups and from single-parent families (Al-Ghutaimel et al., 2014). The prevalence of ECC in Taiwan and Southern Taiwan is reported to be between 56% and 69% respectively, for three-year-old children (Tang et al., 2013). In a recent systematic review and meta-analysis it was reported that the prevalance of ECC in China ranges between 35% to 89% (Uribe et al., 2021).

In Western Australia, dental caries is the fifth and sixth most common cause for hospitalisation of Aboriginal children, who are aged between one to four years old, five to 12 years old, respectively (Chauhan et al., 2012). These international statistics are similar in Australasia, where 50% of six-year-old Australian children (Kimura & Ohara-Nemoto, 2007) and 49% NZ children, aged five to 11 years old, experience dental caries (MOH, 2010).

2.12.1 Dental caries in New Zealand children

Dental care in NZ is free for children from age zero to 18 years old. Dental services are provided through district health boards and private contracting dentists, with the objective being to limit and prevent dental disease in childhood and adolescence (Moynihan & Petersen, 2004; MOH, 2008; MOH, 2010). A recent NZ study in Northland, has reported that up to 40% of children aged five years were affected by ECC (Aung et al., 2019). The study was undertaken to assess if ECC was linked to previous hospitalisations in preschool children related to avoidable medical ailments, including injuries. The study results showed ECC had a significant association with injury related hospitalisations, however, not with respiratory conditions or other medical issues (Aung et al., 2019).

The NZ MOH statistics collected from community dental clinics also show that 40% of five-year-old children living in areas without fluoridated water were affected by dental caries in 2017, and this number was similar for children living in non-fluoridation areas (39%) (MOH, 2017). Māori and Pacific children were found to have higher dmft/DMFT scores on average compared to other ethnicities, when assessed at ages five and eight. Study limitations of data collection were that only children who were enrolled with the community dental clinics were included. The data source also acknowledges that fluoridation status was not known for some children and that some children may have resided in more than one household with these different houses having different water fluoridation status (MOH, 2017). However, these data are helpful in documenting how many school aged children who were seen at community dental clinics had ECC in their first teeth and how risk of caries varies with ethnicity and fluoridation status. Figure ***6*** shows the amount of NZ children who did not have dental caries at age five, per ethnic distribution and by water fluoridation status in 2018.

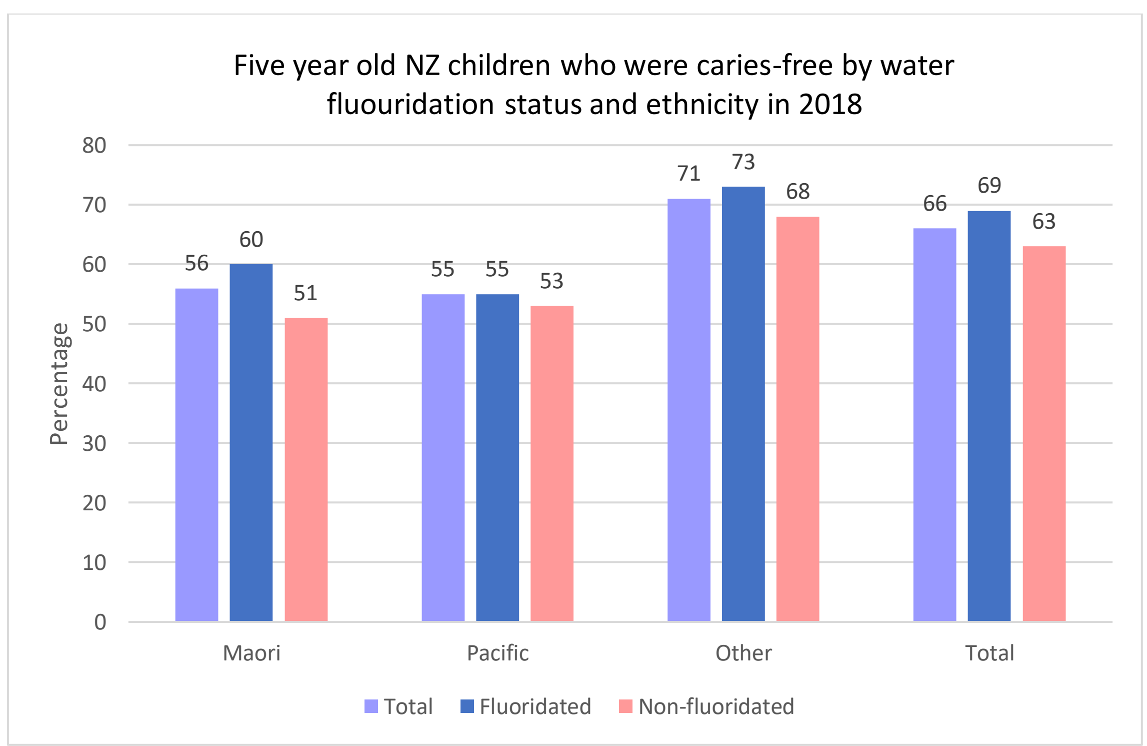


Figure 6

The proportion of NZ children (aged 5) who did not have dental caries by ethnic distribution, and water fluoridation status in 2018

In 2018, the mean dmft score for children of all ethnic groups was lower for those who were living in regions of fluoridated water, compared with those residing in non-

fluoridated water regions. Additionally, higher mean dmft scores by ethnic distribution were observed in Māori (2.9) and Pacific (3.3) children, compared with children of other ethnicities (1.3) (MOH, 2019b). One fifth (20%) of NZ preschool aged children (two to four years old) have experienced dental caries in their primary teeth, as reported by the 2009 NZ Oral Health Survey (MOH, 2010). Further, ethnic differences presented in children who were affected by dental caries, where a larger proportion of children (aged two to 11) were of Māori (52%) and Pacific (53%) ethnicities, compared with Asian (34%), NZ European or those from other (38%) ethnic groups (MOH, 2010). Dental disparities by ethnic group in NZ children are also present for access to regular dental services, where Māori and Pacific children are less likely to visit regularly compared with children of other ethnicities.

There has been little change since the early 1990s when Māori children were three times more likely to be affected by dental caries than non-Māori children (Thomson, 1993). In the present day, statistics for Māori children show that they are thrice more likely to have dental treatment under sedation (GA), and be three time more likely to be non-enrolled with the free community dental service (Broughton et al., 2013).

2.12.2 Improving the current rates of ECC in NZ

The literature review on ECC established and emphasized how prevalent and preventable this disease is, which affects numerous children in NZ (Bach & Manton, 2014). Those at most risk of ECC are young children, children of Māori and Pacific ethnicity and those with limited access to dental care in NZ.

The previous sections in this chapter have covered the risk factors for the initiation and development of ECC, as well as current prevention methods, management, and treatment options available for childhood dental caries. The most current global prevention strategy is by the WHO, who have developed a tool kit for preventing dental caries in young children (WHO, 2019a). Many treatments offered in dentistry for treatment of infectious diseases in a child's mouth can be invasive. As a result, children may endure more discomfort prior to or during dental treatment, or require local or general anaesthesia to undergo treatment, for instance to avoid pain when a dental professional may be filling a tooth or when having a tooth extracted (Fejerskov & Kidd, 2003).

The unfortunate effects and experiences of ECC can lead to decrease in quality of life for children, poor self-esteem, a higher probability of future dental caries and create a fear for future dental visits due to past negative experiences (Moynihan & Petersen, 2004). The prevalence of ECC also increases the economic burden for primary health care providers who often provide treatment which is expensive and/or entails multiple appointments or long waiting lists. Oral health practitioners aim to provide regular dental examinations for high-risk patients, so they can decide timely interventions for early preventative measures or referrals to the hospital or dentist.

To improve ECC statistics and future oral health outcomes, other components that influence the susceptibility of primary teeth to childhood dental diseases need to be considered. For instance, dental defects like EHP that predispose teeth to ECC should be considered, when looking for ways to protect tooth surfaces from childhood dental caries. Approaches that reduce health inequalities are required to provide dental benefits for children before they develop ECC.

Dental defects are known risk factors for ECC (Seow, 1998). Research indicates that vitamin D supplementation may have the potential to reduce dental defects on primary teeth and protect teeth from dental caries (Schroth et al., 2012; Hujoel, 2013). This will be fully reviewed in chapter three.

The purpose of this literature review was to outline the prevalence of childhood dental diseases in NZ, and how ECC can impact upon dental and overall health of young children. Restorative management techniques that exist to treat this disease can only be advised post tooth eruption and after the disease has occurred. The study presented in this thesis proposes an alternative method to prevent ECC, by supplementing pregnant mothers and their children with vitamin D during tooth development periods. In this way, if teeth are more resistant to dental caries with vitamin D supplementation being an effective means to preventing dental disease and dental defects, there may be a reduction in the rates of dental caries and reductions in OH inequalities.

Figure ***7***, summarises in utero (pregnancy), infancy and early childhood risk factors covered in this literature review, which predispose to the development of ECC, and are necessary to address to prevent ECC.

Pregnancy

- Maternal vitamin D status
- Micronutrient deficiencies --> foetal development
- Maternal oral hygiene
- Pre-term birth
- Socioeconomic status
- Mothers nutritional intake

Early infancy

- Newborn vitamin D status
- Ages at tooth eruption
- Neonatal rickets
- Dental defects
- Oral microbiome
- Breast/bottle feeding practices
- Low birth weight
- Parental income
- Ethnicity
- Bacterial transmission
- Sugars
- Oral hygiene

Early childhood

- Vitamin D status
- Dental defects
- Dental plaque
- Salivary flow
- Sugar
- Tooth brushing
- Dental visits
- Fluoride
- Childhood rickets

Figure 7

The potential risk factors for ECC in utero and after birth

The intent of the following chapter is to review the understanding of the relationship of ECC with vitamin D supplementation, to establish if early life vitamin D supplementation may improve future childhood dental outcomes.

Chapter 3 Literature review of vitamin D supplementation and its effect on ECC and developmental dental defects

3.1 Introduction

Much of the literature available today supports the notion that vitamin D supplementation may be beneficial in preventing dental disease (Hujoel, 2013; Schroth et al., 2014). Moreover, vitamin D supplementation has been linked with reducing existing dental caries in children's teeth (Mellanby & Pattison, 1928), as well as preventing the occurrence of developmental dental defects in primary teeth (Norrisgaard et al., 2019; Deng & Niu, 2020). Maternal vitamin D status during pregnancy has also been linked with ECC in their child's primary teeth (Schroth et al., 2014; Tanaka et al., 2015). This has been attributed to vitamin D having a pivotal role in tooth development that begins in utero, as vitamin D impacts tooth formation, like it impacts bone mineralisation (Botelho et al., 2020).

This literature review presents a summary of the published literature on the potential of vitamin D supplementation preventing ECC and developmental dental defects.

Firstly, the role of vitamin D in the normal functioning of the human body, in health and well-being will be described in this chapter. Following this, the different sources of vitamin D will be described. Prior studies on vitamin D and developmental dental defects and dental caries will be critically reviewed, before discussing the incidence of vitamin D deficiency in NZ, and its potential implications for children's oral health.

3.1.1 Aim of the literature review

The aim of this literature review was to summarise the existing knowledge on vitamin D and dental caries and vitamin D and developmental dental defects and identify that there is a need for this dental research.

The prevention of dental caries in the context of supplementing children with vitamin D pre-birth and in early infancy has not been fully explored, particularly never before in a high caries risk population of Auckland, NZ. Therefore, by identifying other studies and research in high caries risk populations and child based studies overseas, this literature review stems as a base for further research in this area in young NZ children

where oral health needs are high and vitamin D supplementation may serve as a caries preventive solution, by protecting against this non-communicable disease, ECC.

This literature review focused on studying research findings for studies that were conducted in children where vitamin D supplementation was given, or where the effects of vitamin D on dental caries or developmental dental defects, and gingivitis were observed.

3.1.2 Methods

There are few studies to date that have investigated the impact of vitamin D on ECC and developmental dental defects in childhood. To ensure that the available published literature in this specific domain was comprehensively reviewed, a detailed review per study has been provided and the effects of vitamin D and dental defects, were compared from similar age groups and study populations.

The following oral health outcomes that were focused on for this literature review were ECC, developmental dental defects and childhood gingivitis.

3.1.3 Approach to the literature search

The primary sources where published literature was sought from for this review, includes the databases: “Google Scholar”, “Science Direct”, “Medline”, “PubMed”, “EBSCO” and “Web of Science”. The reference lists of some selected articles were also reviewed, and relevant cited articles were studied. The key words used to search the literature on vitamin D supplementation and its effects on dental health can be seen in **Table *5***.

Table 5

Key words for the literature search on vitamin D supplementation and its effects on children's dental health

Key words
Vitamin D supplementation and children's dental health
Vitamin D and ECC
Vitamin D and dental caries
Vitamin D and developmental dental defects
Vitamin D and gingivitis in children

The boundaries to the search of the literature included in this chapter, were:

- No studies prior to the 1900s were included
- Only child-based population studies were included (where children were below 15 years old), and
- The focus was on vitamin D and dental caries and/or developmental dental defects in children

Studies that were included in this literature review therefore, had to meet the four following conditions:

5. The study was conducted in child populations (for instance, age relevant studies in infants, toddlers, preschool children, or school aged children).
6. Primary teeth or mixed dentitions were examined and reported in the study.
7. The study investigated the relationship of vitamin D with the health of primary teeth.
8. The study included an investigation of some form of vitamin D, for instance dietary supplementation, vitamin D supplementation, or the effects of sunlight exposure on a dental outcome.

The excluded studies had the following attributes:

- Studies with only older children or adult participants
- Studies in which only the permanent dentition were examined
- Studies in which there was no evidence of vitamin D being investigated in relation to primary dental health

- Studies in which vitamins other than vitamin D were investigated (**Figure *8***).

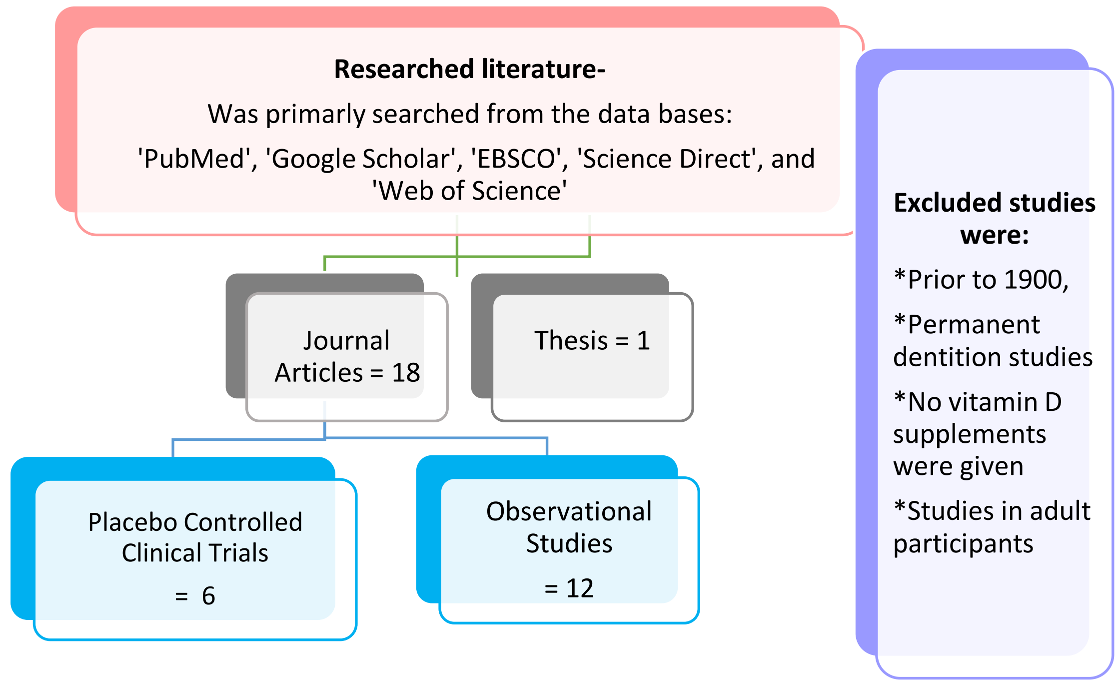


Figure 8

A summary diagram of literature sources identified that investigated the relationship of vitamin D with ECC and developmental dental defects

There have been three systematic reviews and meta-analyses that have investigated the relationship of vitamin D with dental outcomes, with the first one published in 2013 (Hujoel, 2013; Theodoratou et al., 2014; Botelho et al., 2020; Cagetti et al., 2020). Two of the meta-analyses looked at the corelation of vitamin D with dental caries and one at vitamin D and periodontal diseases. The intention of this literature review has primarily been to focus the attention on vitamin D supplementation and its effects on ECC and developmental dental defects in childhood, which is what makes this literature review unique. The studies that were reviewed have been listed in **Figure *9*** as per the scientific hierarchy of best available research. These studies will be individually discussed in section 3.5.3.

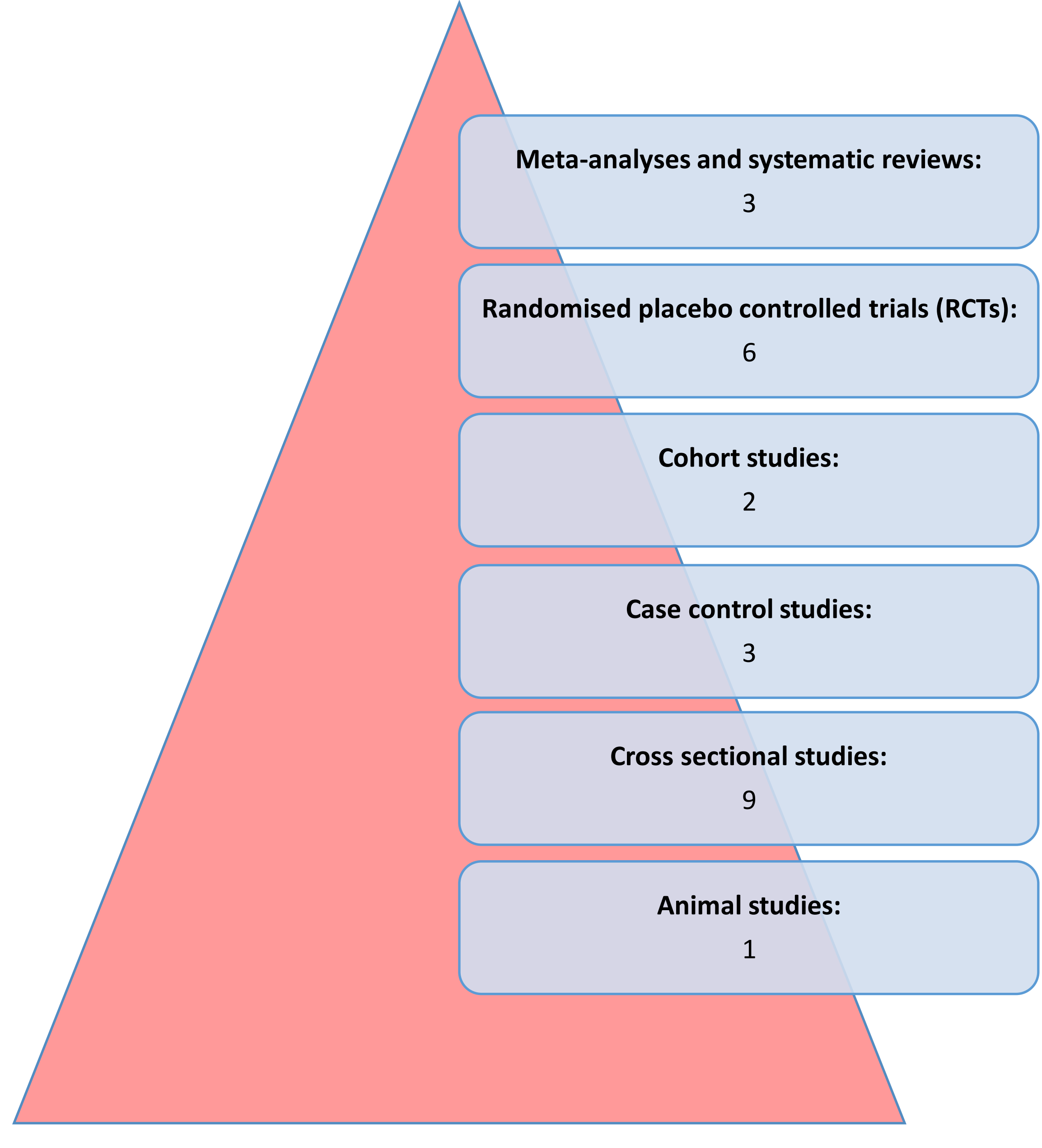


Figure 9

Studies that were included in this literature review, as per the hierarchy of best available scientific evidence

The background on vitamin D in relation to dental health will be presented next, followed by a review of the role of vitamin D in health and its physiology.

3.2 Background on vitamin D and childhood dental health research to date

Historical and contemporary studies have reported that vitamin D supplementation appears to improve childhood dental health by reducing dental caries in children's teeth (Mellanby et al., 1924; Schroth et al., 2012). There have been very few studies that have investigated early life vitamin D supplementation and its effects on children's

dental health outcomes. To date, there have been six placebo-controlled trials that have explored the relationship between vitamin D supplementation and children's dental health. These will be described later in section 3.8.

Contemporary studies have explored the effects of different vitamin D supplementation doses in pregnancy and in early childhood on ECC as well as developmental dental defects. The vitamin D doses used have ranged from 100,000 international units (IU) given in pregnancy to 100 IU given in childhood. However, there have been no investigations to date, exploring the effect two different doses of vitamin D supplementation during both pregnancy (of 1000/2000IU) and infancy (of 400/800 IU) on ECC and developmental dental defects. The following section presents an overview of the role of vitamin D in overall health and well-being.

3.3 Vitamin D in health

Vitamin D is a fat-soluble vitamin that is essential for bodily functions. Vitamin D plays a central role in the metabolism of calcium and phosphorus (Grant, 2011; Williams, 2014) and it is also recognised to be a modulator for the function of the immune and neuromuscular systems (Grant, 2011; Davit-Beal et al., 2014; Natarajan et al., 2014). Calcium is an important mineral for adequate calcification of bones and teeth in the human body as well as assisting other cells in the body to maintain a stable equilibrium.

The benefit of having sufficient vitamin D in the body is that it contributes to the normal development of bones and teeth and plays a crucial function in regulating the immune system by maintaining calcium homeostasis.

A broader role of vitamin D has been proposed in health, where vitamin D deficiency (VDD) has been associated with increasing the risk of a range diseases including diabetes, cardiovascular diseases and certain cancers (Meinerz et al., 2016). VDD additionally adds to the risk of bone fractures and falls, as it worsens osteoporosis, causes osteomalacia and reduces muscle strength (Bener et al., 2013).

VDD is a prevalent health condition worldwide (Amrein et al., 2020). There are individuals in all populations and across all age groups who have an increased risk for vitamin D insufficiency compared to the general population (Palacios & Gonzalez,

2014; Aguiar et al., 2017). These include people who have darker skin tones, those from ethnic minority groups, pregnant women, infants, children with low birth weight, adolescents, older adults and non-Western immigrants (van Schoor & Lips, 2018). Furthermore, the risk of VDD for children is increased by the presence of chronic illnesses, such as kidney disease, cystic fibrosis, sickle cell disease and asthma, as does having darker skin pigmentation, being a purely breast-fed infant, with insufficient vitamin D in breastmilk if the mother has VDD (Pitts et al., 2007; Adeniyi & Odusanya, 2017; Pettifor et al., 2018).

3.3.1 Vitamin D and oral health

It was first discovered by May Mellanby, more than 100 years ago, that dietary vitamin D supplementation provided resistance to dental caries in children, as it aided tooth development by promoting the calcification of tooth enamel (Mellanby, 1918). Mellanby and Pattison (1928) also confirmed that diets high in vitamin D were able to arrest existing carious lesions, by its effect of calcifying tooth enamel as well as being able to stimulate the production of secondary dentine. Thereby vitamin D supplementation was able to have a “hardening” or strengthening effect on teeth. Mellanby’s pioneering research found that vitamins A and D given to children via fortified cereal were able to reduce the probability of dental caries and EHP (Mellanby, 1918; Mellanby et al., 1924).

Since then, Schroth and his team have investigated and reported that lower maternal 25(OH)D levels were associated with higher rates of ECC and S-ECC on children’s teeth (Schroth et al., 2012; Schroth et al., 2014). This is due to VDD in pregnant women having unfavourable effects on the formation of children’s teeth, thus posing higher risks for children to acquire ECC from weaker tooth structure and by having acquired developmental dental defects. Low vitamin D statuses in pregnant and lactating women are known to increase the probability of their children developing developmental dental defects in their primary teeth, such as EHP, which places them at a higher risk of acquiring ECC (Schroth, Lavelle, et al., 2005).

VDD has a direct impact on amelogenesis (tooth enamel formation). When vitamin D deficiency is severe (serum 25(OH)D concentrations are <10 mg/mL (25 nmol/l)), it causes hypocalcaemia. This makes it possible for developmental dental defects to form

on teeth, as with insufficient vitamin D, there are lower concentrations of Ca^{2+} and phosphate ions available for the mineralisation of teeth (Botelho et al., 2020). Therefore, inadequate vitamin D status during periods of tooth development affects the quality of dental tooth tissues formed, and the teeth produced are then more vulnerable to dental caries.

Studies performed in several countries have also found correlations between vitamin D status and dental caries in children, these studies will be discussed in section 3.8. In the next section, the physiology of vitamin D and its functions in the body will be described.

3.4 The physiology of vitamin D

Vitamin D exists in two primary forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is produced in the skin by its exposure to sunlight (UV radiation). Vitamin D_2 or ergocalciferol is generated from ultraviolet irradiation of ergosterol, present in some plants. Both forms of vitamin D are also available from dietary intake (Lips, 2006).

Skin exposure to ultraviolet rays begins the production of vitamin D_3 enabling the synthesis of 7-dehydrocholesterol which exists in epidermal cells of the skin (Pitts et al., 2007). The steroid produced by the dehydrogenation of cholesterol goes through photolysis, which is where pro-vitamin D_3 (7-dehydrocholesterol) in the skin is transformed to pre-vitamin D (DeLuca, 2008; Camargo et al., 2010). Vitamin D_3 is then hydroxylated in the liver to 25-hydroxyvitamin D.

Vitamin D_2 also has a hydroxylation step in the liver to develop into the form of vitamin D (25-hydroxy vitamin D (25[OH]-D, calcidiol, or calcifediol) which can be stored in the body (Pitts et al., 2007) (see **Figure *10***). The bioactive form of vitamin D in circulation is 25(OH)D, which is measured to assess the vitamin D status of individuals (Chen et al., 2010) (see **Figure *10***).

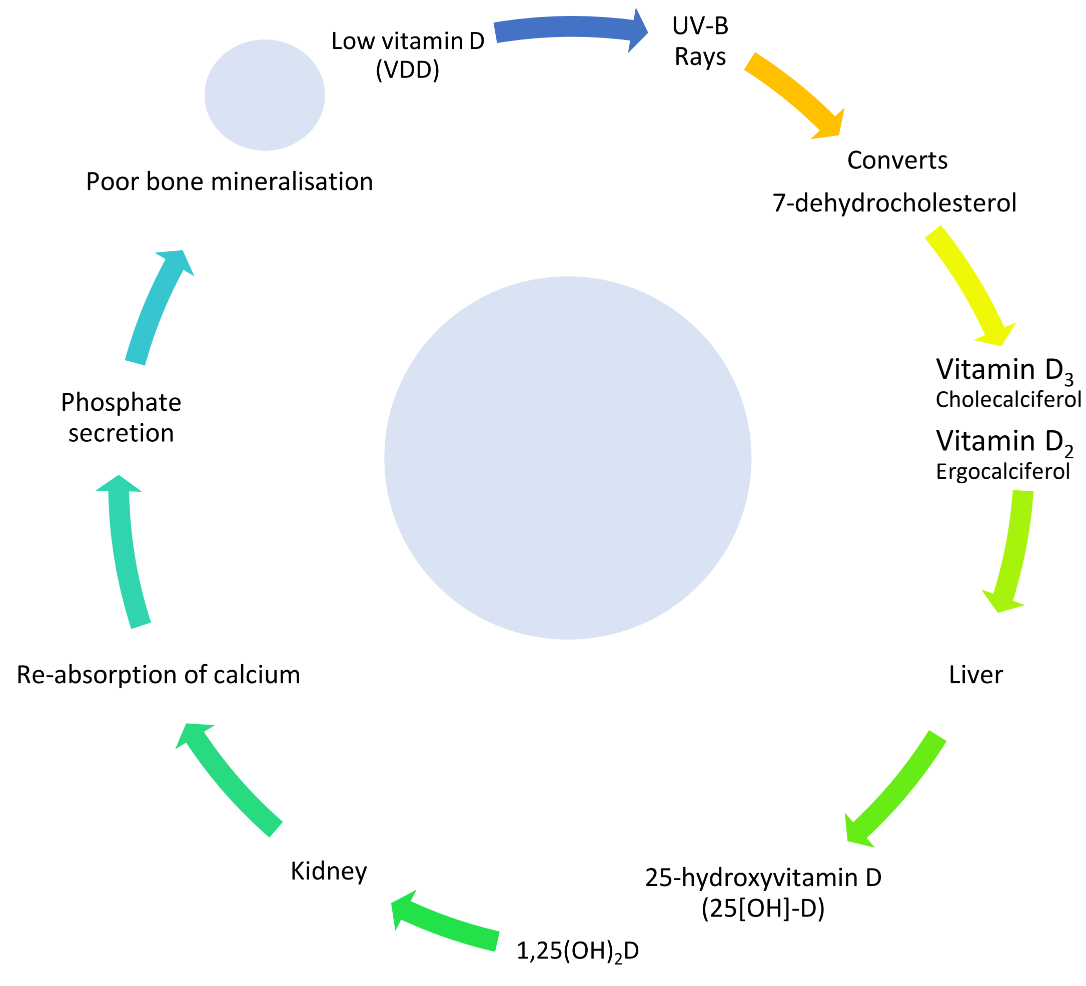


Figure 10

Vitamin D conversion and calcium absorption

The metabolic pathway of vitamin D begins from the pro-hormone 25-hydroxyvitamin D (25(OH)D), which is hydroxylated in the kidney into the functioning form of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D$), or calcitriol. The biologically active form of vitamin D is $1,25(OH)_2D$ and it initiates calcium absorption from the gastro-intestinal tract. The hydroxylation step to produce $1,25(OH)_2D$ is now known to also occur in several other tissues and cells within the body (Dusso et al., 2005; Lips, 2006; Misra et al., 2008; Holick, 2012). In the immune system for instance, 25(OH)D is hydroxylated to $1,25(OH)_2D$ by macrophages and other immune cells. As a result the $1,25(OH)_2D$ produced, regulates the proliferation of T lymphocytes and influences the profile of cytokine production (Adams & Hewison, 2008).

3.4.1 Functions

The absorption of calcium and phosphorous into the small intestine is facilitated by 1,25$(OH)_2$D. If dietary calcium intake is insufficient to meet the body's needs, renal tubular calcium reabsorption is increased by the parathyroid hormone (PTH), and production of 1,25-dihydroxyvitamin D by the kidneys, is increased. The PTH stimulates osteoblasts to initiate the conversion of pre-osteoclasts into mature osteoclasts. Osteoclasts increase the mobilisation of calcium stored in bone. Through these various mechanisms vitamin D is thereby able to maintain calcium and phosphorous homeostasis (Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). Osteoclast induced dissipation of mineralised collagen matrix within bones can result in osteopenia and osteoporosis (Holick & Feldman, 1997). Insufficient calcium intake and vitamin D deficiency in utero and early childhood, may therefore result in bone calcium deficiency (Holick & Feldman, 1997).

3.4.2 Vitamin D deficiency (VDD)

Vitamin D deficiency (VDD) is defined by a serum (25(OH)D) concentrations <50 nmol/L (20 ng/mL) by the NZ MOH and the US Institute of Medicine and in most other countries (Drury et al., 1999; Holick, 2017). However, the serum 25(OH)D concentrations used to define VDD vary in published clinical studies. Serum 25(OH)D concentration is used to measure vitamin D status as it has a half-life of two to three weeks, and demonstrates resiliency to fluctuating PTH concentrations (Pitts et al., 2007).

VDD results in a decrease in dietary calcium intestinal absorption from 30 to 40% down to 10 to 15%, and a decrease in dietary phosphorus intestinal absorption from 80% down to 60% (Holick, 2012). The parathyroid gland responds, increasing the release of PTH in order to increase serum calcium concentration (Pitts et al., 2007). The secretion of PTH, gives rise to increased phosphorus excretion in urine and increases the reabsorption of calcium in the kidneys. There is also an increase in calcium re-absorption from bone. This process maintains serum calcium concentrations, but it also is responsible for causing a reduction in bone mineralisation (Pitts et al., 2007). The combination of decreased calcium absorption from the gut, increasing calcium utilisation from bone and urinary phosphorous excretion, leads to poorer

mineralisation of newly formed bone. The failure of newly formed bone tissue (osteoid) mineralisation manifests over weeks to months, clinically emerging as rickets (Tinanoff & O'Sullivan, 1997). Rickets can only occur if inadequate bone mineralisation happens prior to the fusion of bone epiphyses with their adjacent metaphyses (Herzog et al., 2016). Insufficient vitamin D, calcium and phosphorous absorption occurring after all epiphyses have fused still results in decreased osteoid mineralisation, which occurs at sites of bone modelling and remodelling rather than at epiphyseal growth plates. This pathological process is called osteomalacia (Herzog et al., 2016). See **Table *6*** next, for the symptoms of rickets disease.

Table 6

Body parts affected by rickets disease and the symptoms

Body parts affected by rickets disease	**Symptoms of rickets disease**
Entire body	Pain and/or irritation
Nervous system	Delayed development of gross motor skills
Wrists, ankles, other bony epiphyseal areas	Widening
Skull	Delayed fontanelle closure, thinning/ softening of the skull resulting in craniotabes
Teeth	Delayed tooth eruption, and/or dental defects on teeth
Bones	Poor growth and decreased mineralisation

3.4.3 The effects of vitamin D on the immune system

Vitamin D is essential for normal immunity as it regulates the production of anti-microbial peptides (AMPs) and alters the cytokine response within the body following foreign antigen presentation (Armitage, 2002). The innate immune system, which begins with epithelial cells, serves as the first physical defence barrier against infection or bacterial infiltration into the body. Vitamin D is a component of the innate immune system. Following exposure to pathogens, vitamin D stimulates the production of AMPs to provide protection against bacterial micro-organisms. In addition, $1,25(OH)_2D$ modifies the adaptive immune system function, both via the innate immune system and also via a direct effect on cells of the adaptive immune system (Schwalfenberg, 2011). Cells of the immune system have the capacity to obtain circulating 25(OH)D and metabolise this to the active hormone form of vitamin D ($1,25(OH)_2D$), via the enzyme

CYP27B1 (1α-hydroxylase). Initially CYP27B1 was only believed to be in the kidneys. However, it is now recognised that it can be found in a normal of organs systems and tissues including epithelial cells and immune system cells (McMahon et al., 2011).

It was theorized that vitamin D supplementation would improve tooth tissue formation and provide an on-going protective effect against dental caries, like that of topical fluoride. The potential impact vitamin D would have on changing the properties of saliva were also considered by researchers. As early as the 1930s it was proposed that vitamin D could control dental caries by its immunological functions (Mellanby & Pattison, 1928).

In a review of clinical trials of research on vitamin D and dental caries, Hujoel (2013) observed that vitamin D status during pregnancy may impact upon embryonic stages of tooth development. The meta analysis concluded that “this review of controlled clinical trials suggests that supplemental vitamin D causes a 47% reduced risk of caries” and that vitamin D and calcium supplements were found to collectively lower rates of tooth loss (Hujoel, 2013). This best available evidence suggested a link that VDD in pregnancy increased the risk of developmental dental defects in children and that caries risk was reduced with vitamin D supplementation. However, the quality of evidence presented was low and mostly cross sectional. Some limitations of the reviewed literature were that the researchers were unable to determine the methods utilised in 22 of the 24 studies carried out before the 1950s. Other potential sources of bias include that the diet, fluoride exposure, dental caries, serum vitamin D levels and sunlight exposure, were not reported consistently across the studies included in the review (Hujoel, 2013). Further biases include publication bias, where reports of negative findings for vitamin D on dental health were less likely to be published, as several researchers were strong advocates for vitamin D.

3.4.4 Vitamin D and childhood rickets disease: Epidemiological studies

Deficiency in vitamin D can cause defective bone formation, rickets disease and osteomalacia in childhood (Tinanoff & O'Sullivan, 1997; Misra et al., 2008; Camargo et al., 2010). Ricket disease was first described in Northern Europe and England in the mid-17th century. In the early 20th century rickets had become widespread in Northern Europe and the US, particularly due to industrialisation with pollution and high-density

housing, which restricted exposure to sunlight. It was not until 1918, that it was appreciated that rickets disease could be caused by a nutritional deficiency (Heersche & Kanis, 1990; Norman, 2012). This new understanding followed Sir Edward Mellanby's experiments, who demonstrated the successful treatment of rickets disease in rachitic beagle puppies with cod liver oil (Hawgood, 2010). Foods were, thereafter, fortified with vitamin D, which led to the reduction of rickets disease prevalence in industrialised countries.

A number of countries however stopped vitamin D food fortification after World War II, partly because of incidences of hypercalcemia being observed secondary to the consumption of vitamin D fortified food (Chen et al., 2010). Over the last several decades, there has been a re-emergence of rickets disease, primarily due to VDD (Soros et al., 2007; Natarajan et al., 2014). This is believed to have occurred for several reasons. Population groups where childhood rickets disease has been observed, include children born to women who were VDD whilst pregnant, children with more pigmented skin, exclusively breast-fed children and in mothers and infants with limited sunlight exposure. Rickets disease in this context is reported in infants from many countries, including China, India, and the Middle East. Infants have developed rickets disease in utero, secondary to severe maternal VDD during pregnancy (Misra et al., 2008). Therefore, childhood rickets can be prevented by improving vitamin D status in expectant mothers and children.

VDD and calcium deficiency both contribute to nutritional rickets in children globally. Nutritional rickets disease is prevalent in countries where there is the potential for sufficient sunlight exposure all year-round, such as Africa, Asia, and the Middle East. There has also been a re-emergence of childhood rickets disease in developed countries such as the US and Europe, possibly from increased immigration of darker skinned individuals, breast feeding and limited sunlight exposure and/or use of SPF sunscreens (Grant, 2011). Childhood rickets disease occurs because of limited skin exposure to sunlight for both mothers and children, which can be attributed to a larger proportion of time spent indoors, skin being covered by clothing and atmospheric conditions including those caused by air pollution (Schroth et al., 2014).

In the next section the available sources by which vitamin D is obtainable, will be discussed.

3.4.5 Sources of vitamin D

It is necessary for children to obtain enough vitamin D to avoid conditions linked with VDD, such as rickets. Vitamin D is obtained in three different ways, which have been alluded to earlier.

Vitamin D is attainable from the three following prime sources:

1. Sunlight or UV-B radiation absorption in the skin, results in vitamin D_3 production
2. Dietary sources which contain vitamin D; both natural and vitamin fortified food and drinks included, and
3. Vitamin D supplements (Carroll et al., 2014).

Humans mostly derive vitamin D in two ways, one is by direct synthesis of UV radiation into the skin, recognised as cholecalciferol (or vitamin D_3), and it can be obtained from diet, most commonly as ergocalciferol (or vitamin D_2).

3.4.5.1 Sunlight (ultraviolet [UV] rays)

The most effective way of obtaining vitamin D_3 naturally, is by skin exposure to sunlight or UV-B radiation, as already mentioned. Sufficient sunlight exposure to produce adequate amounts of vitamin D, is however hindered by factors like sun avoidance due to sunburn and skin cancer risk, geographical and seasonal barriers to natural sunlight, clothing, sunscreens, lifestyle and cultural factors (Rajab & Hamdan, 2002).

Vitamin D status of individuals is also influenced by latitude, season, time of day and skin colour, therefore any recommendations to obtain adequate vitamin D would need to consider these factors. Compared with people with fairer skin or minimal pigmentation, people with darker skin pigmentation require exposure to sunlight for a longer period to achieve adequate serum concentrations of 25(OH)D. The estimated sunlight exposure requirement for a darker skinned person is up to six times more than a fair skinned person (Fejerskov, 1997; Munns et al., 2006). The contrast to these

factors, which influence the adequacy of sunlight exposure for vitamin D production, make it very difficult to have fixed recommendations for all children and adults regarding both necessary and safe sunlight expose outdoors. It is also necessary for recommendations that are made about skin exposure to ensure that they do not place children at risk of skin damage and/or cancer in the future. The American Academy of Pediatrics (AAP) recommend that children under six months should be fully kept out of direct sunlight to reduce skin cancer risk (Misra et al., 2008).

According to the current WHO guidelines, there are no recommendations for required amount of sunlight exposure for breastfed infants to achieve and maintain adequate vitamin D status, as defined by a serum 25(OH)D concentration of >50 nmol/L (20 ng/mL) (Misra et al., 2008; WHO, 2019b). According to the WHO new born babies have low serum vitamin D stores at birth, therefore, they require vitamin D attainment from breast milk, sunlight, or supplementation in early infancy (WHO, 2019b). Since mother's vitamin D status can be low during pregnancy and whilst lactating, low vitamin D content in breast milk adds to the risk of infants acquiring VDD. Moreover, other risk factors for infants developing VDD include living at higher latitudes, restricted sunlight exposure or cultural norms (for instance wearing covering clothing), which can all influence the chance of childhood diseases caused by VDD. The current recommendations are to provide vitamin D supplementation to infants and young children who are at risk of VDD, to prevent rickets, these include those who have insufficient sunlight exposure and/or children who have darker pigmented skin (WHO, 2019b).

A study conducted in Ohio by Specker et al. (1985) in infants under six months old, who were followed over 12 months, described sufficient sunlight exposure for 61 fully breast-fed term infants to maintain serum 25(OH)D concentrations greater than 27.5 nmol/L (11 ng/mL), was 30 minutes a week wearing diapers and two hours per week in clothing. Children in this study who had darker skin pigmentation were reported to have lower levels of vitamin D_2 and D_3 in breastmilk and 25-hydroxyvitamin D_3 concentrations, when compared to the children with minimal skin pigmentation. In addition, the study found, 25-hydroxyvitamin D_2 concentration levels did not differ in the study children, but it was thought that despite milk and vitamin D intake being correlated, sunlight exposure still proved to be a more significant source of vitamin D

(Camargo et al., 2010). Currently more research is being completed to investigate and base recommendations about acquiring vitamin D via sunlight, diet or supplementation (MOH, 2019b).

In NZ, due to seasonal variations of sunlight intensity and high skin cancer rates, it is difficult to make population wide recommendations on sunlight exposure. Currently there is not a scientifically validated “safe threshold” for how much one should expose themselves to sunlight (UV) for the necessary vitamin D production without increasing one’s risk for skin cancer (MOH & Cancer Society of NZ, 2012).

In NZ, it is recommended to apply a sunscreen to skin prior to sun exposure, which is known to reduce overall vitamin D production. Sunscreens with a sun protective factor (SPF) of ≥15 will absorb about 99% of the UV-B rays to which the skin is exposed, thereby limiting vitamin D production. NZ guidelines recommend that a sunscreen with a minimum SPF of 15 should be applied to skin if staying more than 15 minutes in the sun (Holick, 2008; MOH & Cancer Society of NZ, 2012). Modern lifestyles, for example working indoors, additionally limits exposure to sunlight as can skin conditions. UV-B radiation is also absorbed by glass (Munns et al., 2006). Sunbeds are not advised to improve vitamin D status, as they are associated with an increased risk of melanoma (MOH & Cancer Society of NZ, 2012).

3.4.5.2 Food sources containing vitamin D

Vitamin D can be obtained by eating foods rich in vitamin D (see **Table 7** *and*
Table *8*), though adequate levels of vitamin D are difficult to attain by diet alone (Axelsson, 2000). Very few foods naturally contain vitamin D, some of which are: oily fish like salmon or tuna, eggs, and animal liver. Dietary intake contributes to a small portion of overall vitamin D consumption, as people generally do not eat many foods which contain vitamin D (see **Table *9***).

Table 7

Foods naturally containing vitamin D

Food types	Varieties
Caviar (fish eggs)	Beluga, starlet, ossetra, and sevruga
Seafood	Oysters, shrimp, fish: mackerel, salmon, sardines, cod liver oil, trout, tuna
Organ meats	Animal liver
Eggs	Egg yolks
Mushrooms	Maitake, morel, chanterelle, oyster and white
Tofu	Silken tofu, sprouted tofu, firm/extra firm tofu
Dairy alternatives	Soymilk, almond milk and rice drinks

Table 8

Natural and fortified vitamin D foods and their vitamin D content

Food	Vitamin D Content in International Units (IU)
Atlantic herring (raw)	1628/100 g
Butter	35/100 g
Calf liver	15–50/100 g
Canned pink salmon with bones in oil	624/100 g
Canned tuna/sardines/salmon/mackerel in oil	224–332/100 g
Cereal fortified	40/serving
Cheddar cheese	12/100 g
Codfish (raw)	44/100 g
Cod liver oil	175/g; 1360/tablespoon
Cooked salmon/mackerel	345–360/100 g
Cow’s milk	3–40/L
Dried shitake mushrooms (non-radiated)	1660/100 g
Egg yolk	20–25 per yolk
Fresh shitake mushrooms	100/100 g
Fortified milk/infant formulas*	400/L
Fortified orange juice/soy milk/rice milk	400/L
Margarine, fortified	60/tablespoon
Parmesan cheese	28/100 g
Shrimp	152/100 g
Swiss cheese	44/100 g
Tofu, fortified	(1⁄5 block) 120
Yoghurt (normal, low fat, or non-fat)	89/100 g

Adapted from: Misra et al. (2008)

It is estimated that in NZ <10% of vitamin D is derived from dietary sources (Misra et al., 2008; MOH, 2013). It is recommended by the NZ MOH that individuals should acquire vitamin D where possible from the few foods that naturally contain it, for instance salmon, herring and mackerel fish, liver and eggs, as well as vitamin D fortified foods like margarine, yoghurt and milk (MOH, 2013). The recommendation from the NZ MOH guidelines is to have the dietary intake of 5 µg (200 IU) of vitamin D per day, for pregnant and lactating women and infants from birth (see **Table *9***), a lower intake than what is recommended in the US, UK and northern Europe (Gussy et al., 2006).

Table 9

The recommended dietary intakes of vitamin D

	Australia and NZ (NHMRC 2006)	**US and Canada** (Institute of Medicine 2011)	**UK** (Dept. of Health 1991)	**Germany, Austria and Switzerland** (Deutsche Gesellschaft für Ernährung 2012)
Pregnancy	5 µg/day (200 IU) or 10 µg/day (400 IU) supplement	15 µg/day (600 IU)	10 µg/day (400 IU)	20 µg/day (800 IU)
Infants	5 µg/day (200 IU)	10 µg/day (400 IU)	7–8.5 µg/day (280–340 IU)	10 µg/day (400 IU)

Adapted from the Companion Statement on Vitamin D and Sun Exposure in Pregnancy and Infancy in NZ (MOH, 2013)

The way food is cooked or prepared additionally impacts on the amount of vitamin D that is available from the dietary source; for instance, frying fish reduces its vitamin D content, whereas baking does not. Since young children often do not consume foods with natural sources of vitamin D, it becomes necessary to fortify their food and beverages with vitamin D, particularly when sunlight exposure may be scarce (Misra et al., 2008).

Vitamin D fortification of milk and milk formula

The amount of vitamin D in breast milk is not sufficient to meet daily vitamin D intake requirements for an infant (200 IU per day), therefore vitamin D supplementation is likely to be necessary where breast milk is the sole food source (MOH, 2013). Breast milk delivers a concentration of 5 to 80 IU of vitamin D per litre (Wagner et al., 2006).

Infant milk formula in NZ and Australia is fortified with 400 IU of vitamin D_3 per litre. The guidelines for vitamin D fortification of infant formula are based upon a minimum daily intake of vitamin D of 200 IU from milk formula per day. This assumes a minimum consumption of 500 mL of vitamin D fortified milk formula, therefore infant formulas are required to contain a minimum of 400 IU/L vitamin D (Munns et al., 2006).

As a contrast, in Ireland, there are no vitamin D food fortification policy, however a vitamin D supplementation policy exists for infants who are recommended to have 200 IU of vitamin D per day, using approved products from birth to age 12 months (Carroll et al., 2014). This policy is also followed in several European countries, where there is no food fortification policy and there is limited access to adequately fortified foods (Calvo et al., 2005).

Vitamin D fortified food and drink

Foods that are commonly fortified with vitamin D include margarine, juices, cereals, cheeses and breads (Holick, 2008). Vitamin D food fortification practices vary between countries. In the UK, for instance, vitamin D fortification is compulsory in margarine and many other varieties of commercially available foods. In Canada, it is mandatory to fortify milk and milk alternatives with vitamin D (Misra et al., 2008).

In NZ there are no mandated practices for vitamin D food or drink fortification, however from 1996 the fortification of margarines, spreads, milk and other beverages has been permitted at the manufacturers discretion (Wagner et al., 2006). Most imported dairy food sources from Australia are vitamin D fortified, such as milk, formula and margarine (Rockell et al., 2005).

3.4.5.3 Vitamin D supplementation

Authorities worldwide recommend vitamin D supplementation for children with insufficient vitamin D intake. Vitamin D supplements are available in either liquid or tablet forms. Vitamin D_2 and D_3 are both used for supplementation, however vitamin D_3 (25(OH)-D3) is more effective for increasing serum 25(OH)D concentration (Misra et al., 2008).

Variations exist for vitamin D supplementation policies between different countries. In the US for instance, the AAP endorses vitamin D supplementation in infants as early as

two months of age, as vitamin D obtained by children from their mothers via placental transfer is usually sufficient to maintain adequate vitamin D status to age eight weeks, assuming the mothers have adequate vitamin D status while pregnant. The current Food and Drug Administration (FDA) and AAP guidelines recommend all infants (fully and partially breast fed) and children should be given 400 IU per day of vitamin D, to prevent rickets and maintain serum vitamin D concentrations >20 ng/mL (50 nmol/L) (Wagner & Greer, 2008; FDA, 2010; O'Callaghan et al., 2019). As it is believed there is not enough transmission of maternal vitamin D to child to provide adequate 25(OH)D concentrations, differing guidelines exist on infant vitamin D supplementation. For instance, in the US, guidelines for pre-term infants with low birth weight (<1500g) are between 200 to 400 IU of vitamin D supplementation per day, whereas in Europe infants are recommended to be supplemented with from 400 to 800 IU of vitamin D per day with a safe upper limit of 1000 IU per day, and differs from that recommended for infants in Central Europe (between 400-800 IU per day) (Kołodziejczyk et al., 2017).

The WHO (2017) cites, that vitamin D supplementation for infants around the world, can range between 200 IU per day (5 micrograms daily) to 1200 IU per day (30 micrograms daily), in addition to breast feeding . In some cultures, it can be introduced via alternative milk options such as cow's milk or formula intake, in cereals, or by maternal vitamin D supplementation, however, there are no public health policies currently for mandatory vitamin D supplementation in new born children (WHO, 2017). Despite the current consensus for breast fed infants to receive vitamin D supplementation, the prevalence of infants receiving supplementation is relatively low in most parts of the world (Sotunde et al., 2019). In the US, of infants with severe VDD, who were breast fed in winter, only 22% were reported to have received vitamin D supplementation. Other barriers that exist for infants not being given vitamin D supplementation include, mothers of lower SES, poorer educational backgrounds, non-European ethnicity, and immigrant status. Further, infants who are fed milk formula are required to have one litre of formula per day, to obtain the recommended daily intake of 400 IU vitamin D, which is a volume of milk consumption that is often not achieved (Sotunde et al., 2019)

According to the recent Scientific advisory committee on nutrition (SACN), in the UK authorities currently advise that safe daily vitamin D supplementation for infants from

birth to one should be between 8.5 to 10 micrograms per day, and 10 micrograms for children aged between one and four (SACN, 2016). Policies in Ireland, also advise vitamin D supplementation for infants (Carroll et al., 2014).

Pregnant and breastfeeding women also also recommended to have 10 micrograms of vitamin D supplementation per day in the UK (SACN, 2016). Maternal vitamin D supplementation is also beneficial for new-borns to ensure they have adequacy in vitamin D status at birth. In a RCT of vitamin D_3 supplementation where mothers were given a daily dose of 400, 2000 or 4000 IU from 12-16 weeks pregnancy until delivery, this was within a safe limit and no mothers were found to have hypercalcemia or increased urinary calcium to creatinine ratios (Portaro et al., 2008). In a NZ RCT of vitamin D supplementation during pregnancy, it was found that a daily vitamin D_3 supplementation dose of 1000 IU or 2000 IU from 27 weeks' pregnancy until delivery, was defined as safe based upon maternal serum calcium concentrations (Ekeroma et al., 2014; Grant et al., 2014).

The current NZ MOH guidelines state that pregnant women should have a daily dietary vitamin D intake of 200 IU or take vitamin D supplementation of 400 IU per day, for those who have little exposure to natural sunlight (MOH, 2013). Upon comparison, this is relatively lower than other westernised countries where the recommendations for pregnant women are to have a dietary vitamin D intake of 600 IU per day in the US and Canada, and 800 IU per day in Germany, Austria and Switzerland (MOH, 2013).

3.4.6 Vitamin D status of pregnant and lactating women

In NZ, as well as other Westernised and non-westernised countries, VDD is prevalent in pregnant or lactating women and their infants and children (Judkins & Eagleton, 2006; Grant et al., 2014; Natarajan et al., 2014). A systematic review and meta-analysis summarised that globally 54% of pregnant women were reported to have VDD as defined by 25(OH)D concentration <50 nmol L, and this figure was 75% for VDD in new borns (Saraf et al., 2016).

According to a systematic review and meta-analysis by Saraf et al. (2016), it was summarised that globally the prevalence of VDD (25(OH)D <50 nmol L) in pregnant women ranged from 42-72% in America, 18-90% in Europe, 46% in the Mediterranean

regions, 66-96% in South East Asia, and 41-97% in Western and Pacific regions. Before this, the prevalence of VDD in pregnancy has been reported for up to 61% of NZ women, 18% of pregnant women in the UK, 25% in the United Arab Emirates (UAE), 80% in Iran, and from 60-84% of non-western women living in the Netherlands (Dawodu & Wagner, 2007). VDD in pregnant and breast-feeding women affects their child both in utero and after birth (Grant et al., 2014). If maternal VDD is severe during pregnancy, abnormal foetal skeletal metabolism patterns can occur (Murguía-Peniche, 2013).

Maternal and foetal (cord blood) 25(OH)D concentrations are closely correlated (Saraf et al., 2016), as 25(OH)D readily moves through the placenta and is metabolized in the foetus from 24 weeks' gestation, where it converts into its active form of 1,25-dihydroxycholecalciferol (Murguía-Peniche, 2013). There is an increased risk of unfavourable pregnancy outcomes for pregnant women who have VDD. These adverse outcomes include an increased risk of gestational diabetes, pre-eclampsia, premature gestation and giving birth to low birth weight new borns (Aghajafari et al., 2013; Wei et al., 2013). VDD could also contribute to periodontal diseases in pregnant women, such as increasing the risk of gingivitis and periodontitis (Seow et al., 2005). Further, VDD during pregnancy has also been linked with poorer dental outcomes for children. The role of vitamin D in childhood dental health will be discussed in the next section.

3.5 Vitamin D and childhood dental health

In the context of vitamin D and dental diseases, the risk for dental caries is increased by the presence of developmental dental defects, therefore as EHP is in the pathway of mechanisms for increasing caries risk this topic will be discussed first.

3.5.1 Vitamin D and developmental dental defects (EHP)

EHP or hypomineralisation is one of the most widespread developmental abnormalities caused by defective enamel development, due to disturbances to the enamel matrix during formation periods (amelogenesis) (Patel et al., 2019). These can be attributed to nutritional factors, preterm births, low birth weight or the ingestion of fluoride. Sometimes the cause is idiopathic and developmental enamel defects can have a genetic cause (White et al., 2012). Enamel hypomineralisation is seen in two to

four-year-old children, as well as in older children who have permanent teeth. EHP is associated with VDD, hereditary vitamin D dependent rickets, hypoparathyroidism, and neonatal tetany in childhood (Zerofsky et al., 2016).

Post tooth eruption, pitting and opacities, brown specks and mottling become obvious on teeth with EHP. Teeth with EHP attract more dental plaque, which exposes them to a higher risk of ECC. Low maternal vitamin D status during tooth development is linked with development dental defects in children’s primary teeth (Schroth et al., 2014), this will be discussed further in the following section.

3.5.2 Maternal vitamin D status is associated with childhood ECC and developmental enamel defects

Vitamin D status of mothers during pregnancy and post birth determines their infant’s vitamin D status, and thereby the quality of the infant's developing tooth enamel. In this way, low maternal vitamin D status is linked with whether a child develops developmental enamel defects or ECC in early life (Holick, 2007; Chen et al., 2010; Schroth et al., 2014).

Since VDD in pregnancy affects the formation of primary teeth, due to inadequate vitamin D status, this in turn causes insufficient calcium transfer from the mother to the fetus, directly affecting the fetal development of bones and teeth. Thereby, tooth enamel is affected by VDD and results in EHP in some children, which cannot be reversed (Davit-Beal et al., 2014). For this reason, vitamin D remains a crucial building block for the formation of strong teeth, and studies support that its adequacy in formative years, could protect teeth from dental developmental defects and ECC.

3.5.3 Studies on vitamin D and childhood dental outcomes

This following section focuses on studies that have investigated the relationship between vitamin D status and dental health outcomes, such as dental caries, developmental dental defects, and/or periodontal diseases. The studies on vitamin D and dental health are discussed in this context and listed in order of scientific hierarchy, with study results summarised for each.

3.5.3.1 Meta-analyses and systematic reviews

1. *Vitamin D and dental caries in controlled clinical trials: Systematic review and meta-analysis*

In a review of 24 controlled clinical trials (CCTs) it was concluded that vitamin D may be able to reduce the occurrence of dental caries (Hujoel, 2013). The systematic review included diverse studies, whereby the effects of vitamin D, in the form of vitamin D_2, vitamin D_3 or ultraviolet radiation, were explored on preventing dental caries. One form was nor more effective than another. Some of the systematic review limitations included studies without randomisation, examiner blinding or placebo supplementation, as well as possible researcher bias. The review did, however, highlight study strengths in identifying research which included clinical dental examinations, diverse population groups, inclusion of older and newer studies, highlighted the long-time frame over which research of the relationship of vitamin D status with oral health has been conducted. In keeping with previous studies, the author acknowledged that dental caries may be prevented by the action of vitamin D as it contributes to the mineralisation of teeth, as well as acting as a preventative agent against active caries, similar to topical fluoride and being able to alter the composition of saliva, as well as having immunological properties. This systematic review and meta-analysis described the contradicting stances held on vitamin D for the prevention of dental caries in the mid-1900s. The American Dental Association (ADA) for instance concluded that vitamin D supplementation was not a positive agent in preventing or treating dental caries, whereas the American Medical Association (AMA) found vitamin D was valuable for the management of dental caries. This systematic review concluded that there was a reduced risk of dental caries of 47% to 54% (in studies where the vitamin D supplementation dose was given without bias). Study methodologies were varied, there were different doses and approaches to how vitamin D supplementation was given, different age groups (zero to 16 years old), mixed or primary dentitions studied and single sex and both sex studies (Hujoel, 2013).

This systematic review study also found that deficient vitamin D status during pregnancy were reported to be associated with developmental dental defects, such as EHP, thus increasing caries risk (Hujoel, 2013). Most of the studies around the topic of VDD and developmental dental defects referred to in this study were however

conducted between 1934 and 1975 and did not have the methodological rigour of modern-day clinical trials. It was unable to find more relatively current studies, where sunlight exposure may have differed from the studies performed during the first half of the 20th Century studies. The review noted that with the recent increases in rates of dental caries, vitamin D supplementation warrants contemporary investigation if its role in preventing dental caries and periodontal diseases.

2. *Vitamin D and multiple health outcomes: Umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials*

The focus of this systematic review and meta-analysis was to look at existing research which identified the effects of vitamin D (as measured by either 1,25-dihydroxyvitamin D or 25-hydroxyvitamin D serum concentration, or vitamin D supplementation), on various health outcomes, including dental caries (Theodoratou et al., 2014). The study concluded that there was a positive association for birth weight, maternal vitamin D status or supplementation with childhood dental caries, however it indicates further RCTs in this area should be completed as definitive information on the relationship of vitamin D status or supplementation with childhood dental caries was lacking (Theodoratou et al., 2014). In this umbrella review, although the focus was not solely the effects of vitamin D on dental health, both observational studies and RCTs were included that assessed the relationship with dental health. Strengths of the review were that it included quantitative studies, there were large number of systematic reviews (a total of 107) and observational studies (a total of 74) included, and vitamin D was observed in some capacity with the relevant health outcome(s) in all studies. The study concluded that it is probable that vitamin D supplementation decreases childhood dental caries, it is also likely to raise maternal vitamin D concentrations during pregnancy and lead to higher birth weight (Theodoratou et al., 2014).

Limitations of this review of systematic review and meta-analysis were: that the effect size of vitamin D in the studies could not be measured; there were many health outcomes considered, for example cardiovascular disease, gestational diabetes, hypertension, and stroke, to name a few; and for some conclusions could not be drawn about the relationship of them with vitamin D. This study revealed that lower levels vitamin D are associated with several adverse health outcomes and were suggestive that

higher vitamin D concentrations may have health benefits, such as reducing odds of colorectal cancer, cardiovascular disease, hypertension, or diabetes (Theodoratou et al., 2014). A strength of this review of systematic reviews and meta-analyses is that a diversity of population groups were included, with a wide range of age groups (mostly adults), various vitamin D supplementation doses and forms were assessed. However, the review concluded that highly convincing evidence for a clear role of vitamin D did not exist for any outcome, and that further studies were needed before firm conclusions about causal relationships could be reached. Overall, this study was able to show that there is data that suggests that vitamin D supplementation could reduce the risk of childhood dental caries, without any health risks (Theodoratou et al., 2014).

3. The role of vitamins in oral health. A systematic review and meta-analysis

This recently published systematic review and meta-analysis critically assessed the scientific literature on the role of vitamin D in relation to dental pathological conditions and specific periodontal diseases (Cagetti et al., 2020). The review included 334 articles out of 407 identified, where the selection criteria were papers published from 2000 to 2019 and that studied the relationship between serum vitamin D concentrations and/or vitamin D supplementation, with dental conditions (Cagetti et al., 2020). The central dental outcomes that this review focused on were periodontal diseases, and dental caries in relation to vitamin D. The authors recognised that while VDD can initiate reduced bone density and osteoporosis, it may well impact the advancement of periodontal diseases, in addition. This study found higher dose vitamin D supplementation (of 2000 IU) was able to improve gingival outcomes, compared to lower doses of vitamin D supplementation (of 500 or 1000 IU) (Hiremath et al., 2013). In another study, by Woelber et al. (2016) it was reported that in a dietary vitamin D supplementation experiment (with vitamin C and Omega 3, fatty acids, and antioxidant intake), it was found that gingival index scores, bleeding on probing and periodontal inflammation were reduced by 50% over a four-week intervention period. In diabetic patients, supplementary vitamin D for six months, decreased the likelihood of mouth ulcers and gingivitis occurring compared with patients who received placebo ($p<0.05$) (Cagetti et al., 2020). Adequate serum vitamin D statuses were observed to have reduced the rates of periodontitis in five studies, and six studies showed serum vitamin D concentrations had statistically significant associations with the presence of

dental caries in children. Moreover, low maternal vitamin D statuses during pregnancy were associated with the presence of developmental dental defects (EHP) in their children's teeth (Cagetti et al., 2020). Limitations of this review include that the age ranges of the children differed, there were variations in the type of vitamin D supplementation used, and more studies on vitamin D and periodontitis were included. Serum vitamin D concentrations were found in two studies, one in children aged up to eight years old and the other 10-12 years, to be associated with dental caries, DMFT rates, and impacted upon future caries risk, therefore, adequate vitamin D concentrations (of >50 nmol) were recommended for reducing the risk of dental caries in children (Gyll et al., 2018; Kim et al., 2018; Cagetti et al., 2020). Other vitamins were also investigated in relation to caries risk but are out of the scope of this present PhD study, therefore, will not be elaborated upon in this literature review.

This review re-affirms that there is no definitive answer as to whether vitamin D prevents dental caries or developmental dental defects. The authors concluded that more scientific evidence is needed on the relationship between vitamin D supplementation and dental conditions like dental caries, developmental dental defects, and periodontal diseases.

The limitations identified in this systematic review were that there were no longitudinal studies identified, the role of vitamin D remained unclear and there were differences in vitamin D supplementation methods used in the studies. The strengths of this study were that it considered the relationship of vitamin D with multiple dental diseases, for instance periodontal diseases as well. It incorporated contemporary literature, however the available data still remains inconclusive for recommending vitamin D as a preventative caries agent according to this review (Cagetti et al., 2020).

3.5.3.2 Randomised placebo control trials (RCTs)

In this next section I have summarised studies that were undertaken in the UK in the early 1900s to 1980, examining the effect of vitamin D supplementation on dental health.

1. *The action of vitamin D in preventing the spread and promoting the arrest of caries in children*

A study by Mellanby and Pattison (1928), showed that vitamin D_2 supplementation was able to arrest and prevent new dental caries lesions from forming and spreading in children. The 21 children aged under six years old, had dental examinations before and after vitamin D supplementation was given and they were monitored for 28 weeks. The study showed that carious lesions arrested after a diet abundant in vitamin D through supplementation with cod-liver oil, extra milk, and eggs (exact amount of vitamin D not stated), and dental outcomes were better for three to four-year-old children than for the seven to eight-year-old children. Mixed dentitions were examined in this study and children had two dental examinations, which made the findings more reliable to draw conclusions from, for the changes observed post the vitamin D intervention were carefully documented.

2. *The fat-soluble vitamins and dental caries in children*

In another earlier study by Day and Sedwick (1934) the effects of vitamin A and D supplements given to children over a year were examined, where the daily vitamin D supplementation dose was 1400 IU. The children were aged 13 to 14 years, and there were no apparent effects of vitamins A and D observed on existing dental caries. The strengths of this study were that the study had a larger sample size, with 430 participants and their teeth were dentally examined by visual exams and clinical radiographs. Another strength was that the subjects were compared with matched control patients who did not receive vitamin D supplementation. The study was unable to determine the effects of vitamin D supplementation on erupting teeth as the supplements were given post tooth eruption, and many of the teeth would have been permanent teeth. Further investigation was needed after this study, to determine if there was a relationship between vitamin D supplementation and dental caries reduction.

3. *Vitamin D studies, 1933–1934*

A study in the early 1930s by McBeath (1934) recruited 425 children from orphanages in New York and nearby, who were prospectively examined to test the effects of vitamin D supplementation on their dental health. The children were aged from eight to 14 years old and given three different daily doses of vitamin D supplementation (of

100, 150, and 300 IU). One form of vitamin D was cod liver oil in milk, and the other was irradiation with UV light, these were the two experimental groups and the third the control group. It was found that the children who received 300 IU (higher dose) ff vitamin D daily had fewer dental caries.

The study strength were that it compared control participants to the children who received vitamin D supplementation and the children were examined thrice in 12 months, by the same examiner. Another strength if the study was that the children had fully erupted teeth and older children may have been more cooperative for dental examinations. However, the limitations of this study were that the effects of vitamin D supplementation could not be assessed on the developing dentition during tooth eruption times, and mixed dentitions were examined.

4. *The role of vitamin D in the control of dental caries in children(McBeath & Zucker, 1938)*

In another study, by McBeath and Zucker (1938), 871 children who were aged from six to 14 years were recruited and prospectively examined once every year for three consecutive years. The study assessed the effects of vitamin D supplementation on their dental health. A total of 601 children were given vitamin D supplementation by artificial UV radiation or in dietary milk, and 270 were given placebo. Both forms of supplementation showed moderate success in reducing dental caries in the children's teeth, nevertheless, artificial UV radiation proved to be more effective. The strengths of the study included having a placebo group, a longitudinal study design and three dental examinations of the children.

Study limitations included that mixed dentitions were examined, for instance in the 1934 study by McBeath, and vitamin D supplements were given post tooth development and after dental caries had been initiated in the teeth of some study children, therefore not being able to see the pre-eruptive effects of vitamin D on teeth.

5. *Maternal vitamin D intake and mineral metabolism in mothers and their new born infants*

A study in Edinburgh in the 1980s by Cockburn et al. (1980) investigated whether vitamin D supplementation provided to pregnant women had a beneficial effect on the tooth tissues of their children. This study extended previous investigations by

supplementing pregnant women with 400 IU vitamin D from their 12th week of gestation. This study sought to establish if vitamin D had an impact on the dental tissues of the children born to these women. In this study, 506 pregnant women took vitamin D supplementation, and 61 of their infants were dentally examined post birth. The merits of this study were that it was a quantitative, placebo controlled, double blind study where a paediatric dentist examined the children at two to six years of age, when their primary teeth were fully erupted. Examiners in this study were blind to maternal and infant vitamin D status. It was found that many of the mothers were hypocalcaemic during pregnancy and 48% of infants had prenatally determined enamel defects. The study showed that women who did not receive vitamin D supplementation from the 12th week of pregnancy until giving birth, had an increased risk of their children having dental defects and hypocalcaemia compared with children whose mothers took vitamin D supplementation during pregnancy. Results from this study showed that VDD in pregnancy was linked with neonatal hypocalcaemia and the formation of developmental enamel defects in the offspring.

6. *Association of high-dose vitamin D supplementation during pregnancy with the risk of enamel defects in offspring: A 6-year follow-up of a randomized clinical trial*

A study from Denmark that was undertaken from 2009 until 2018, found that high dose vitamin D supplementation in pregnant women resulted in a 50% reduced risk of enamel defects in their children's teeth at age six. The study recruited 623 pregnant women from their 24th week of pregnancy and 496 of their children. The women were originally from the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort recruited from 2008 to 2010, where 315 women were given a vitamin D_3 supplementation of 2400 IU daily, till delivery and 1-week post birth, and 308 women were given a placebo supplementation. This was additional to the recommended 400 IU of vitamin D supplementation which children had per day, via nutritional or supplementary form (Norrisgaard et al., 2019). This study therefore compared the effect of 2400 IU, and 400 IU of vitamin D supplementation versus placebo during pregnancy, on the dental health of children aged six years old. The children were dentally examined by an examiner who remained blind to their vitamin D supplementation or placebo statuses before birth, and the presence of developmental enamel defects and childhood dental caries were recorded.

It was found in this study that children whose mothers had received higher dose vitamin D supplementation (of 2400 IU) were less likely to have developmental enamel defects on their teeth at age six. Therefore, high dose vitamin D supplementation in pregnancy had a causal relationship with the presence of fewer developmental enamel defects in the primary and permanent dentition as indicated by this RCT (Norrisgaard et al., 2019).

The strengths of this study were that it was a randomised, double blind, placebo-controlled study. The study mothers had good adherence to the study medicine and the children who attended for dental examinations had a high follow up rate (84%) for dental examinations, from being enrolled at one week old to being seen at age six years. There was also only one examiner to collect dental data, which minimised discrepancies. The study did however have limitations, as data were not collected on other confounding variables that may have impacted on dental caries and enamel defects, such as diet, home care, and lifestyle habits.

As vitamin D plays an important role in tooth enamel mineralisation, based on the study findings, vitamin D supplementation in pregnancy could be a potentially safe and effective intervention to protect children's teeth from enamel defects (Norrisgaard et al., 2019).

3.5.3.3 Cohort studies

1. *Prevalence of caries among preschool-aged children in a northern Manitoba community*

The first study by Schroth and team initiated in 1999, found no association between vitamin D supplementation and the presence of dental caries and/or enamel defects (Schroth, Smith, et al., 2005). This study was undertaken to assess caries prevalence, risk factors for ECC and the effects of modified Stoss therapy, which is a high dose of vitamin D supplementation (100,000) given to pregnant women, to assess its effects on children's primary dental health (Schroth, Smith, et al., 2005).

The aim of this study was to see if maternal high dose vitamin D supplementation is associated with an decreased odds of developmental dental defects (EHP) in primary teeth and if it lowered the risk of ECC in children (Schroth, Smith, et al., 2005). Pregnant women were given 100,000 IU of vitamin D (ergocalciferol) supplements in

their third trimester, and their babies were supplemented with the equivalent vitamin D dose as the mothers (of 400 IU), at six weeks of age (Schroth, Smith, et al., 2005). In this cross-sectional, retrospective cohort study, 98 children were able to be recruited from a sample of 179 children, between 1994 to 1999, some children had left the community and some parents did not wish to participate. The study children underwent dental examinations and their mothers answered questions on their children's oral hygiene habits, their feeding habits in infancy, and their own health and nutrition during pregnancy. Clinicians remained blinded to the participants' vitamin D supplementation status. On average children were three years old when their teeth were examined. The mean age for the first tooth to erupt was six months and most children visited a dentist for the first time at age two or three years. Most of the children had dental caries on at least six teeth, with these being diagnosed at age two and three years, 40% of the children were diagnosed with dental caries by age two. Thirty-nine percent of the children who required dental treatment had this treatment under GA (Schroth, Smith, et al., 2005).

In this study, higher rates of dental caries and higher decayed, extracted, and filled teeth (deft) scores were seen in children who had milk or a higher amount of sugar in their diet, no refrigeration and were from low-income families. This additional information was collected in the mothers self-reporting questionnaires. There was no relationship between maternal vitamin D supplementation and the presence of enamel dental defects (EHP) or dental caries on the children's teeth. Potential reasons for not finding a relationship between vitamin D supplementation and dental caries or dental defects on primary teeth included: variation in timing of the vitamin D supplements given and examining the subjects at under 12 months of age when many of their primary teeth may still be unerupted, or dietary sugars having overridden any protective effect of vitamin D. The intake of vitamin D_2 also varied across the sample population and the dental information that mothers reported retrospectively may have not always been accurate, due to the time lapse.

Later tooth eruption times were present in infants whose mothers did not receive vitamin D supplementation in pregnancy (Schroth, Smith, et al., 2005). The sample of children included in this study had poorer oral health compared to the general population. Several factors variables affected the dental outcomes, including poor oral

hygiene, bottle feeding, sugar containing beverages, and low maternal intake of vitamin D rich foods (Schroth, Smith, et al., 2005).

2. Vitamin D and dental caries in children

In this study, Schroth and team studied the relationship between serum 25(OH)D concentration and dental caries in 1,017 Canadian children aged between 6 and 11 years (Schroth et al., 2016). They enrolled children from the Canadian Health Measures Survey from 2007 to 2009. Calibrated dentists undertook the dental examinations, and dental caries in the primary and permanent teeth were noted. Dmft scores were recorded and patients' vitamin D status was assessed by measurement of serum 25(OH)D concentration. Children with optimal serum 25(OH)D concentrations (≥75 nmol/L) were at significantly lower risk of dental caries. Children with serum 25(OH)D concentrations of ≥50 nmol/L were also at low risk of dental caries than children with serum 25(OH)D concentrations of <50 nmol/L (Schroth et al., 2016).

Strengths of this study include measuring serum 25(OH)D levels and controlling for other additional risk factors for caries, by collecting data on fluoridated water exposure, dietary intake including how often they had sugar containing drinks and milk intake, as well as tooth brushing and how often they went to a dentist. Study weaknesses included that only cross-sectional data was available from the Canadian Health Measures Survey, the serum 25(OH)D concentrations during tooth development were not known, and the season when serum 25(OH) concentrations were measured were not reported. The study concluded that optimal vitamin D status is protective against dental caries.

3.5.3.4 Case control studies

1. The role of vitamin D deficiency in early childhood caries

A case-control study in the US (North Carolina) measured serum 25(OH)D concentrations in 10 children with ECC who were undergoing dental treatment in hospital under GA, and compared them with caries free control patients (Williams, 2014). The children were aged zero to six years old and the inclusion criteria was that the children had to be medically fit or had only a mild systemic disease. Data collection took place from November 2013 to February 2014. Children had blood samples taken

to measure serum 25(OH)D concentrations and each child had a dental examination to assess dmft and dmfts scores. Parents also answered behavioural questionnaires on their child's other biological and non-biological caries risk factors (Williams, 2014).

The study showed associations of ECC with vitamin D deficiency of various levels (serum 25(OH)D <10 ng/ml to 29.9 ng/ml, (<25 nmol/L to <75 nmol/l)) as well as elevated PTH levels, upon comparison with children who had no dental caries and whose serum 25(OH)D levels were >30 ng/ml (75 nmol/l). Strengths of this study were that it included control patients, the study gathered additional information on risk factors for ECC (eating habits, frequency of snacks, fluoride exposure, tooth brushing and visits to the dentist) and measured the serum vitamin D concentrations of the children. It also obtained information on the parents' dental history and oral hygiene habits.

Study weaknesses were that it was not conducted with an ethnically diverse sample and many of the children were from higher caries risk groups (low-income families, higher sugar intake, and parents from low educational backgrounds).

2. *Suboptimal vitamin D associated with dental caries at an urban pediatric hospital*

Another case control study undertaken by Jumani (2016) in Seattle between 1999 and 2014, investigated the relationship between serum 25(OH)D concentrations and dental caries in children who were receiving dental care in a hospital setting. The study recruited 276 children aged from one to six, who had a higher susceptibility to dental caries. This was because over 75% of the sample were medically compromised children, with severe systemic diseases. The prevalence of dental caries in this sample of children was 33% overall, and the children with VDD (25(OH)D <30 nmol/L) were twice more likely to have dental caries compared to children with optimal (>75 nmol/L) and sub-optimal (<75 nmol/L) serum 25(OH)D concentrations (Jumani, 2016).

A strength of this study was the children only had their primary teeth assessed, allowing the association of vitamin D status on ECC to be examined. The study weaknesses were that the children had varying degrees of physical health, ranging from physically healthy to mild and severe systemic diseases, as well as the study sample having several additional risk factors for ECC that were not accounted for.

3. Higher vitamin D intake during pregnancy is associated with reduced risk of dental caries in young Japanese children

In an on-going prospective study in Japan, beginning in 2007, it was found that higher maternal dietary vitamin D intake in pregnancy was associated with reduced odds of dental caries during childhood (Tanaka et al., 2015). This study recruited 1210 mother-infant dyads where data were collected by the mothers transcribing their child's dental examination data onto study questionnaires, from their children's health handbooks, containing dental examination information from ages 36-46 months old.

It was considered that Japanese diets would naturally contain a higher vitamin D content, than most other countries. Dairy, shellfish, and fish intake was measured with a dietary questionnaire given to the mothers. The study showed that confounding variables such as child's dietary vitamin D intake, did not affect the risk reduction for dental caries associated with the mother's vitamin D intake (Tanaka et al., 2015).

The resistance of teeth to dental caries, was thought to be attributed to the influence of vitamin D during tooth development in utero, as opposed to postnatally. However, skin colour, sun exposure, lifestyles, latitude, season, and cultural routines could affect vitamin D status in this population also. The study did have of number of limitations, including each mother transcribed the dental data from their child's health handbook records, and there was no consideration of natural sunlight exposure of study participants. Moreover, mothers were also of higher educational backgrounds, compared to the general population and the dental caries information solely relied on mothers transcribing the information into study forms, which left room for error. Also, serum 25(OH)D concentration was not measured on the mothers or the children (Tanaka et al., 2015).

3.5.3.5 Cross sectional studies

A series of cross-sectional studies in Canada have explored the association of maternal and child vitamin D status and childhood dental caries. I detail the relevance and key findings from each here.

1. Influence of maternal prenatal vitamin D status on infant oral health

In the second study by Schroth (2010), higher maternal vitamin D concentration during pregnancy was associated with a reduced odds of dental caries and developmental

dental defects in children's primary teeth. The study investigated if low maternal vitamin D status during pregnancy, predisposed children to developmental dental defects such as EHP, and therefore increasing the risk of ECC (Schroth, 2010).

This study recruited 207 pregnant women, in which their serum 25(OH)D concentrations were assessed during their third trimester. Dental examiners assessed their children's teeth for the presence of EHP and ECC when they were 12 months old and were blinded to the vitamin D status of the mothers. The mothers answered questionnaires pertaining to other risk factors for dental caries, such as infant feeding practices, oral hygiene habits and the mother's dental history. The participants of this study were predominantly Aboriginal, who are a vulnerable population group for dental disease in Canada (Schroth, 2010).

There was an association was observed between women having low vitamin D status during pregnancy and increased odds of their child having ECC and dental defects in their primary teeth. While the vitamin D concentrations were not significantly lower for women whose children had EHP compared with women whose children had normal teeth, they were for women whose children had ECC. Low maternal vitamin D status was also associated with increased odds of their children having untreated dental caries. Infants of women with low serum calcium concentrations during pregnancy, or who did not know about vitamin D, or did not use margarine every day were at increased odds of having EHP in their primary teeth (Schroth, 2010).

2. The relationship between vitamin D and severe early childhood caries: A pilot study

This study in Canada took place in 2008, where the association of vitamin D status of young children and the occurrence of S-ECC was investigated (Schroth et al., 2012). The study recruited 38 children under six years of age, who were undergoing dental treatment under GA for S-ECC and compared them to age-matched caries-free control children. Parents were asked to complete questionnaires on demographic factors, their child's nutritional habits, dietary intake, additional supplementation, sun exposure and other risk factors of ECC, including oral hygiene and visits to the dentist.

It was found in this study that there was a positive association for children who had S-ECC to have lower vitamin D concentrations than caries free control patients (p =

0.032). There was also an association between the presence of S-ECC with higher PTH levels in comparison with caries free control children (p = <0.001). Additional risk factors for ECC such as tooth brushing (later) and low SES status were also investigated but were found to not be associated with the presence of S-ECC (Schroth et al., 2012).

3. *Vitamin D status of children with severe early childhood caries: a case-control study (Schroth et al., 2013)*

A larger study that was undertaken in Canada from October 2009 to August 2011, assessed the relationship between vitamin D levels in young children and S-ECC in their teeth. The study researchers recruited 144 preschool children with S-ECC who were having dental treatment under GA, and 122 caries free, control patients. The children were aged between one and three and cord blood draws were taken for measurement of their serum vitamin D concentrations, PTH and calcium levels. Parents and caregivers were also asked to complete questionnaires describing demographics and risk factors of ECC. This study showed that serum 25(OH)D concentrations were lower in children with S-ECC, when compared with age-matched control patients. The study also showed that children who had S-ECC also had lower serum calcium and albumin levels (all p values were $p<0.001$) (Schroth et al., 2013).

Additional risk factors that contributed to lower serum 25(OH)D concentrations were infrequent milk intake, season of recruitment being winter, low SES and poor overall health of the study children.

4. *Prenatal vitamin D and dental caries in infants*

Schroth et al. (2014) investigated whether maternal 25(OH)D levels during pregnancy, may affect their children's EHP and ECC rates by influencing tooth calcification. A total of 207 pregnant women took part in this study. Their serum vitamin D concentrations were assessed in the second or third trimesters and their children had a dental examination at 12 months old. Strengths of this study included that the examiners remained blind to the mother's vitamin D statuses and parents completed additional questionnaires providing information on their demographic backgrounds, infant feeding practices and their child's overall health status.

Overall, 33% of pregnant women in the study had VDD (serum 25(OH)D concentration <35 nmol/L). Significant associations were found between low maternal vitamin D

levels and their children having ECC (p = 0.02). The presence of ECC was significantly associated with infants increasing age (p = 0.002) and EHP present on teeth (p = 0.001) (Schroth et al., 2014).

Weaknesses of this study included that the study sample was already at higher risk of dental caries then the general population and the infants were only examined at 12 months of age, therefore all 20 primary teeth may not have erupted. Study strengths included having only one dental examiner, mother's prenatal serum vitamin D status being measured and obtaining additional information on influencing factors for ECC.

This study showed that inadequate maternal vitamin D concentrations do place children at a higher risk of developing ECC and EHP during infancy.

Cross sectional studies from other developed countries will be discussed next.

5. Serum vitamin D, PTH, and calcium levels in patients with and without early childhood caries

A cross-sectional study undertaken in the US by Meinerz et al. (2016) between 2015 to 2016, investigated if there were differences in serum 25(OH)D, PTH and calcium levels for children with ECC, compared with children who were caries free. The study recruited 60 children with ECC and 30 caries free (control) children. The children were aged six and under with the majority being from African American ethnic backgrounds. Parent/guardians answered questionnaires on the child's demographic information, medical and dental history, and exposure to sunlight for their child. Study participants were examined at paediatric dental clinics and enrolled into this study if they had ECC. Control patients without ECC were enrolled from a hospital where they were undergoing other surgical procedures under GA (Meinerz et al., 2016).

Serum 25(OH)D concentrations did not differ between those with ECC and caries free control patients in this study. Study results suggested that vitamin D status did not have any effect on the odds of ECC, however ethnic differences were evident between the case and control groups. It is necessary for studies to consider additional influencing factors such as darker skin pigmentation, season of vitamin D measurement, sunlight exposure, additional vitamin D intake via diet or supplements,

fluoride exposure, sugar intake and tooth brushing to assess the correlation between vitamin D and occurrence of dental caries better (Meinerz et al., 2016).

6. Impact of cord blood vitamin D level on early childhood caries in infancy: A pilot study

A prospective cross-sectional study by Korun et al. (2017) in Cyprus, investigated if low cord blood 25(OH)D concentration (25(OH)D <20 ng/ml (<50 nmol/l)) was associated with increased odds of ECC and developmental dental defects (EHP). The study took place from 2013 to 2016. Ninety pregnant women from a hospital obstetric clinic were recruited, their children were then dentally examined between one and two years of age. Initially, all the expectant mothers agreed to the collection of their baby's cord blood samples at delivery for analysis of vitamin D status, however 40 infants were excluded due to the study exclusion criteria. This was of having congenital anomalies, infections, metabolic diseases, premature birth (<34 weeks gestation), low birth weight (<2500) and/or loss to follow-up (Korun et al., 2017).

The dentist who examined the children's teeth remained unknown to the children's vitamin D status and parents completed questionnaires on demographic information, as well as the child's sun exposure (< than or ≥ 15 minutes per day), medical information, feeding habits, duration of breast feeding, frequency of sweet food intake and oral hygiene habits at 12 to 24 months of age. The study showed that deficient cord blood 25(OH)D levels (<30 ng/ml L (<75 nmol/l)), were associated higher odds of with EHP ($p = 0.018$) and ECC ($p = 0.001$). It was also found that poor oral hygiene habits of both parents and children, were associated with the occurrence of dental caries in this study ($p = <0.05$) (Korun et al., 2017).

The study strengths included assessing 25(OH)D cord blood sample concentrations, examiner blinding and obtaining information on the external factors related with ECC. Drawbacks of the study were that dental examinations were undertaken whilst teeth were still erupting therefore there may not have been an accurate representation of EHP and ECC may not have had enough opportunity to develop and progress.

7. Association of maternal vitamin D deficiency with early childhood caries

Another recent cross-sectional study completed on Alaskan children in the US, studied the relationship between mother's serum vitamin D status during pregnancy and ECC rates in their children (Singleton et al., 2019). The study assessed a total of 76 pregnant

women, and 57 children, who were aged between 12 and 59 months. The study population comprised pregnant women who were part of the Maternal Organics Monitoring study (MOMs) from 2010 to 2013 in a rural region of Southwestern Alaska, where diets and pollutant factors in foods were monitored and vitamin D concentrations were measured on maternal blood samples collected at 16 weeks gestation and cord blood samples collected at birth. The purpose of the MOMs study was to establish if nourishment from subsistence diets were adequate to outweigh the risks of exposure to environmental pollutants (Singleton et al., 2019).

The children in this dental study were primarily of Yup'ik Eskimo background that lived in remote areas without fluoridated water. Study researchers found that 91% of children in this study were affected by ECC. Further to this, children aged 12 to 36 months who had deficient vitamin D levels at birth (25(OH)D <30 nmol/L (75 nmol/l)), were significantly more likely to have higher mean dmft scores compared children with adequate serum vitamin D concentrations at birth ($p = 0.002$). Other variables the study also assessed were breastfeeding, maternal smoking and tobacco use, and dental treatment received outside of this study for the children. The researchers concluded that VDD in pregnancy and early infancy does correlate with ECCs in young children (Singleton et al., 2019). Limitations of this study were that other potential risk factors for dental caries were not accounted for; the study sample size was small and dental records of children were used to assess ECC retrospectively. The authors of this study proposed improving vitamin D sufficiency in pregnant women to reduce the risk of ECC in their children (Singleton et al., 2019).

8. Vitamin D and dental caries in healthy Swedish children

A cross sectional study published in 2018 by Gyll et al. (2018), showed that vitamin D statuses were associated with an increased odds for children having ECC who were from Northern and Southern Sweden, with dark and light skin complexions. In this randomised controlled trial, 206 children were given vitamin D supplementation during infancy and again at age six. Vitamin D supplementation of 400 IU/day (10 ug) in the form of vitamin D_3 drops, were recommended from birth to two years and, for children with darker skin, up to age five. At six years old, vitamin D supplementation was given to the children in the form of daily vitamin D fortified milk. They were divided into three groups who were given: vitamin D high dose 1000 IU/day (25 ug),

vitamin D low 400 IU/day (10 ug) or placebo supplementation, for a duration of three months (Gyll et al., 2018). Two years post the vitamin D supplementation, children were dentally examined, and data were collected on dietary habits and SES. The examiners in this study were trained dentists who measured dental caries and developmental dental defects (EHP) present on the children's teeth. Parents answered questionnaires about their child's oral hygiene habits (such as tooth brushing) and dietary intake (sugar). Plaque biofilm and saliva samples were also taken from the children.

The study showed that vitamin D status was comparable for children in the vitamin D supplementation versus placebo groups. Children who had inadequate vitamin D (25(OH)D <50 nmol/L) status, were however, more likely to have been affected by dental caries. Darker skinned children had significantly more caries reported, than children with fairer skin complexion (Gyll et al., 2018).

Vitamin D status did not appear to impact upon developmental dental defects (EHP) on the children's permanent teeth. The innate immunity peptide (LL37) found in saliva, was however, found to be lower in children with VDD (<50 nmol/L) (Gyll et al., 2018).

Strengths of this study include vitamin D or placebo assignment was randomised, and examiners were blinded to vitamin D statuses. Both permanent and primary teeth were assessed in this study for dental caries and dental defects. The shortcomings of this study were that dentitions were only examined at one time point and vitamin D supplementation after birth, was recommended but not necessarily given, therefore the design was not intention to treat VDD. Therefore, there was no way of ensuring participants took additional vitamin D supplements during infancy (when teeth are developing).

9. *Vitamin D deficiency and risk of dental caries among young children: A public health problem*

In a study by Bener et al. (2013), the incidence of childhood dental caries was measured and compared in relation to vitamin D status and other influencing demographic and lifestyle risk factors. In this cross-sectional study, 1,249 participants aged between 7 and 16 years were recruited in Qatar from August 2009 to June 2010, from primary health care centres. Data were collected on vitamin D status, dental

outcomes, and sociodemographic and lifestyle factors. The WHO criteria of recording decayed teeth as code 1, filled teeth due to decay as code 2, filled teeth with no decay as code 3 and missing teeth as no code and erupted teeth as code 8, were used to record the children's dental statuses. The incidence of dental caries was higher in those with VDD (as defined by a serum 25(OH)D concentration of <20 ng/ml (50 nmol/l) ($p = 0.01$), compared with control participants. DMFT scores were also lower in children with optimal vitamin D concentrations of 20-80 ng/ml (50-200 nmol/l) (Bener et al., 2013).

Children with a family history of diabetes mellitus had higher DMFT scores, compared to children without a history of diabetes mellitus in their family. Blood samples were collected from the children to measure vitamin D status. Low serum 25(OH)D levels were defined as <20 ng/ml (50 nmol/l) (mild to moderate VDD) and <10 ng/ml (25 nmol/l) (defined as severe VDD). It was found that children with VDD were 1.13 times (95% confidence interval [CI] of 1.05-1.21, $p<0.01$) more likely to develop dental caries than those who had optimal vitamin D levels (Bener et al., 2013).

Strengths of this study were having a validated scoring system for decayed, missing, and filled teeth, and measurement of other contributing factors for dental caries such as socio-demographic determinants, parental education, and home care habits such as tooth brushing. Study weaknesses included not examining for dental defects, which would influence caries experience, and clinical dental examinations did not include blowing air to dry the tooth surface, or an overhead light for better visibility.

3.5.3.6 Animal studies

1. An experimental study of the influence of diet on teeth formation

Sir Edward Mellanby (1918) was the first researcher to prospectively examine the effect of dietary vitamin D on the dental health of puppies. Mellanby's research published in 1918 reported vitamin D supplementation improved tooth calcification and reduced the occurrence of dental defects in dogs (Mellanby, 1918).

The study findings showed that dogs who had taken adequate amounts of vitamin D in their diet had stronger teeth with fewer developmental dental defects, due to adequate tooth calcification and mineralisation of tooth tissues (Mellanby, 1918).

Some of the limitations of Mellanby's study were that the effects of vitamin D was investigated in dogs in this first study, and the amount of dietary supplementation given may have been varied. The study examiner was also aware of the dog's baseline vitamin D status that could have influenced the results. To improve the strength of this study, the dogs who were given vitamin D supplementation could have been compared to control dogs who did not take vitamin D. However, a diet rich in vitamin D was able to reduce the occurrence of dental defects, and lower the risk of ECC, showing positive effects of vitamin D supplementation in this study.

This initial research by Mellanby established that vitamin D was able to stimulate tooth tissue calcification in dogs. Mellanby's experiments were primarily focused on animals to begin with, in which it was found that diets deficient in vitamin D led to improper dentine formation and to dental defects. After 1921, when it was recognised that vitamins A and D were distinct from one another, more attention was given to vitamin D due to its properties of enhancing tissue calcification.

3.5.3.7 Summary of studies on vitamin D and childhood dental outcomes

Studies have shown a relationship between VDD in pregnant women and young children and increased odds of ECC and S-ECC. However, there are only a small number of studies that have been conducted to examine the effects of vitamin D supplementation on childhood dental outcomes, such as caries and developmental dental defects, and very few studies have used a randomised controlled trial design.

Table *10* and **Table *11*** provide summaries of the above-mentioned studies that have investigated the effects of vitamin D supplementation on childhood dental caries and dental defects. The studies have been separated into observational studies (**Table *10***) and randomised placebo-controlled clinical trials (**Table *11***).

Table 10

Observational studies which investigated the effects of vitamin D supplementation on dental caries and developmental dental defects in children

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
• **Animal Studies**						
Mellanby (1918)	1918	UK	Dietary vitamin D supplementation in dogs.	To describe the physiology and modifications in tooth structure following vitamin D supplementation in dogs, to see if it protects teeth from dental defects.	A prospective observational study n = 3 dogs Dentally examined after being killed	• Diets poor in vitamin D were more likely to promote teeth with abnormal calcification and teeth with dental defects. • Adequate vitamin D intake promotes sound tooth calcification. • *+ findings of low dietary vitamin D intake and more teeth with dental defects*
• **Human Studies**						
Mellanby and Pattison (1928)	1928	UK	Dietary vitamin D supplementation in three groups of children, with different vitamin D_2 doses.	To examine the effects of vitamin D supplementation on childhood dental caries.	A prospective observational study n = 21 children Ages = Five to nine years Duration = 28 weeks Children dentally examined before and after vitamin D supplementation. Teeth: Mixed dentitions	• Dietary vitamin D_2 supplementation appeared to prevent new dental caries and limited the spread of and arrested existing carious lesions. • Most teeth showed arrested caries after vitamin D supplementation. Vitamin A had no effect on dental caries. • Vitamin D worked better in 3½ & 4-yr-old children, compared to 7½ year-old children. • One tooth extracted at 35 weeks, was divided into three and showed no active decay when stained. • *+ findings of vitamin D supplementation and fewer dental caries*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Schroth, Smith, et al. (2005)	2005	Canada	Pregnant women were given vitamin D_2 supplementation in the form of Stoss therapy of 100,000 IU at first diagnosis of pregnancy and in their third trimester. Their babies were given the same dose at 6 weeks old. A comparison group of children who did not receive the vitamin D supplementation (from a year before Stoss therapy was given)	To assess the effects of pregnancy and infancy vitamin D supplementation on the dental health of pre-school children.	A cross-sectional and retrospective, quasi-experimental cohort study n = 98 children Ages = 3-4-year-olds Pediatric dentist was kept blind to vitamin D statuses when dentally examining children. Other additional risk factors of ECC were considered by mothers answering questionnaires on feeding practices, children's oral hygiene, dental visits, fluoride, maternal dental health, dietary habits, and vitamin D exposure. This group was already vulnerable to dental disease. Teeth: Primary teeth	• No relationship between vitamin D supplementation and enamel hypoplasia (EHP). • Vitamin D supplementation showed a strong association with early tooth eruption (of significance) • The population group had a high risk of dental disease, 98% had ECC and 50% had EHP. • Other risk factors for ECC were tooth brushing habits, visits to a dentist and the amount of sugar intake. • ***0** findings of vitamin D supplementation and improving dental defects*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Schroth (2010)	2010	Canada	Serum vitamin D (25(OH)D) levels were measured on pregnant women in their 2nd trimester. No vitamin D supplementation	To determine if there is an association between serum 25(OH)D levels in pregnant mothers and ECC and EHP in their children's teeth.	A prospective observational study n = 206 pregnant women and 135 infants Most women were of Indigenous Canadian (aboriginal) ethnicity. Ages = after infants were 12 months old. Children were dentally examined to assess the presence of ECC and EHP. Teeth: Primary teeth Data was also collected on the additional risk factors for ECC.	• Vitamin D concentrations were related to the frequency of mother's milk consumption and prenatal vitamins. • Mothers of children with EHP had mean 25(OH)D concentrations during pregnancy that were not significantly lower. • Mothers of children with ECC had significantly lower 25(OH)D levels than those whose children were caries-free. • Infants with EHP were significantly more likely to have ECC. • VDD levels in pregnancy may possibly affect tooth calcification in offspring and predispose them to ECC. • + findings of maternal vitamin D and dental caries • ***0*** *findings of maternal vitamin D statuses and the presence of EHP (dental defects)*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Schroth et al. (2012)	2012	Canada	Serum samples to measure children's 25(OH)D and PTH were collected. No vitamin D supplementation.	To assess the associations between serum vitamin D levels in children and S-ECC on their teeth.	An observational study n = 38 children Duration: July-September 2008 Ages = children under six years old with S-ECC. Children were dentally examined whilst under GA for dental treatment. The measured outcomes were then compared with aged matched and caries-free control children. Parents completed questionnaires on demographic factors, nutrition, diet intake, supplements, sun exposure, oral hygiene, visits to the dentist and other demographic factors i.e. SES status.	• Low vitamin D and higher PTH levels in children were associated with S-ECC. • On average children were 3.5 years old. • Children with S-ECC had significantly lower 25(OH)D concentrations than caries-free children. • Children with S-ECC had significantly higher PTH levels compared to caries-free children. • Children with S-ECC were two times more likely to have low 25(OH)D levels (< 75 nmol/L) and 8 times more likely to have elevated PTH levels (≥ 50 cm/L) than caries free children. • Only 13% of children had a dental visit by 12 months of age, and 63% did not have dental examinations whilst under 2 years of age. • There was a trend for later tooth brushing in children with S-ECC, but this was not statistically significant. • Low SES levels had an impact on S-ECC presence • *+ findings of low serum vitamin D concentrations and S-ECC in children*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Schroth et al. (2013)	2013	Canada	Serum 25(OH)D levels, calcium, albumin and PTH levels measured in young children. No vitamin D supplementation	To assess whether children with S-ECC have lower serum 25(OH)D, calcium, albumin, and higher parathyroid hormone (PTH) levels.	An observational study N = 266 children Ages = under 6 years old Duration = 2009 – 2011 Children having dental treatment for S-ECC under GA were dentally examined and compared with caries free control children. Teeth: Primary teeth. Parents/Caregivers completed questionnaires on dietary intake, use of supplements, sun exposure and skin pigmentation, oral hygiene habits, and socioeconomic factors including household income, parental education level and government assistance.	○ Children with S-ECC, were significantly more likely to have low vitamin D, calcium, and albumin status and higher PTH levels. ○ Children with caries in early childhood were more likely to have poor nutritional intake compared to caries-free controls. ○ Children with S-ECC had significantly lower mean vitamin D, calcium, and albumin levels, and significantly higher PTH levels than those caries-free. ○ On average children were 3 years old. ○ Children with S-ECC were significantly more likely to have vitamin D levels below the optimal thresholds for adequate vitamin D status (25(OH)D <75 and <50 nmol/L respectively). ○ S-ECC, infrequent milk consumption, and winter were significantly associated with lower vitamin D levels. ○ Low serum 25(OH)D levels, low household income, and poorer ratings of the child's general health were associated with S-ECC. ○ *+ findings for low serum vitamin D concentrations and higher rates of S-ECC*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Schroth et al. (2014)	2014	Canada	Prenatal 25(OH)D levels were assessed in pregnant women in their 2nd and 3rd trimesters.	To determine the relationship between prenatal 25(OH)D concentrations and dental caries in infants	A prospective, observational study N = 207 Pregnant women A dentally vulnerable and low socioeconomic population. Ages = 12-month-old infants Children were dentally examined by one examiner. Mothers answered questionnaires on demographic information as well as the use of prenatal vitamins, health conditions, nutritional intake, and awareness of ECC. Caregivers asked about child's additional risk factors for ECC, the age of child when their first tooth erupted, oral hygiene practices, and visits to the dentist.	○ EHP, infant age, and lower prenatal 25OHD levels were significantly associated with ECC. ○ Mothers of infants with ECC were significantly more likely to be Aboriginal, rated their own health as average or poor, had other children with ECC, and consumed milk less frequently during pregnancy. Furthermore, they were more likely to use food banks and had low incomes. ○ Infants with EHP were significantly more likely to have ECC. ○ Children with ECC were significantly older than those who were caries-free. ○ ECC was not significantly associated with bottle-feeding or breastfeeding. ○ *+ findings of prenatal vitamin D and post-natal ECC and dental defects*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Williams (2014)	2014	U.S.	No vitamin D supplementation given; serum 25(OH)D concentrations were measured for children undergoing dental treatment under GA.	To assess if vitamin D deficiency was associated with ECC presence	A prospective observational case control study N = 10 children Ages = zero to six years old were recruited. Data collected from 2013-2014 by collecting blood samples to assess vitamin D concentrations of children who had ECC. Parents answered self-reported questionnaires on other risk factors for ECC. Parents self-reported dental and oral hygiene habits were also recorded.	○ ECC was associated with VDD in children (<10 to 25nmol/L), and increased PTH levels. ○ Weaknesses of the study were that most participants were already at high risk for dental caries (low SES families, high sugar intake in diets and parents with low educational backgrounds). However, it recruited control patients to compare with, where dmft scores were measured and compared for the children with sufficient vitamin D statuses (>30ng/ml). ○ *+ findings found for low vitamin D statuses being linked with higher rates of ECC in these study children.*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Tanaka et al. (2015)	2015	Japan	No vitamin D supplementation given; only pre-natal dietary intake was recorded by questionnaires to account for foods containing vitamin D.	To determine if a higher maternal dietary vitamin D intake during pregnancy results in a reduced caries risk for children	A prospective observational study n = 1210 mother-infant pairs Duration = on-going since 2007. Mothers transcribed their 3-4-year-old children's dental exam data from the child's health record book. Dietary questionnaire's given to mothers to answer, as Japanese diets naturally contain high vitamin D content, so dairy, shellfish and fish intake could be recorded during pregnancy.	○ Pregnant women with a higher dietary vitamin D intake during pregnancy, is associated with a reduced risk of dental caries in their children. ○ The child's dietary vitamin D intake did not affect risk reduction associated with mother's vitamin D intake. ○ This supports the theory that vitamin D influence during tooth development periods could improve resistance to dental caries. ○ *+ findings of adequate maternal vitamin D during pregnancy, and dental caries in their offspring*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Meinerz	2016	U.S.	No vitamin D supplementation given; serum 25(OH)D concentrations were measured in children	To assess if there were differences in serum vitamin D, PTH and calcium levels amongst children who had ECC and without ECC	A cross sectional study from 2015-2015 N = 60 children Ages – below six years old African American background children were recruited from a hospital setting where they were having surgery under GA.	○ There were no serum vitamin D concentration differences in those patients who were caries free control patients and those who had ECC. ○ However, ethnicity was a strong factor in determining caries risk, other additional factors for dental caries were important to consider such as sunlight exposure, sugar intake and tooth brushing. ○ ***0*** *findings indicated that vitamin D concentrations were not a factor for increased caries risk in these study children*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Schroth et al. (2016)	2016	Canada	25(OH)D levels of the children were obtained.	Assessing the relationship between vitamin D status and dental caries in childhood.	An observational study n = 1,017 children Ages = six to 11-years. Data obtained for children from a national, cross-sectional health surveys from 2007-2009. Teeth = Mixed dentitions. Oral health–related variables included tooth brushing, dental visits, and water fluoridation. Socioeconomic variables included education, household income and presence of dental insurance. Dietary variables included the frequency of sugar sweetened drinks and milk drinking.	○ ECC was significantly associated with 25(OH)D levels of <50 nmol/L. ○ Additional factors that impacted on dental caries were, low SES status, not brushing teeth twice daily, and not having yearly dental visits. ○ An average of 2 primary teeth affected by caries and 0.49 permanent teeth affected by caries, for a total dmft/DMFT score of 2.4. ○ Children with 25(OH)D concentrations ≥75 nmol/L had significantly lower odds for caries Children with 25(OH)D levels ≥50 nmol/L had lower odds for caries The odds of ECC were lower for children whose mothers had higher levels of education. ○ Children who brushed twice daily had significantly lower odds of ever having experienced caries. Children with higher 25(OH)D levels had lower caries scores. ○ Visiting the dentist at least once a year associated with higher DMFT scores, while brushing twice daily was associated with significantly lower caries scores. ○ *+ findings of low 25(OH)D levels in children and higher caries scores*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Jumani	2016	U.S.	No vitamin D supplementation given; serum 25(OH)D concentrations were assessed in children	To investigate the relationship between serum vitamin D concentrations and dental caries in children	A case control study, N = 276 Ages = zero to six years old Medically compromised children receiving dental treatment in hospital who had VDD (<30nmol/L) were compared with case controls who had sub optimal (<75 nmol/L) and optimal (>75nmol/L) serum vitamin D concentrations. Primary dentitions were assessed	○ Medically compromised children with ECC were twice more likely to have dental caries, compared to those who did not have VDD. ○ *+ findings were found for VDD being associated with a higher caries risk in these study children*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
Gyll et al. (2018)	2018	Sweden	Vitamin D supplementation given during infancy, and at six years old of: ○ 10 nmol/L ○ 25 nmol/L and, ○ placebo, daily for 3 months.	To assess the relationship of vitamin D supplementation during infancy and at age six years old with childhood dental caries.	A prospective study. N = 206 children Ages = Eight years old Children dentally examined. Teeth: Mixed dentitions Cord blood 25(OH)D was measured. Plaque biofilm and saliva samples were obtained. Oral health–related variables included tooth brushing, sugar intake and SES status.	○ There was no difference in dental caries for children who had vitamin D supplementation, compared with those who did not receive vitamin D supplementation. ○ However, children with below adequate vitamin D levels (<50 nmol/L) had a higher rate of dental caries present. ○ Vitamin D status at age 6 years was associated with identification of the antimicrobial peptide LL37 in saliva. ○ Vitamin D supplementation had no effect on enamel defects on permanent teeth. ○ ***0*** *findings of vitamin D supplementation on childhood dental caries, and dental defects* ○ ***+*** *findings of low vitamin D status and the presence of dental caries*

Singleton et al. (2019)	2019	U.S.	Vitamin D statuses measured for pregnant women prenatally at delivery and their children at birth.	To assess the relationship between maternal prenatal vitamin D concentrations with ECC and S-ECC in children under five years of age.	An observational study n = 76 women and 57 children Ages = 12 to 59-month-old children were examined Alaskan Native children and their mothers from a prior Maternal Organics Monitoring (MOMs) study were recruited. A vulnerable rural population group with no fluoridated water. Sufficient vitamin D levels were 25(OH)D >50nmol/L - insufficient were <30nmol/L for the children and <50nmol/L prenatally for the mothers. Local dental health records were obtained to compare each child's mean dmft scores with theirs and their mother's vitamin D statuses. Teeth = primary dentitions Infant birth weight and gestational factors and	○ Study findings showed a significant association of VDD with dmft scores in children under three years old ($p = 0.002$). ○ Deficient vitamin D (<30nmol/L) levels were associated with presence of ECC in children. ○ Limitations: A small sample size and other risk factors such as diet and tooth brushing were not measured. ○ *+ findings of low maternal vitamin D during pregnancy being positively associated with ECC*

Author	Year	Country	Vitamin D supplementation or vitamin D statuses	Study Objectives	Methods	Findings
					dental treatment that may have been provided were all considered. Maternal factors considered were tobacco smoking, chewing tobacco and if they were breastfeeding.	

Table 11

Placebo controlled clinical trials on the effects of vitamin D supplementation on dental caries and developmental dental defects in children

Note* n = number of participants

Title	Author	Year	Country	Vitamin D supplementation	Study Objectives	Methods	Findings
○ **Human Studies**							
The action of vitamin D in preventing the spread and promoting the arrest of caries in children	Mellanby and Pattison	1928	UK	Vitamin D supplementation was given in the form of cod-liver oil and dietary vitamin D supplementation by extra milk and eggs intake	To examine if dental outcomes (for caries) were improved with vitamin D supplementation in children	A prospective, interventional study was undertaken on N = 21 children Ages= three to eight years old Two dental examinations were undertaken on children who had vitamin D supplementation over 28 weeks. Mixed dentitions	○ Children who took vitamin D supplementation were found to show improvements in dental caries lesions (existing and new lesions forming) ○ *+ findings that vitamin D supplementation may prevent the progression of dental caries in children*

Title	Author	Year	Country	Vitamin D supplementation	Study Objectives	Methods	Findings
Vitamin D studies	McBeath (1934)	1933-1934	US	Vitamin D supplementation was given to three groups, in different doses: 100 IU, 150 IU and 300 IU daily, in the form of cod liver oil in milk Another group was given irradiated UV light. 2 control groups were given different diets: one group had a regular diet (with vitamin D) and the other group received a placebo diet (with no vitamin D)	To examine the effects of vitamin D supplementation on dental caries, and is a larger dose more beneficial?	A prospective clinical study n = 425 children Ages = Eight to 14 Children were dentally examined thrice over 12 months. Teeth: Mixed dentitions	○ There were fewer decayed teeth in children supplemented with a higher dose of vitamin D (300 IU daily). ○ However, the lower doses of vitamin D fortified milk (100 IU, and 150 IU) were also effective in reducing the incidence of caries. ○ Therefore, the researcher concluded that a larger dose was not necessarily more beneficial. ○ *+ findings of higher dose vitamin D in children and fewer teeth with dentine caries*
The fat-soluble vitamins and dental caries in children	Day and Sedwick (1934)	1934	UK	Vitamins A & D supplements were given (in the form of tablets) over one year. Another group was given placebo supplements	To examine the effect of fat-soluble vitamins, A & D on the progression of dental caries	A prospective clinical study n = 430 children of which 210 were placebo. Ages = 13 to 14 Ethnically diverse Similar SES backgrounds Children were dentally examined with radiographs. Teeth: Mixed dentitions	○ There was an improvement in the incidence of dental caries with vitamin A and D supplementation in the study children. ○ *+ findings of vitamin D supplementation in children and a reduction in their caries rates*

Title	Author	Year	Country	Vitamin D supplementation	Study Objectives	Methods	Findings
The role of vitamin D in the control of dental caries in children	McBeath & Zucker	1938	UK	Vitamin D supplementation was given to children in the form of artificial UV radiation exposure, or dietary milk and placebo controls	To assess if vitamin D supplementation was able to reduce dental caries in children	A prospective, clinical placebo-controlled study N =871 children Ages = six to 14 years old 601 children were given vitamin D supplementation and 270 placebo, dental examinations were conducted once a year. Mixed dentitions examined.	○ Some success was shown in reducing dental caries by vitamin D supplementation (artificial UV radiation seems to be more effective) ○ Study limitations were that different forms of vitamin D supplementation were given. ○ *+ findings of vitamin D supplementation were observed for reducing dental caries in children*

Title	Author	Year	Country	Vitamin D supplementation	Study Objectives	Methods	Findings
Maternal vitamin D intake and mineral metabolism in mothers and their new-born infants	Cockburn et al. (1980)	1980	UK	Pregnant women were given dietary vitamin D_2 supplementation of 400 IU daily (10 micrograms), from 12 weeks pregnant till delivery. 633 pregnant women were given placebo supplementation.	Investigating the effects vitamin D supplementation given to pregnant mothers, on the tooth tissues of their children	A prospective clinical study n = 506 pregnant women who took vitamin D supplementation and 633 who took placebo. The two groups were comparable for social class, SES status and mothers ages. Ages = 2-6-year-old children dentally examined. Pediatric dentist was kept blind to vitamin D/calcium levels on the sixth day post birth.	○ Mothers who did not receive vitamin D supplementation during pregnancy had a higher risk of their children being born with: hypocalcaemic and dental defects on teeth. ○ Pregnancy vitamin D supplementation in this study of 400 IU daily, reduced the frequency of dental defects ($p<0.01$). ○ Vitamin D supplementation is recommended for mothers whose vitamin D intake is <500 IU daily, from diet and skin sunlight, for their babies to have better dental health. ○ Almost half (48%) of infants had prenatally determined enamel defects and mothers had lowered calcium levels. ○ 93% of infants in the vitamin D group did not have enamel defects. ○ *+ findings of maternal vitamin D supplementation during pregnancy and improvements in dental defects on their children's teeth*

Title	Author	Year	Country	Vitamin D supplementation	Study Objectives	Methods	Findings
Association of high-dose vitamin D supplementation during pregnancy with the risk of enamel defects in offspring: A 6-year follow-up of a randomized clinical trial	Norrisgaard et al. (2019).	2019	Denmark	Vitamin D supplementation high dose and placebo (2400IU/400IU) was given to women at 24 weeks pregnant till 1 week postnatal.	To assess the effect of vitamin D high dose supplementation on enamel defects and dental caries in the primary and permanent dentition.	An observational study n = 623 women and 496 children Ages = 6-year-old children were dentally examined Pregnant women were recruited from the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort (COPSAC2010). One dental examiner who was calibrated and remained blind to vitamin D/placebo statuses of participants. Other risk factors for enamel defects or dental caries were not assessed. Teeth = Mixed dentitions	○ High dose (2400IU) vitamin D supplementation versus placebo (400IU) vitamin D supplementation in pregnancy, showed an approximate of 50% odds of reducing enamel defects in children aged six years old. ○ No effect of vitamin D supplementation during pregnancy was observed on dental caries in this study (primary or permanent dentitions) ○ *+ findings of high dose maternal vitamin D supplementation and fewer dental caries in children*

Note* n = number of participants and keys to denote study findings of vitamin D improving dental outcomes in the studies by **+** = positive, **0** = null and **-** = and negative in the findings column

3.5.4 A summary of studies investigating the relationship between vitamin D and childhood dental caries and/or developmental dental defects

Overall, there were 24 studies included in my literature review of vitamin D and childhood dental health. There were three systematic reviews and meta-analyses that were included on the topics of vitamin D and its effects on dental conditions, including dental caries and developmental dental defects. There were 14 observational studies that assessed the relationship between vitamin D levels of pregnant mothers and/or children with childhood dental outcomes. There were six placebo-controlled clinical studies, which investigated the effects of vitamin D supplementation on dental caries or dental defects, and there was one animal study, where dogs were given dietary vitamin D supplementation to assess its effects on their dental health.

The RCTs reviewed, showed associations of vitamin D status with childhood dental caries. The dose of vitamin D supplementation given for preventing ECC was above 400 IU in most of the studies for pregnant women and children.

Children affected by ECC and included in these studies were predominantly between one to four years old. The consistent findings were that children whose mothers were given vitamin D supplementation during pregnancy, had primary teeth with fewer dental defects and had lower rates of childhood dental caries. Children who additionally were given vitamin D supplementation in early life or post tooth eruption also displayed fewer teeth with dental caries.

Limitations in this field of research are that most of the published studies are observational and have not followed children over a longer period but have assessed children using a one-off dental examination to collect study data. Therefore, considering these factors, in this PhD study I have purposefully followed the cohort of children over three consecutive years when they were aged between two to four years old.

My research proposal is well supported by the findings of prior studies, with my hypothesis that 'vitamin D supplementation during pregnancy and infancy protects primary teeth from dental caries and dental defects'. My thesis will help fill the current

gap in knowledge about whether pregnancy and infancy vitamin D supplementation protects the primary dentition against ECC and dental defects in early childhood.

The above-mentioned studies provide some evidence to support the notion that vitamin D supplementation during pregnancy and early infancy, has the potential to improve primary tooth formation by better mineralisation of teeth, and thus reduce the risk of dental caries and dental defects.

3.6 Vitamin D status in New Zealand

Population vitamin D status in NZ is poorer than that described from a number of other developed countries (Rockell et al., 2006). The 1997 national nutritional survey revealed that serum 25(OH)D concentrations were on average lower by 20 nmol/L in NZ individuals, compared with people in the US living at similar latitudes. The 2008/09 NZ adult nutrition survey, showed that 30% of New Zealanders had serum 25(OH)D concentrations <50 nmol/L (Drury et al., 1999). On average, vitamin D status in NZ is poorer for women compared with men. In NZ, people of Pacific or of Māori descent are at increased risk of VDD compared to NZ people of European ethnicity (Ozdemir, 2013).

3.6.1 Poorer vitamin D levels in pregnant New Zealand women

The 2008/2009 NZ Nutrition Survey showed that one third of women who were of child bearing age, were deficient in vitamin D as defined by a serum 25(OH)D concentration of <50 nmol/ (University of Otago & MOH, 2011). A study that was conducted in Auckland and enrolled an ethnically diverse sample of women during pregnancy from April 2010 to July 2011, showed that 42% of them had VDD as defined by serum 25(OH)D concentrations of <50 nmol/l, with 11% of the women having severe VDD (25(OH)D <25 nmol/l) (Ekeroma et al., 2014).

3.6.2 Vitamin D deficiency in NZ born children

“Vitamin D deficiency and nutritional rickets are again emerging as major paediatric health issues in Australia and New Zealand” (Munns et al., 2006). Only 27% of infants born in NZ from 1997 to 2001, had optimal vitamin D levels of 75 nmol/L or higher (Camargo et al., 2010). In a study of urban NZ children enrolled between 1999 and 2002, VDD (25(OH)D <25 nmol/l) was more prevalent in Pacific (24%) and Māori (11%)

children aged six to 23 months and in non-European children (16%) compared to NZ European children (3%) (Grant et al., 2009). Factors that were associated with an increased odds of VDD were winter births, non-European children with darker pigmented skin, cultural practices within Pacific Island communities, lower socioeconomic groups and household crowding (Grant et al., 2009). Other risk factors associated with VDD during childhood included longer gestational periods, younger maternal age, parental asthma and/or darker skinned individuals (Rockell et al., 2005; Camargo et al., 2010).

3.6.2.1 Barriers to obtaining vitamin D in NZ

It is difficult in NZ to obtain adequate vitamin D from sunlight exposure alone, due to large seasonal differences in sunlight intensity and duration. The risk of skin cancer, eye diseases and premature aging of the skin from skin exposure to powerful UVB-rays, are factors that limit sunlight exposure in NZ. In NZ, individuals are advised to wear sun protection such as hats, sunglasses, sun protective clothing and stay in shaded areas where possible, when the ultraviolet (UVB) rays are at their highest penetration which is from 10 a.m. to 4 p.m. from September to April. Snow and water additionally reflect 80% of sunlight; therefore, individuals need to apply sunscreen all year round.

Melanoma incidence rates in NZ are one of the highest in the world. This further makes individuals wary of obtaining vitamin D through skin exposure to sunlight, without adding to their risk of skin damage. The guidelines for defining vitamin D status based upon serum vitamin D concentrations in NZ are given in **Table *12*** (Drury et al., 1999; Munns et al., 2006).

Table 12

Guidelines for serum vitamin D concentrations to determine vitamin D status

Vitamin D Status	Serum concentration measurements of vitamin D
Moderate to severe deficiency	<25 nmol/L
Mild deficiency/insufficiency	25–50 nmol/L
Optimal	50–100 nmol/L
Associated with adverse effects	>100–150 nmol/L
Toxicity	>250 nmol/L

Adapted from the: Ministry of Health, 2013

3.6.3 The need for innovative research on vitamin D supplementation in NZ children and its effects on childhood dental health

This comprehensive literature review presented suggests vitamin D supplementation has an important role in mitigating ECC. This has not been explored in NZ children before, and particularly in a high-risk group who are already vulnerable to dental disease.

In NZ, children of Māori and Pacific ethnicity and those from poorer socioeconomic backgrounds have higher rates of dental caries (MOH, 2010). Dental caries remain a prominent issue that requires greater focus on early prevention methods, to reduce rates of this disease, particularly in young children. To determine exactly how vitamin D supplementation may assist in the prevention of dental caries and enamel defects, larger and more robustly designed RCT's are required (Bener et al., 2013).

3.6.4 Investigating the effects of vitamin D supplementation during pregnancy and infancy and childhood dental caries in NZ

The original Pregnancy and Infancy Vitamin D (PIVID) RCT study formed the basis of this PhD study. It was undertaken to find out if daily vitamin D supplementation with two different doses given to pregnant women and their babies, was able to achieve serum 25-hydroxyvitamin D (25(OH)D) concentrations above 50 nmol/L after birth (Grant et al., 2014). The intervention provided was for pregnant women to take one drop of vitamin D_3 of 1000 or 2000 IU, or placebo per day during the last trimester of pregnancy, and their infants were to take vitamin D_3 doses of 400 or 800 IU or placebo per day from birth to age 6 months (Grant et al., 2014). More detail about the PIVID study is provided in section 4.2.

The PIVID study provided the opportunity for this Doctoral project. The PIVID study enrolled pregnant women during 2010 to 2011. The study aimed to identify the optimal vitamin D supplementation dose required to achieve appropriate 25-hydroxyvitamin D concentrations above 50 nmol/l during infancy.

The primary objective for this current study was to compare the dental health of the PIVID study children, who were randomly assigned to vitamin D (higher or lower dose) or placebo supplementation, by measuring the presence of dmft and teeth with

development dental defects. This PhD study collected repeated measures, by dental examinations of children every year for three years and the data collected will be outlined in the following chapter, four.

This is the first randomised controlled clinical trial in NZ to have examined the effects of early life vitamin D supplementation (during pregnancy and infancy) on early childhood dental health. In the PIVID study on which this thesis is based, mothers from ethnically diverse and different socioeconomic backgrounds were recruited, many of whom were from high-risk groups for dental disease and VDD. Vitamin D supplementation could help improve the current rates of dental disease within NZ children and reduce existing oral health disparities between ethnic groups. Thus, vitamin D supplementation is a potential cost-saving intervention for preventing excessive dental disease, especially in Maori and Pacific children who are at increased risk of both VDD and dental caries. As a result, vitamin D could prospectively reduce the current ethnic disparities in oral health.

3.6.5 Vitamin D and ECC: A relationship summary

Teeth are very important for children's overall health and vitamin D plays an important part in the development of primary teeth. At birth, children have 20 primary teeth already formed below their gingiva or gums, which start to erupt usually around six months of age. Teeth help babies to chew food, facilitate speech, form the shape of their face, and hold placement for their permanent teeth in the jawbone. Studies also show there may be links between VDD and delayed tooth eruption (Speidel & Stearns).

This literature review has provided evidence that vitamin D has a positive influence on childhood teeth in the following ways:

a) **Preventing** childhood dental caries in primary teeth
b) **Preventing** developmental dental defects in primary teeth

A number of studies and reviews have established that low vitamin D status in pregnant women and children does impact childhood dental caries and dental defects (Lips, 2006). Maternal VDD in pregnancy is recognised to contribute to poorer tooth structure formation that begins in utero. Therefore, there is a higher probability of children having dental defects and ECC development if their mothers have VDD. There

has been a limited amount of research done to date, which has investigated vitamin D supplementation and its effects on primary dental health.

It is worth noting from the overview of VDD provided in this chapter, that VDD is prevalent, it affects tooth formation (by insufficient mineralisation), the development of dental defects and ECC. I have also outlined in this literature review how vitamin D fortified foods in diet, as well as vitamin D supplementation, are associated with improved childhood dental caries and reduced odds of dental defects. The results from the above-mentioned studies therefore show that there is a higher risk of dental disease in children with VDD, and/or pregnant women with VDD (Hujoel, 2013).

The purpose of this literature review was to provide evidence to support the need for this dental research and summarise the published literature which has investigated the potential for vitamin D to prevent ECC.

In conclusion, to date there is not enough compelling evidence available, from published studies due to smaller study samples or lack of longitudinal prospective studies, to indicate that vitamin D supplementation reduces the risk of dental disease in children or that it deters the occurrence of developmental dental defects. Further, there was no existing data from NZ on vitamin D supplementation and childhood dental health. This is the first study from NZ to have explored the effects of early life vitamin D supplementation on dental health in children, which will add to the current research of whether early life vitamin D supplementation may reduce ECC and developmental dental defects in children.

Previously completed studies on vitamin D supplementation and its effects on children's dental health have been discussed and critiqued in this chapter. Chapter four, explains the design and methodology of this prospective OH study.

Chapter 4 Study design and methodology

This chapter outlines the study design and methodology for this oral health (OH) study. It will first state the study hypothesis, followed by the study aim and objectives. It will also provide background information on the pre-existing population and will discuss the methodology, recruitment processes, data collection, data entry and statistical analyses used for each sub-study of this PhD.

4.1 Hypothesis, study aim and objectives

The overarching hypothesis, specific aims and objectives for this PhD study are stated as follows.

4.2 PhD hypothesis

Vitamin D supplementation in pregnancy and early infancy protects the primary dentition against dental caries and enamel defects.

4.3 PhD study aim

To investigate if two different doses of antenatal and early life vitamin D supplementation provide oral health benefits in children at ages two, three and four years old and protects the primary dentition against dental caries and defects.

4.4 Study research questions

In accordance with the study objectives, the primary and secondary research questions are listed next (and mentioned in sections 1.5.1 and 1.5.2).

Primary research question:

1. What were the effects of vitamin D supplementation during pregnancy and infancy on early childhood dental health?

Secondary research questions:

2. What were the effects of other potential risk factors on dental caries in this study?

3. Were the mother's oral health statuses in all three study groups equal?

4.5 Specific research objectives

This study implemented a repeated measures study design in which data was collected via a separate sub-study when children from the PIVID study were aged two, three and four years – each constituting a time-specific dataset.

i. **Phase one:** assessed the effects of early life vitamin D supplementation on child OH outcomes.
ii. **Phases two and three:** assessed the effects of early life vitamin D supplementation on child OH outcomes. It also assessed the relationship between the OH of the mother and child with a focus on their oral health habits.

4.6 The Pregnancy and infancy vitamin D (PIVID) study overview

The PIVID study was designed to investigate the most appropriate vitamin D dose in pregnancy and infancy for new borns to achieve a serum 25(OH)D concentration >50 nmol/L (Grant et al., 2014). During the period from April 2010 to July 2011, the PIVID study recruited 260 pregnant women. The design used was a randomised, placebo-controlled, double blind, multi-arm parallel study, with three randomised study groups: higher dose vitamin D_3 (maternal/infant: 2000/800 IU per day), lower dose vitamin D_3 (maternal/infant: 1000/400 IU per day), or placebo (maternal/infant: placebo).

A placebo is a pharmacologically inactive agent, assigned to some participants in an experimental study to form a control group. A block randomisation method was utilized to assign study participants in equal proportions to one of the three study arms: placebo, or lower dose vitamin D_3 or higher dose vitamin D_3. The double-blind approach ensured that participants, investigators, and health care providers remained unaware of which study group the participants were assigned. Postnatally, the children received a continuation of the same group assignment as their mother (higher dose vitamin D, lower dose vitamin D, placebo). This continued from birth until six months of age. The mother and their infants were recipients of one of the three following doses:

i. Lower dose vitamin D: of daily 1000 IU for mothers and 400 IU for infants, or
ii. Higher dose vitamin D: of daily 2000 IU for mothers and 800 IU for infants, or

iii. Placebo dose: for mothers and infants, see **Figure *11***.

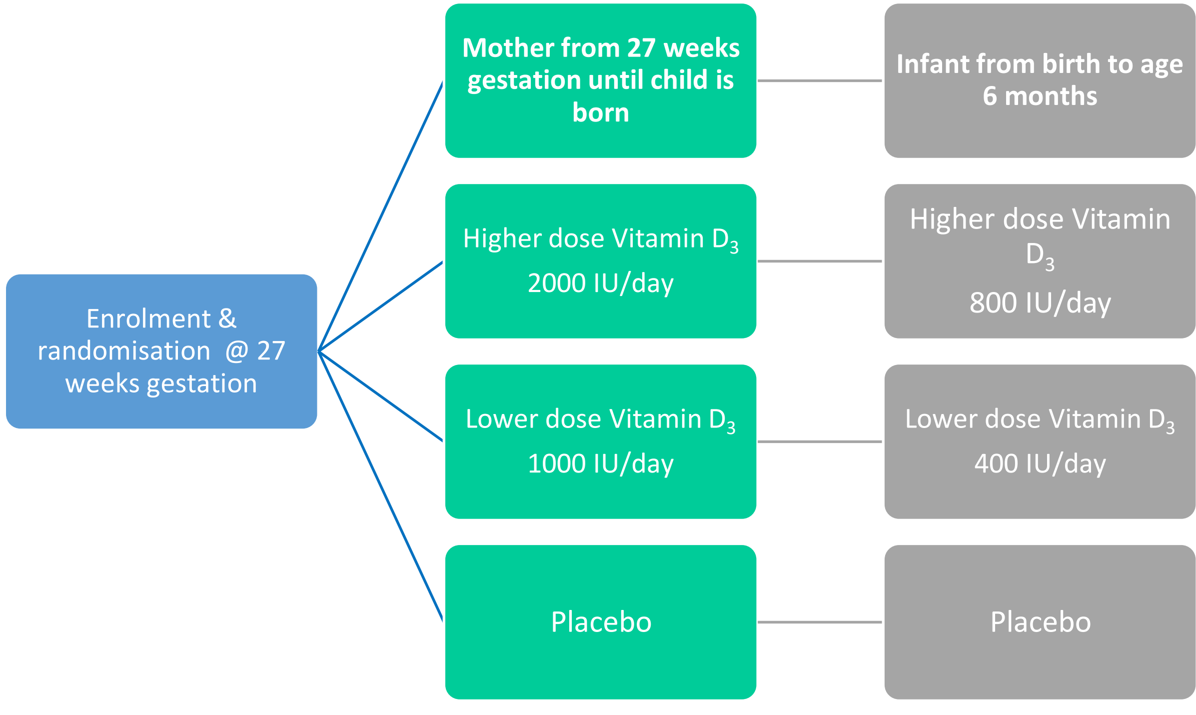


Figure 11

Mother-child pairs allocated to vitamin D and placebo groups in the PIVID study

(Grant et al., 2014)

4.6.1 Vitamin D supplementation and safety in PIVID

Vitamin D supplementation was not administered in this OH study, therefore, 25(OH)D concentrations were not measured of the mothers and children upon enrolment into this study. This study sought to explore the effects of the initial vitamin D supplementation given prenatally and after birth (during the PIVID study), on the primary teeth of study children.

In the PIVID study, the study mothers were also assessed for vitamin D (25(OH)D) concentrations in PIVID at 36 weeks pregnancy, and all were found to be equal upon enrolment.

During the PIVID study, measures were also taken and recorded for the baseline vitamin D statuses in the infants at two, four, six, 12 and 18 months old. No vitamin D doses (higher (2000 IU mothers and 800 IU children) or lower (100 IU mothers and 400 IU children) were found to cause hypercalcemia in the infnants upon the completion of

the PIVID study. Other studies have shown safe upper limits of up to 4000 IU during pregnancy, without causing toxicity (Grant et al., 2014).

4.7 Study design and methodology

In this study I attempted to contact all 260 mother-infant pairs from PIVID and reviewed these children across three consecutive years, constituting both a cross-sectional dataset at each year (children at ages two, three and four years old) and a longitudinal dataset across all three years (see **Figure *12***).

Figure 12

Study design of the OH PhD study which extended the PIVID study

In the next section, the ethical approval, recruitment processes, data collection methods and statistical analyses used in this PhD study are outlined.

4.7.1 Ethical approval

The NZ Health and Disability Ethics Committee (HDEC) granted ethical approval for this OH study, as an extension of the PIVID study, in August 2013 (NTX09/11/101/AM03) (see Appendix A). HDEC is the body created to ensure that proposed health research meets the ethical standards of the National Ethics Advisory Committee - Kāhui Matatika o te Motu (New Zealand Association of Clinical Research (NZACRes). The acquired ethical approval ensured patients had complete autonomy in the study and that the study design protected their rights and safety. Approval also ensured that the expected outcomes from this study had the potential to be of overall benefit to the wider public. This study commenced after researchers had obtained ethical approval.

The ethical approvals and amendments for the ethics of this study were approved by HDEC, ensuring:

- There were no risks of the study to the participants involved.
- Patient rights, beliefs, ethnic and cultural backgrounds would be respected.
- The research is of benefit across all ethnic groups in the community.

The site locality approval was given by the Auckland University of Technology Ethics Committee (AUTEC), which granted permission for the data collection for this research study to take place at AUT's oral health clinics. This is detailed below and in E5 found in Appendix B.

AUTEC approved the ethics application based upon the following criteria:

1. The project information was provided, including the study description, details of the primary researcher Professor Anita Nolan and oral health therapist Smita Keshoor undertaking the study data collection.
2. Evidence of external ethical approval by the HDEC.
3. There was acknowledgement of the partnership component between the researcher and study participants. The study therapists supported partnership by providing a summary of the child's dental status at the time of dentally examining children and providing appropriate advice on oral care to mothers/caregivers at appointments. This information was given to help participating families to aim for good oral and overall health of those involved.

4. The study results will be provided to the participants at the end of the research study.

4.7.1.1 Research consultation with Māori

The implementation of this research had been designed to fulfil and adhere to the needs of Māori participants in NZ. Prior to ethical approval and commencement of this study, Māori feedback and responsiveness were sought and received in 2011. This study adopted the same approach for engaging with our Māori participants as the PIVID study (Grant et al., 2014). The He Korowai Oranga: Māori Health Strategy was followed (Hsu et al., 2010), by respecting the three principles of the Treaty; partnership, participation and protection. It also adheres to maintaining patient privacy and confidentiality with *Aroha* – care.

1. Partnership: by working with whānau who may attend at dental appointments in addition to mothers and children.
2. Participation: the interaction between the researchers and participants. The child's dental conditions were explained post examination, to the parents by the oral health therapist who examined their child.
3. Protection: no researcher or examiner implied judgment about or forced any information from a participant. The researchers were also culturally sensitive to the ethnic diversity of participants and thereby respected any specific needs of the mothers and infants.

Interacting positively promotes growth for relationships between dental professionals and patients; to facilitate this a kohā (gift) was offered to participants at the end of their appointment to show appreciation for their involvement and contribution.

The following specifications were applied to define the inclusion and exclusion criteria of the PIVID study participants to partake in this PhD study. Refer to **Table *13*** and **Table *14***.

Table 13

Inclusion criteria for PIVID and OH study participants

Inclusion criteria for PIVID study:	Inclusion criteria for PhD study:
Singleton pregnancy	Child aged over two years
Prior to recruitment the estimated gestation was 26 to 30 weeks	Participated in the PIVID study
To be of any ethnicity	Written consent given by mothers
Mother of any age	

Table 14

Study exclusion criteria for PIVID and OH study participants

Exclusion criteria for the PIVID study:

- Maternal; history of renal stones, hypercalcaemia or being hypercalcaemic when enrolling in the PIVID study
- Any major pregnancy complications
- Women receiving >200 IU per day vitamin D supplementation per day
- Twins or more than one child expected (Grant et al., 2014).

Exclusion criteria for the OH study:

- Heart murmur or any bleeding condition of the child.

Reason: If certain bacteria enters the bloodstream, bacteremia may lead to infective endocarditis, an infection of the endocardium or the heart valves, which may damage or even destroy heart valves. It occurs when certain bacteria in the bloodstream lodge on and infect abnormal heart valves or other damaged heart tissue, it can be life threatening (Błochowiak, 2019).

4.7.2 Recruitment for this PhD study

The eligible sample population of this PhD study included all the children who had participated in the previous PIVID study (Grant et al., 2014). The original PIVID study sample comprised 260 participants, of whom there were 180 mothers who permitted further contact in future, beyond completion of the PIVID study. The PIVID study manager helped in contacting the PIVID study mothers by phone or email, when their children were aged over two years old and they were provided an explanation of this study. The mothers were offered participation in this oral health follow-up study. Upon acceptance into the study, the purpose of the study and study procedures were explained in depth and an information pack was emailed to those who wished to have

further information. For this oral health study, recruitment took place from August 2014 until February 2016.

Dental examinations to assess children's teeth were carried out at clinics in South Auckland, near the homes of most participants. Those participants who were unable to attend during weekdays were provided with weekend or late afternoon appointments, to maximise attendance rates. At the time of dental appointments, parents and caregivers had the opportunity to ask questions about the purpose of the research. This was explained verbally. Written information sheets were also provided, detailing the procedures involved (see Appendix C for participant study information sheet). Mothers and family members had sufficient opportunities to discuss any queries relating to the study, beforehand or during appointments, before they decided whether to participate in the study. Study consent forms and information sheets were given prior to mothers formally committing to being involved, which granted them overall autonomy (Misra et al., 2007). Mothers who had agreed to participate, were asked to sign a written consent form (see Appendix D). Participants who were recruited into this PhD study were provided with a new identifier (ID) number, in addition to the existing PIVID ID number, which enabled us to follow individuals across the three oral health assessments.

4.8 Data collection

The different types of data that were collected and the methods that were applied, will be detailed in this section, preceding this, clinical calibrations that took place for examiners will be explained.

4.8.1 Examiners standardization and calibration

Prior to the clinical data collection for this study, 13 dental therapists who were dentally examining children over three phases, each individually underwent clinical calibration sessions.

The clinical calibration sessions were employed to ensure consistency across the therapists' findings and for inter-examiner reliability (Horowitz, 1972). Study therapists were calibrated to the accepted 'gold standard' in order to minimize discrepancies and ensure the margin of differences were under 5% (Hefti & Preshaw, 2012). Dental

examinations were performed on patients separate to the study, for minimising error and standardisation of examination procedures between all therapists, to ensure validity of the results would not be adversely affected (Nelson et al., 2011).

Every participant was assigned a new identification (ID) number to ensure patient confidentiality, privacy, and anonymity. The new ID numbers were aligned to the original PIVID study ID numbers provided by the study statistician. In this way, all examining dental therapists remained blind to the vitamin D status of the child and only the study statistician was aware of the vitamin D supplementation group the child belonged to in the PIVID study.

4.8.2 Data collection and bias

The overall evaluation of child dental health outcomes was quantitative. There was potential for recall bias in this study. However, bias in the collection of data was minimised by using quantitative methods to assess and record the dental findings of the study children. The study questionnaires were also coded so the answered questions on risk factors and mother's dental health, were quantifiable. The information collected included oral hygiene habits, frequency of sugars rich items, fluoride exposure and visits to the dentists (see **Table *15***).

The following four main methods were used to assess OH outcomes for children in this longitudinal, quantitative study:

1. One dental examination was undertaken of each child annually, to assess the effects of early-life vitamin D supplementation on childhood dental health, at ages two, three and four years.
2. PIVID study mothers completed self-reporting questionnaires on their child's dental and dietary practices, frequency of sugar-rich snacks and their use of dental services within NZ.
3. PIVID study mothers completed self-reporting questionnaires, which were clinically validated, detailing their own OH habits and their use of OH services within NZ.

Table 15

Study variables that data were collected on in this OH study

Variables	Child			Mother		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Covariates Demographic Data						
Age	✓	✓	✓	✓	✓	
Sex	✓				✓	
Ethnicity	✓				✓	
Socioeconomic status	✓				✓	
Oral Health Outcomes						
Decayed teeth	✓	✓	✓			
Missing teeth	✓	✓	✓			
Filled teeth	✓	✓	✓			
Dental defects present	✓	✓	✓			
Plaque present	✓	✓	✓			
Plaque index score	✓	✓	✓			
Gingival index score	✓	✓	✓			
Child Oral Health questionnaire						
Dietary intake	✓	✓	✓			
Oral hygiene home care	✓	✓	✓			
Use of oral health services	✓	✓	✓			
Mother's self-reported oral health questionnaire (OHIP)						
Oral hygiene habits				✓	✓	✓
Use of oral health services				✓	✓	✓

Information on dietary (sugar) intake, oral hygiene habits, and dental visits, was not measured nor collected in this study, as it was not part of the original hypothesis. Therefore, the data that was collected was not used to be adjusted for analyses due to small sample sizes.

4.8.3 Covariate measurement

The age, sex, ethnicity, and SES specifics for each child were previously obtained in phase one of this study, from the data set created from the PIVID study.

4.8.3.1 Measuring child dental health outcomes

The dental examination was purely observational, where oral health therapists (OHTs), solely noted the positive dmft scores (<0) for children.

To establish the benchmark for dental caries prior to analysis, this study matched the Ministry of Health's 2010 Oral Health Survey for decayed teeth in preschool aged children (**Table *16***).

Table 16

Threshold for establishing a baseline measure for dental caries in this study

Country	Age	Average	Year	Supporting literature
New Zealand	2-4 years	0.80 decayed, missing, or filled primary teeth (dmft)	2010	Our Oral Health Key findings of the 2009 New Zealand Oral Health Survey

The mothers' self-reporting questionnaires for data collection were in a survey form, with mostly closed-ended questions to collect information on the oral health habits of mothers and their children, as well as some dietary knowledge information.

4.8.3.2 Data collection instruments

Consistent measurement tools were used throughout the phases of this study. These comprised the dental examinations by a calibrated dental therapist and the mothers self-reporting oral health questionnaires with the same closed ended questions per subject. The dates that the data were collected was recorded, including the dental examinations undertaken, to ensure precision of the data collected. By using unchanged instrumentation internal validity was maintained.

Each child in this study had single dental examinations with oral health therapists, when they were two, three and four years old, thereby they had three in total. The dental equipment for collecting clinical dental data consisted of dental chairs for the study children and oral health therapists, dental instruments including mirrors, tweezers, dental probes, and air/water syringes (triplex) and dental visual projection lights for visible and tactile examination of teeth and gingival or soft tissues.

The dental examination procedure for each child involved the following:

i. A full mouth clinical dental base charting of the primary dentition for each child, including defective teeth and dmft scores (see Appendix I)
ii. Dental plaque and gingival index scores (see **Table *17*** and **Table *18***)

4.8.3.3 Dental base charting

The dental base chart consisted of measuring and recording teeth with dental defects and the dmft scores obtained from the clinical dental examination findings. The dmft index is an internationally recognised measuring tool, used to record the presence of dental caries on teeth by clinical examination only. The dmft index has been used in previous dental studies by WHO and NZ MOH (Moynihan & Petersen, 2004; Misra et al., 2008).

4.8.3.4 Plaque and gingival index scores

Dental plaque associated with dental caries can be quantified and measured by plaque index and gingival index scores that have been developed by Löe and Silness (1963) with their appropriate scoring criteria (see **Table *17*** and **Table *18***). Plaque index and gingival index scores were used to assess gingival health and the presence of inflammation in this study.

Table 17

Criteria for plaque index scores

Plaque index scores	Scoring measures:
0	No plaque
1	Plaque present on some but not on all interproximal, buccal, and lingual surfaces of the tooth.
2	Plaque present on all interproximal, buccal, and lingual surfaces, but covering less than one-half of these surfaces.
3	Plaque extending over all interproximal, buccal, and lingual surfaces, and covering more than one-half of these surfaces.

Table 18

Criteria for gingival index scores

Gingival index scores	Scoring measures:
0	No inflammation
1	Mild inflammation, slight change in colour, slight oedema, no bleeding on probing
2	Moderate inflammation, moderate glazing, redness, bleeding on probing
3	Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding

4.8.3.5 Brief report of the child's dental status

Each parent or caregiver was provided a short verbal summary of the child's dental health status and appropriate home oral hygiene instructions at the end of each appointment. Dental referrals were made to Auckland regional dental services for children not enrolled and those who required further dental treatment.

4.8.4 Child oral health questionnaires

The child's dental health information was gathered using a two-part, self-reporting, multi-choice questionnaire answered by mothers. This questionnaire recorded the children's dental characteristics. The first part of the questionnaire collected information that described home oral care habits and the second part collected information that described feeding practices. The child dental health and feeding questionnaires were developed to gain an insight into how oral health, dietary habits and home care practices of the children may affect their dental health. The mother's

self-reporting questionnaires for the children focused on the additional risk factors that may impact dental caries. These were:

- **Tooth brushing habits**

  How frequently do you brush your child's teeth?

  Does someone help the child with tooth brushing?

  Does the child's toothpaste contain fluoride?

  Who else shares the childs toothbrush?

  Are your child's teeth brushed after snack before they go to bed?

- **Visits to a dentist**

  Has your child ever visited a dentist?

  How often have they been?

- **Pacifier use**

  Does your child use a pacifier?

  How is it cleaned?

- **Dietary sugar intake**

  Have you ever added milo or sweetener to your child's milk?

  Does your child usually have a drink (other than water) or snack before bed?

  Does your child sleep with a bottle?

  Have you ever added sweetener/sugar to their drinks?

  Covariate questions on the frequency of sugar intake was collected by looking at the number of times the child had a sugar snack per day

  Does your child have one, two or three or more sugar snacks per day?

See Appendices E and F for the full mother's self-reporting child OH questionnaires.

4.8.5 Mothers self-reporting questionnaires

Study mothers were asked to complete OHIP self-reporting questionnaires on their own dental health and habits to gauge the mother's dental health status and to compare this between the three groups given vitamin D (higher dose, lower dose, and placebo). The mothers commented on their own perception of their dental behaviours and how frequently they visited the dentist (see Appendix G). It was not possible or

part of this research to physically examine the study mothers teeth and gingival health statues, therefore, a validated dental health questionnaire was utilized to measure maternal dental and gingival health (Slade & Spencer, 1994).

Mothers completed the self-reporting OHIP questionnaires (OHIP-14) to report on their personal dental health. The OHIP questionnaire asked mothers to provide information regarding their own oral health habits. This information included their frequency of accessing dental services, rating of their own dental health, and highlighting any current problems with their teeth or dentures. The clinical dentistry features measured by the OHIP questionnaire included the mother's ability to eat/chew foods, pain experienced in the mouth and their ability to taste and/or speak (Slade & Spencer, 1994).

The reasoning behind using a questionnaire was that it was not possible or practical to undertake dental examinations on the study mothers, as that was outside the scope of this study. The OHIP-15 questionnaire is a self-reporting, multi-choice, validated questionnaire from Natarajan et al. (2014) which relates oral health to quality of life. It is a dependable assessment tool which is a reliable proxy measure of what would be found from a dental examination of the patient's actual mouth. Its reliability has been established in other studies (DeLuca, 2008) and the OHIP questions were deemed appropriate to gauge the mothers' oral health habits, perception and access of dental services. The OHIP questionnaire was utilized in the NZ Dunedin Multidisciplinary Health and Development Study, where it was reported that the survey responses were consistent with the experience of disease within populations (Vargas-Ferreira et al., 2014). The OHIP-14 questionnaire provided insight into the oral health related quality of life for the mothers and allowed for an investigation of the relationship of the oral health of each mother and child pair.

The OHIP-14 questionnaire comprised the following seven domains: functional limitations, physical pain, psychological discomfort, physical disability, psychological disability, social disability, and handicap. This simple method measures the individual's perception of their own oral health and if any current dental problems impact on their daily QOL (Chen et al., 2010). It evaluates the frequency and impacts of dental conditions. Studies using the OHIP questionnaire have shown that poor oral hygiene

practices, barriers to accessing dental services and dental problems all inevitably affect general health (Dusso et al., 2005). See Appendix H.

4.9 Methods for data entry

All data collected was on paper forms then transferred to an Excel spreadsheet. The data was recoded in numerical format for the purpose of statistical analysis. To ensure the accuracy of the data entered, an independent researcher conducted random data checks on 20% of all data samples at the end of each of the phase. This endorsed scrutiny allowed for identification of any irregularities present in the entered data. Data discrepancies that were found were corrected and cross checked at another time by the independent researcher to ensure the accuracy of data entries.

4.10 Statistical analysis

A thorough check of the data was completed before commencing any analysis to ensure accurate data collection and entry and to identify adequacy of planned missing data strategies. Observations more than three SDs from the mean were flagged as outliers. Logic checks were also employed.

4.10.1 Descriptive statistics

The actual number and proportion of each important covariate (demographic characteristics, child's dietary practices, dietary intake and oral hygiene habits and maternal oral health were reported overall and by ethnic subgroup. The outcome variables for the child's feeding and dietary practises were collected by the mother's self-reported survey on their childs consumption of sugar sweetened beverages. These were reporting if the mother added sugar, sweetener, or Milo to the childs milk or other drinks, and/or give a sweetened beverage to their child before going to bed. The variables that made up dietary intake consisted of their childs daily intake of sugar snacks (for instance one, two, three or more than three snacks per day which were containing sugar).

Means (95% CI), standard deviations, medians and quartiles were reported for continuous variables and analysed using Student's t-test (for parametric data) and Mann-Whitney U-test for non-parametric data. Cross-tabulations were reported for

categorical variable comparisons between each vitamin D supplementation group and analysed using Chi-Square statistic (or Fisher's exact test when expected values for any individual cells in a 2X2 table were less than 5, or for ≥20% of cells in an n x r table).

4.10.2 Inferential analysis

General linear models were used to identify any demographic, oral hygiene, feeding practice/dietary intake and maternal oral health risk factors for child oral health at each follow-up measure (phases one, two and three).

A generalized mixed effects model was used to model the repeated measures data (across all phases; one through three) to identify the longer-term influence of demographics, oral hygiene habits, dietary intake and maternal oral health risk factors on child oral health. Variable selection was also undertaken with each outcome based on information criteria, primarily using standard selection heuristics. A priori identified confounding variables were adjusted for in the analysis and others were assessed empirically. Multiple imputation of missing covariate data was used for final inferences (Adams & Hewison, 2008). Inferences, which ensued, were based on a 5% significance level and two-sided alternatives. Data analysis was performed using RStudio (R Core Team, 2017).

The study results will be given next in chapter five.

Chapter 5 Study results

In this chapter, the study results will be shown in two sections: descriptive statistics will be presented first in section A (5.1), followed by inferential study results in section B (5.2).

5.1 Section A: Descriptive results

5.1.1 The demographic profile of the study population

In this section, the representative analysis to compare the study population with the original PIVID study cohort is described, followed by a demographic profile of the study sample population in this OH study.

Below is a description of the exploratory statistical analyses that were completed to evaluate and compare the PIVID study participants to the sample population of this OH study.

5.1.2 Comparing participants of this OH study with the PIVID study

This OH study comprised 50% of the PIVID study participants. Descriptive statistics were used to determine if the PIVID study participants who were enrolled in the oral health study, differed from PIVID study participants who were not enrolled in the oral health study. The methodology has been described in section 4.6.

5.1.2.1 Maternal demographics

The maternal demographic factors compared between this OH study and the PIVID study included: the mother's age, educational status, body mass index (BMI), ethnicity, gestational diabetes, smoking during pregnancy, doctor diagnosed high blood pressure and household crowding (see **Table *19*** and **Table *20***).

5.1.2.2 Mother's age

There were statistically significant differences in the age of mothers at enrolment into the PIVID study when those enrolled in the oral health study were compared with those not enrolled in the oral health study ($p = 0.0003$, see **Table *19***).

5.1.2.3 Mother's education

Almost two thirds of the study mothers had a Diploma or a bachelor's level degree in this study (60-62%), which was like the PIVID study (64%). There were no statistically significant differences seen in the mother's education over the study phases (p = 0.994, **Table *20***).

5.1.2.4 Mother's Body Mass Index (BMI)

There were no statistically significant differences in the BMI of mothers in the PIVID study, compared to this study (p = 0.374, see **Table *19***).

5.1.2.5 Mother's ethnicity

The ethnic composition of the PIVID study sample and the proportion of the women who enrolled in the oral health study were of Māori (PIVID = 33% versus oral health study 31%) or Pacific (PIVID = 43% versus OHS = 47%) ethnicity and NZ European (PIVID = 8% versus OHS = 9%), or Asian and other ethnicities (PIVID = 15% versus OHS = 12%). There were no statistically significant differences for mother's ethnicity in the PIVID study compared to the OH study (p = 0.994, see **Table *20***).

5.1.2.6 Maternal gestational diabetes

There were no statistically significant differences in mothers who had diabetes in the PIVID study, compared to this study (p = 0.863, see **Table *19***).

5.1.2.7 Maternal smoking during pregnancy

There were no statistically significant differences in the proportion of mothers who smoked during pregnancy in the PIVID study (during pregnancy), compared to the OH study upon recruitment (p = 0.931, see **Table *19***).

5.1.2.8 Mothers with doctor diagnosed high blood pressure

There were no statistically significant differences in the proportion of mothers who had hypertension in this OH study, compared to the PIVID study (p = 0.265, see **Table *19***for baseline comparison).

Intake of additional vitamin D supplementation

The proportion of mother's who took additional vitamin D supplements in the PIVID study (during pregnancy) versus this study was not statistically significant (p = 0.832).

The proportion of children who were given additional vitamin supplements after birth (at six months old), did not differ in a comparison for the children enrolled in the PIVID study, compared with children enrolled in this study (p = 0.513, see **Table *20***).

Mothers' self-reported sun exposure

Self-reported sun exposure for mothers in the PIVID study (during pregnancy) versus this study was not statistically significant (p = 0.498). However, maternal self-reported sun exposure for their children at six months old, approached statistical significance (p = 0.063) for the PIVID study versus this study, but was not statistically significant at eighteen months old (p = 0.414, see **Table *20***).

5.1.2.9 Household crowding

Household crowding for women enrolled in the PIVID study sample compared to the oral health study was slighter lower, for 1-5 people (PIVID = 30.5% versus oral health study 36.5%) and 6-8 people (PIVID = 24% versus OHS = 26%). It was much the same for 9 or more people residing in one household for both PIVID and the oral health study (PIVID = 9% versus OHS = 10%), and others remained unspecified (PIVID = 36% versus OHS =28%). Descriptive analyses of this study showed there were no statistically significant differences in household deprivation for the PIVID study participants compared to OH study participants (p = 0.962, see **Table *20***).

Table 19

The demographic characteristics of mothers who were In the PIVID study versus mothers who were in the OH Study

Variable	Categories	Phase 1	Phase 2	Phase 3	PIVID	Test of difference (p-value)
Average mother's age at enrolment into the PIVID study					27 (±6)	
Average mother's age when had first child (years)		22.19 (4.2)	22.51 (4.17)	22.16 (4.2)	22.43 (4.46)	0.962†
Mother's education	No Secondary School Education	20 (15.3%)	18 (17.0%)	13 (17.6%)	33 (12.7%)	0.994‡
	NCEA Level 1 or 2	18 (13.7%)	13 (12.3%)	11 (14.9%)	33 (12.7%)	
	NCEA Level 3 or 5	4 (3.05%)	4 (3.77%)	1 (1.35%)	14 (5.38%)	
	Other/overseas education	8 (6.11%)	7 (6.6%)	4 (5.41%)	13 (5.0%)	
	Post school qualification	81 (61.8%)	64 (60.4%)	45 (60.8%)	167 (64.2%)	
Mother's BMI at enrolment into the PIVID study					209 (87.80%)	
Married/De facto/Living together relationship	Yes	108 (90.8%)	89 (90.8%)	59 (89.4%)	29 (12.2%)	0.945‡
	No	11 (9.24%)	9 (9.18%)	7 (10.6%)		
Mother's gestational diabetes					14 (17%)	
Mother's smoking status	Yes	27 (32.1%)	20 (29.4%)	14 (31.8%)	50 (26.7%)	0.931‡
	No	57 (67.9%)	48 (70.6%)	30 (68.2%)	137 (73.3%)	
Household crowding	1-5 people	40 (30.5%)	31 (29.2%)	21 (28.4%)	95 (36.5%)	0.962‡
	6-8 people	32 (24.4%)	27 (25.5%)	17 (23.0%)	67 (25.8%)	
	9 or more people	12 (9.16%)	10 (9.43%)	6 (8.11%)	25 (9.62%)	
	Not specified	47 (35.9%)	38 (35.8%)	30 (40.5%)	73 (28.1%)	
Socioeconomic status						Not in the PIVID dataset

Note*:* † = ANOVA statistical tests used to compare the distributions for continuous data and ‡ = Chisq-test used to compare distributions for categorical measures across the three phases of OHS and the overall PIVID study.

Table 20

Maternal variables compared for this OH study versus the PIVID study

Maternal variables	Categories	OHS		Not in OHS		Test of difference (p-value)**
Age at enrolment into the PIVID study	Means [range]	29.1	[19.1-43.7]	26.3	[14.6-43.3]	0.000†
BMI at enrolment into the PIVID study		31.9	[19.2-49.0]	32.7	[19.8-52.7]	0.374†
Doctor diagnosed diabetes	Yes		2 (1.7%)	3	(2.2%)	0.863‡
	No	112	(96.6%)	134	(97.1%)	
Gestational diabetes	Yes		4 (4.6%)	3	(3.2%)	0.574‡
	No		82 (94.3%)	90	(96.8%)	
Doctor diagnosed high blood pressure	Hypertensive		8 (6.7%)	5	(3.6%)	0.265‡
	Normotensive	108	(93.1%)	133	(96.4%)	
Gestational high blood pressure	Hypertensive		2 (1.7%)	5	(3.6%)	0.459‡
	Normotensive	114	(98.3%)	133	(96.4%)	
Mother's self-reported adherence with study medicine during pregnancy	Yes	106	(96.4%)	109	(93.1%)	0.377‡
	No		4 (3.6%)	8	(6.8%)	
Child’s self-reported adherence with study medicine at age 6-months	Yes		95 (86.4%)	72	(66.7%)	0.000‡
	No		15 (13.6%)	36	(33.3%)	
Mother’s additional vitamin supplements use during pregnancy	Yes		11 (10.0%)	13	(11.1%)	0.832‡
	No		99 (90.0%)	104	(89.9%)	
Child’s additional vitamin supplements at age 6-months	Yes		4 (3.7%)	3	(2.9%)	0.513‡
	No	105	(96.3%)	101	(97.1%)	
Mother’s self-reported sun exposure (during pregnancy)	Mins/week	460.1	[0.0-2940.0]	511.2	[0.0-2760.0]	0.498†
Child’s self-reported sun exposure (at 6-months)	Mins/week	155.2	[2.0-1680.0]	245.0	[0.0-2100.0]	t-test p=0.063
Child’s self-reported sun exposure (at 18-months)	Mins/week	520.7	[0.0-3360.0]	595.5	[10.0-2520.0]	t-test p=0.414

Note: †= statistical t-tests used to compare the distributions for continuous data and ‡= Fisher-exact tests used to compare the distributions for categorical measures across the three phases of OHS and the overall PIVID study.

5.1.2.10 Study Population

The first phase of this OH study recruited from a total of 180 of the PIVID study sample, who had permitted further contact at the end of the study. In phases two and three, the study participants were then subsequently recruited from the first phase of this OH study. The first phase recruited 131 participants, the second phase recruited 105 participants and the third phase recruited 74 participants for this PhD study (see **Figure *13*** next).

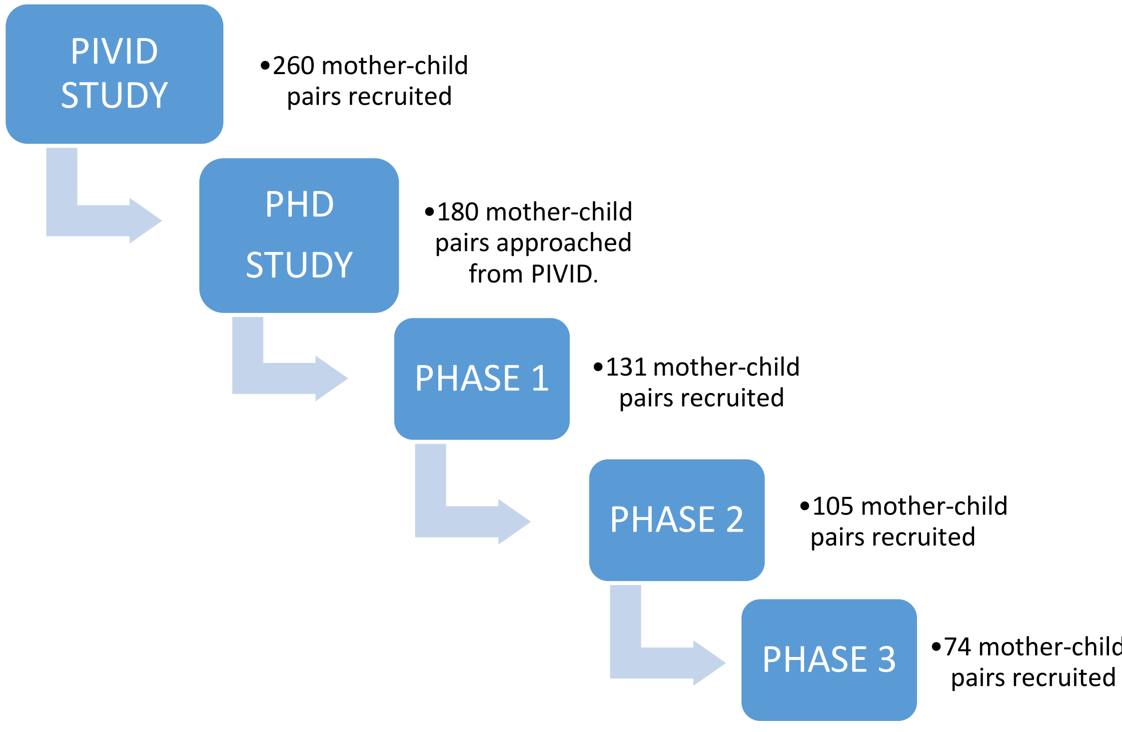


Figure 13

Flow chart summarising the participant numbers moving through from the PIVID study to this OH PhD study

5.1.3 Study demographics

The participants in this study comprised children under five years old, who were predominantly residents of South Auckland, NZ.

5.1.3.1 Average age of the study children

The average ages of children by phase in this study were 2.9 years old (34 months) in phase one, 3.7 years old (44 months) in phase two, and 4.6 years old (55 months) in phase three.

5.1.3.2 Child's sex

Overall, there were slightly more females than males who participated in this OH study. In phase one, there were 67 girls and 64 boys (ratio = 1:05) who participated. In phase two, there were 55 girls and 51 boys (ratio = 1.08) who participated and 38 girls and 36 boys (ratio = 1.06) who participated in phase three. The ratio of female to male participants was not statistically significant over all three study phases (p = 0.993, **Table *21***).

5.1.3.3 Child's ethnicity

The overall study sample was ethnically diverse, with the largest proportion of participants being of Pacific (47%, 48% and 46%) ethnicity in phases one, two and three, respectively. This was followed by Maori (31%, 30% and 35%) participants being the second largest group, then Asian or other ethnicity children (12%, 11% and 11%). NZ European (9%, 10% and 8%) children were the smallest representative group by ethnicity in this study sample. The above reported ethnic distributions were closely representative of the ethnic profile reported for the PIVID study (p = 0.993, as seen in **Table *21***). The ethnic distribution in this study was also consistent with the child ethnic representation in South Auckland NZ (from where the PIVID study sample were recruited), where the proportion of ethnicities for children aged zero to 14 years old were reported as 36% Pacific, 34% Māori, 25% Middle Eastern, Latin American or African and 21% NZ European according to 2014 census statistics (Statistics NZ, 2014).

Table 21

The demographic characteristics of child participants in the PIVID versus this OH study

Note: † = ANOVA statistical tests applied to compare the distributions for continuous data and ‡ = Chi square-test applied to compare the distributions of categorical measures across the three phases of OHS and the PIVID study overall.

Variable	Categories	Phase 1	Phase 2	Phase 3	PIVID	Test of difference (p-value)
Average child's age (months)		34.8 (6.78)	44.23 (7.18)	55.46 (6.12)	No data for PIVID	8.982e-62†
Average child's age (years)		2.9 (0.565)	3.686 (0.598)	4.622 (0.51)	No data for PIVID	8.982e-62†
Child's sex	Female	67 (51.1%)	55 (51.9%)	38 (51.4%)	128 (51.1%)	0.993‡
	Male	64 (48.9%)	51 (48.1%)	36 (48.6%)	121 (48.9%)	
Child's ethnicity	NZ European	12 (9.16%)	11 (10.4%)	6 (8.11%)	21 (8.08%)	0.994‡
	Maori	41 (31.3%)	32 (30.2%)	26 (35.1%)	87 (33.5%)	
	Pacific	62 (47.3%)	51 (48.1%)	34 (45.9%)	113 (43%)	
	Asian/Other	16 (12.2%)	12 (11.3%)	8 (10.8%)	39 (15.0%)	
Child's birth weight (grams)		3525 (538)	3563 (516)	3566 (539)	3536 (670)	0.578†

5.2 Section B: Inferential Results

The inferential results of this PhD study will be presented in the next section. I have provided a summary table below with the study objectives and the measures taken to achieve these, with summary of the findings for each before going into greater detail (see **Table *22***).

Table 22

Inferential study results summarised

Objectives	Measures	Findings
To determine the effects of early life vitamin D supplementation on child dental outcomes	Dental examinations of each child to collect clinical examination data on: • Teeth present • Decayed teeth • Missing teeth • Filled teeth • Dmft scores <0 • Developmental dental defects • Plaque presence • Plaque index scores <0 • Gingival index scores <0	In phase one, the children in the higher dose vitamin D supplementation group had earlier tooth eruption patterns, compared to placebo. Children in the higher dose vitamin D supplementation group had fewer dental caries and a greater amount of dental plaque, compared to the children in the placebo group.
To determine other factors which impacted on childhood dental disease	Mothers self-reporting questionnaires on child dental health and dietary habits: these included • feeding habits, • frequency of sugar snacks (1, 2 or 3 or more sugar drinks or foods per day), • visits to a dentist, • fluoridated water exposure and • oral hygiene habits (tooth brushing frequency)	Visits to a dental professional impacted upon dental outcomes. Children who did not regularly visit a dentist had more dental caries in phase one, compared to those who were visiting a dentist regularly. Fluoride exposure was not significantly associated with a lower rate of childhood dental caries, most participants were from the same living area (South Auckland) where water supplies are fluoridated. Having one or more sugar snack per day, was associated with the presence of dental caries.
To determine maternal responses to the self-reporting questionnaires on their own oral health	Mothers validated self-reporting 'Oral Health Impact Questionnaire' (OHIP-14)	Maternal oral health was the same between the three study groups: higher dose vitamin D, lower dose vitamin D and placebo.

5.2.1 Intervention group, sex, and ethnic distribution of children in the three study phases

5.2.1.1 The proportion of children in each intervention group (placebo, lower dose, higher dose vitamin D) across the three study phases

The proportion of children in the higher dose vitamin D, lower dose vitamin D and placebo groups did not differ for the children participating across the three phases of the study (p = 0.991).

Table 23

Distribution of children by vitamin D intervention group across the three study phases

Vitamin D Intervention Group	Study phase			Comparison of three phases
	Phase 1	Phase 2	Phase 3	
Higher dose	33 (25.2%)	25 (23.8%)	18 (24.4%)	$\chi^2 = 0.286$ p = 0.991
Lower dose	48 (36.6%)	42 (40.0%)	28 (37.8%)	
Placebo	50 (38.2%)	38 (36.2%)	28 (37.8%)	
Total	131 (100)	105 (100)	74 (100)	

5.2.1.2 Child sex distribution by intervention group in the three study phases

The proportion of girls versus boys in the three oral health study phases (by vitamin D status)

Both sexes were equally represented across the three study phases. In phase one there were 51% girls and 49% boys; in phase two 52% were girls and 48% boys; and in phase three there were 51% girls and 49% of boys (see **Table *23***).

5.2.1.3 Child ethnic distribution by intervention group in the three study phases

The proportion of participants who were Pacific, Māori, Asian or other ethnicities and NZ European

Overall, in the three study phases the largest proportion of participants were of Pacific (47%) and Māori (32%) ethnicity, followed by Asian or other ethnicities (12%) and the smaller proportion being NZ European children (9%), in each phase (p= 0.994). See **Table *23***, where it shows that the ethnic distribution stayed similar across all study phases.

5.2.1.4 Study OH outcomes by sex and ethnic group across three study phases

Across the three study phases, there were no differences between sexes in the proportion of males versus females for having all primary teeth present (OR = 0.88, 95% CI = 0.44, 1.76, p = 0.717), decayed teeth present (OR = 1.26, 95% CI = 0.78, 2.03, p = 0.337); filled teeth present (OR = 1.25, 95% CI = 0.57, 2.75, p = 0.578); missing teeth present (OR = 1.22, 95% CI = 0.43, 3.57, p = 0.704); or teeth with developmental dental defects (OR = 1.08, 95% CI = 0.66, 1.77 p = 0.745). Across the three study phases there was no difference between sexes for the dmft scores (OR = 1.33, 95% CI = 0.84, 2.12, p = 0.226, see **Table *24***).

Across the three study phases, there were no differences between sexes in the proportion of males versus females, for having plaque present on teeth (OR = 1.67, 95% CI = 0.88, 3.25, p = 0.120), or plaque index scores (OR = 1.6, 95% CI = 0.83, 3.01, p = 0.168). In comparison to girls, the odds of boys having a gingival index score ≥ 0 were increased (OR = 2.0, 95% CI = 1.26, 3.31, p = **0.004**). This difference was present in phases two (OR = 3.9, 95% CI = 1.74, 9.26, p = **0.001**) and three (OR = 1.5, 95% CI = 0.47, 5.14, p = 0.489), but not in phase 1 (OR = 1.4, 95% CI = 0.68, 2.90, p = 0.356). Differences in diet between sexes were not analysed.

Table 24

Child dental outcomes by sex (males versus females), per phase and over all three phases

Variable	Phase 1		Phase 2		Phase 3		All Phases	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
All 20 teeth present	0.85 (0.34-2.11)	0.726	1.41 (0.23-11.07)	0.711	0.73 (0.17-3.00)	0.659	0.88 (0.44-1.76)	0.717
Any decayed teeth present	1.06 (0.48-2.37)	0.881	1.33 (0.60-2.93)	0.482	1.54 (0.60-3.99)	0.368	1.26 (0.78-2.03)	0.337
Any filled teeth present	0.69 (0.09-4.29)	0.688	0.63 (0.12-2.69)	0.535	2.54 (0.80-9.01)	0.125	1.25 (0.57-2.75)	0.578
Any missing teeth present	1.05 (0.12-8.96)	0.963	1.08 (0.19-6.10)	0.924	1.64 (0.26-13.01)	0.602	1.22 (0.43-3.57)	0.704
Any teeth with developmental dental defects present	0.61 (0.25-1.42)	0.256	2.17 (0.98-4.89)	0.058	0.83 (0.30-2.27)	0.721	1.08 (0.66-1.77)	0.745
Dmft Score > 0	1.06 (0.49-2.33)	0.877	1.44 (0.67 ,3.14)	0.356	1.71 (0.68 ,4.37)	0.252	1.33 (0.84 ,2.12)	0.226
Plaque present on any teeth	1.21 (0.54-2.76)	0.638	Model did not converge	0.994	0.94 (0.16 ,5.41)	0.945	1.67 (0.88 ,3.25)	0.120
Plaque Index Score > 0	1.12 (0.49-2.56)	0.784	8.51 (1.48-160.97)	0.047	1.29 (0.27 ,6.99)	0.748	1.57 (0.83 ,3.01)	0.168
Gingival Index Score > 0	1.40 (0.68-2.90)	0.356	3.93 (1.74-9.26)	0.001	1.52 (0.47 ,5.14)	0.482	2.03 (1.26 ,3.31)	0.004

5.3 Child ethnic distribution for this OH study

5.3.1 The ethnic distribution for each dental outcome

The distribution of the children's ethnicity per dental outcomes were reported for each study phase and across all three phases (see **Table *25***). For all comparisons, the reference group is NZ European. The results are presented by each study phase and across all three study phases, where possible (see **Table *27***). Please note due to small sample sizes, some of the ethnic models for specific oral health outcomes did not converge.

5.3.1.1 All 20 primary teeth present by ethnicity

Across all three study phases and separately in phases one, two and three, the association of ethnicity with all 20 teeth being present could not be reported because the models did not converge.

5.3.1.2 Any decayed teeth present by ethnicity

In comparison with NZ European children there were no statistically significant differences in any decayed teeth being present, for Māori (OR = 0.74, 95% CI = 0.32, 1.76, p = 0.488), Pacific (OR = 0.65, 95% CI = 0.29, 1.49, p = 0.293), Asian/other ethnicity (OR =0.47, 95% CI = 0.16, 1.35, p = 0.164) across all three study phases, nor within any of the phases.

5.3.1.3 Any filled teeth present by ethnicity

In comparison with NZ European children there were no statistically significant differences in the odds of any filled teeth being present, for Māori (OR = 0.42, 95% CI = 0.13, 1.50, p = 0.160), Pacific (OR = 0.39, 95% CI = 0.13-1.32, p = 0.105), Asian/ other ethnicity (OR = 0.60, 95% CI = 0.14, 2.50, p = 0.480) across all three study phases, nor within phase one or phase three.

In phase two, the odds of havening filled teeth present were decreased for Māori (OR = 0.09, 95% CI = 0.00, 0.77, p = **0.044**) and Pacific (OR = 0.11, 95% CI = 0.01, 0.75, p = **0.025**), but not for Asian/other children (OR = 0.53, 95% CI = 0.06, 3.99, p = 0.541).

5.3.1.4 Any missing teeth present by ethnicity

Across all three study phases and separately in phases one, two and three, the association of ethnicity with any missing teeth present could not be reported because the models did not converge.

5.3.1.5 Any teeth with developmental dental defects present by ethnicity

In comparison with NZ European children there were no statistically significant differences in the odds of any teeth with dental defects being present for Māori (OR = 0.68, 95% CI = 0.28, 1.69, p = 0.388), Pacific (OR = 0.76, 95% CI = 0.33, 1.83, p = 0.524) or Asian/other ethnicity (OR = 0.95, 95% CI = 0.34, 2.70, p = 0.922) across all three phases nor within each phase.

5.3.1.6 Elevated dmft scores by ethnicity

In comparison with NZ European children there were no statistically significant differences in the proportion with an elevated dmft score for Māori (OR = 0.61, 95% CI = 0.26, 1.42, p = 0.250), Pacific (OR = 0.57, 95% CI = 0.25, 1.28, p = 0.169), Asian/other ethnicity (OR = 0.54, 95% CI = 0.19, 1.46, p = 0.224) across all three study phases, nor within each phase.

5.3.1.7 Plaque present on teeth by ethnicity

In comparison with NZ European children there were no statistically significant differences in the odds of plaque being present on teeth, for Māori (OR = 1.51, 95% CI = 0.45, 4.52, p = 0.477), Pacific (OR = 1.25, 95% CI = 0.39, 3.43, p = 0.682), Asian/other ethnicity (OR = 0.86, 95% CI = 0.23, 3.05, p = 0.820) over all three study phases nor within each phase.

5.3.1.8 Elevated plaque index scores by ethnicity

In comparison with NZ European children there were no statistically significant differences in the proportion with plaque index scores, for Māori (OR = 1.51, 95% CI = 0.45, 4.52, p = 0.477), Pacific (OR = 1.32, 95% CI = 0.41, 3.65, p = 0.609), Asian/other ethnicity (OR = 0.62, 95% CI = 0.17, 2.07, p = 0.452) over all three study phases nor within each phase.

5.3.1.9 Elevated gingival index scores by ethnicity

In comparison with NZ European children there were no statistically significant differences in the proportion with elevated gingival index scores, for Māori (OR = 1.59, 95% CI = 0.61, 4.66, p = 0.363), Pacific (OR = 2.16, 95% CI = 0.87, 6.15, p = 0.115), Asian or other ethnicity (OR = 2.17, 95% CI = 0.72, 7.09, p = 0.179) over all three study phases nor within phase one or two. In phase three, the models could not converge for gingival index scores and ethnicity.

Table 25

Child OH outcomes by ethnicity, in comparison to NZ European children

Variable	Ethnic group	Phase 1		Phase 2		Phase 3		All phases	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
All 20 teeth present	Maori Pacific Asian/Other	Model did not converge		Model did not converge		Model did not converge		Model did not converge	
Any decayed teeth present	Maori	1.10 (0.27, 5.63)	0.899	0.26 (0.06, 1.06)	0.066	2.00 (0.33, 16.3)	0.466	0.74 (0.32, 1.76)	0.488
	Pacific	0.87 (0.22, 4.34)	0.855	0.37 (0.09, 1.38)	0.148	1.09 (0.18, 8.70)	0.926	0.65 (0.29, 1.49)	0.293
	Asian/Other	1.00 (0.18, 6.16)	1.000	0.19 (0.03, 1.06)	0.070	0.67 (0.06, 7.55)	0.733	0.47 (0.16, 1.35)	0.164
Any filled teeth present	Maori	0.56 (0.05, 12.8)	0.652	0.09 (0.00, 0.77)	**0.044**	1.19 (0.14, 25.48)	0.885	0.42 (0.13, 1.50)	0.160
	Pacific	0.37 (0.03, 8.29)	0.429	0.11 (0.01, 0.75)	**0.025**	1.30 (0.17, 26.94)	0.825	0.39 (0.13, 1.32)	0.105
	Asian/Other	Model did not converge		0.53 (0.06, 3.99)	0.541	1.67 (0.12, 42.45)	0.708	0.60 (0.14, 2.50)	0.480
Any missing teeth present	Maori Pacific Asian/Other	Model did not converge		Model did not converge		Model did not converge		Model did not converge	
Any teeth with dental defects present	Maori	1.21 (0.25, 8.90)	0.825	0.50 (0.12, 2.00)	0.327	0.60 (0.09, 5.09)	0.603	0.68 (0.28, 1.69)	0.388
	Pacific	1.46 (0.33, 10.21)	0.650	0.42 (0.11, 1.57)	0.194	0.96 (0.16, 7.66)	0.962	0.76 (0.33, 1.83)	0.524
	Asian/Other	1.67 (0.27, 13.89)	0.597	0.60 (0.11, 3.09)	0.538	1.2 (0.13, 12.83)	0.872	0.95 (0.34, 2.7)	0.922
Dmft sore > 0	Maori	0.83 (0.21, 3.57)	0.787	0.30 (0.07, 1.21)	0.098	1.00 (0.16, 6.31)	1.000	0.61 (0.26, 1.42)	0.250
	Pacific	0.58 (0.16, 2.44)	0.430	0.43 (0.1, 1.62)	0.224	0.79 (0.13, 4.81)	0.790	0.57 (0.25, 1.28)	0.169
	Asian/Other	0.67 (0.12, 3.57)	0.630	0.41 (0.07, 2.13)	0.296	0.60 (0.06, 5.24)	0.641	0.54 (0.19, 1.46)	0.224

Variable	Ethnic group	Phase 1		Phase 2		Phase 3		All phases	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Plaque present on any teeth	Maori	1.03 (0.20, 4.30)	0.966	1.50 (0.07, 17.36)	0.751	Model did not converge		1.51 (0.45, 4.52)	0.477
	Pacific	1.14 (0.23, 4.46)	0.855	1.17 (0.06, 9.07)	0.890	2.07 (0.09, 20.37)	0.562	1.25 (0.39, 3.43)	0.682
	Asian/Other	1.00 (0.16, 5.69)	1.000	1.10 (0.04, 30.37)	0.949	0.60 (0.02, 8.25)	0.708	0.86 (0.23, 3.05)	0.8201
Plaque index score > 0	Maori	1.03 (0.20, 4.30)	0.966	1.50 (0.07, 17.36)	0.751	Model did not converge		1.51 (0.45, 4.52)	0.477
	Pacific	1.39 (0.28, 5.52)	0.657	1.17 (0.06, 9.07)	0.890	1.50 (0.07, 13.19)	0.7392	1.32 (0.41, 3.65)	0.609
	Asian/Other	0.73 (0.12, 3.86)	0.718	0.50 (0.02, 6.06)	0.595	0.60 (0.02, 8.25)	0.7085	0.62 (0.17, 2.07)	0.452
Gingival index score > 0	Maori	1.24 (0.31, 6.32)	0.773	1.60 (0.38, 8.39)	0.541	Model did not converge		1.59 (0.61, 4.66)	0.363
	Pacific	2.17 (0.58, 10.48)	0.279	1.72 (0.44, 8.57)	0.460			2.16 (0.87, 6.15)	0.115
	Asian/Other	1.36 (0.26, 8.16)	0.718	2.67 (0.49, 17.18)	0.270			2.17 (0.72 ,7.09)	0.179

5.4 Binary analyses of associations between vitamin D dosages and the nine dental outcomes

Listed below are the dental outcome variables that were described in section 4.8.

The dental outcome variables assessed were:

1. All 20 primary teeth present
2. The number of decayed teeth present
3. The number of missing teeth present
4. The number of filled teeth present
5. Decayed missing and filled teeth (dmft) score
6. Teeth with developmental dental defects present
7. Plaque present on teeth
8. Plaque index score
9. Gingival index score

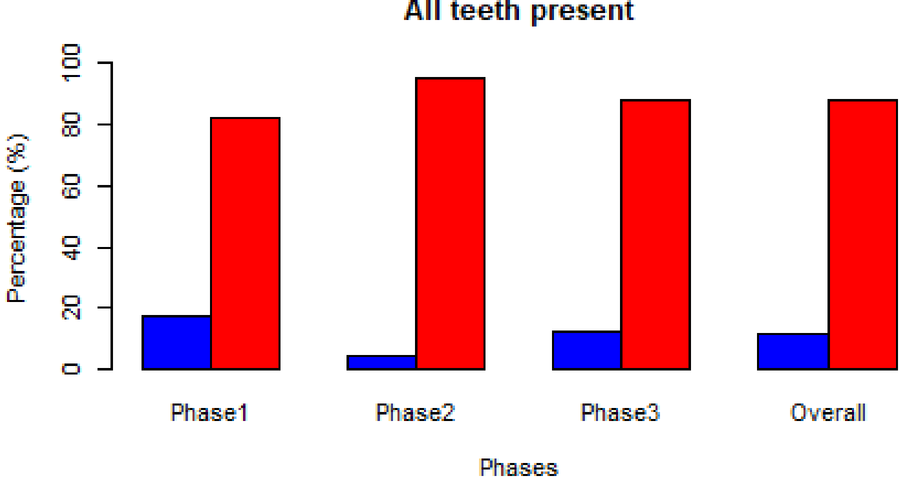
Each child dental health outcome was coded as a binary variable that was analysed using a logistic regression model. **Table *26*** shows the coding of “Yes” and “No” categories for the above variables.

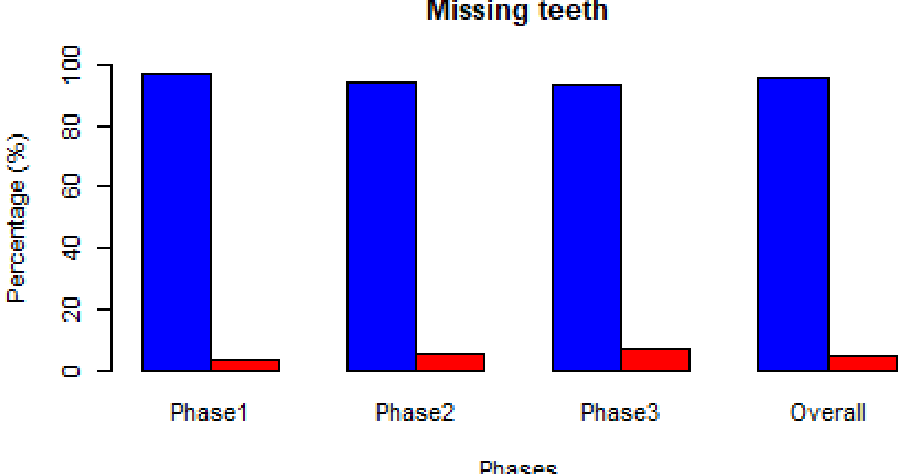
Table 26

Binary dental variables and their "yes" and "no" categories

Binary Variables	**“Yes”**	**“No”**
1. All 20 teeth present	= 20 teeth primary present	= 1 or more missing tooth
2. Decayed teeth present	= 1 or more decayed teeth	= no decayed teeth
3. Missing teeth present	= 1 or more missing tooth	= no missing teeth
4. Filled teeth present	= 1 or more filled tooth	= no filled teeth
5. Dmft score	= 1 or more decayed, missing or filled teeth	= no decayed, missing or filled teeth
6. Teeth with dental defects present	= 1 or more teeth with dental defects	= no teeth with dental defects
7. Dental plaque present on teeth	= plaque on any teeth	= no plaque present on any teeth
8. Plaque index score	= plaque on one or more teeth	= no plaque on teeth
9. Gingival index score	= gingival inflammation in one or more areas	= no gingival inflammation

The dental outcomes were plotted per phase and over all three phases (**Error! Reference source not found.*, 15 and 16***).





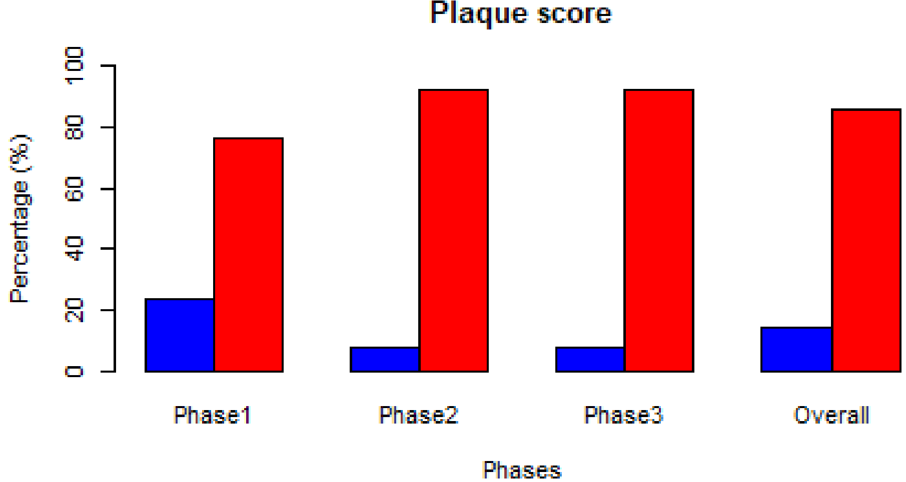
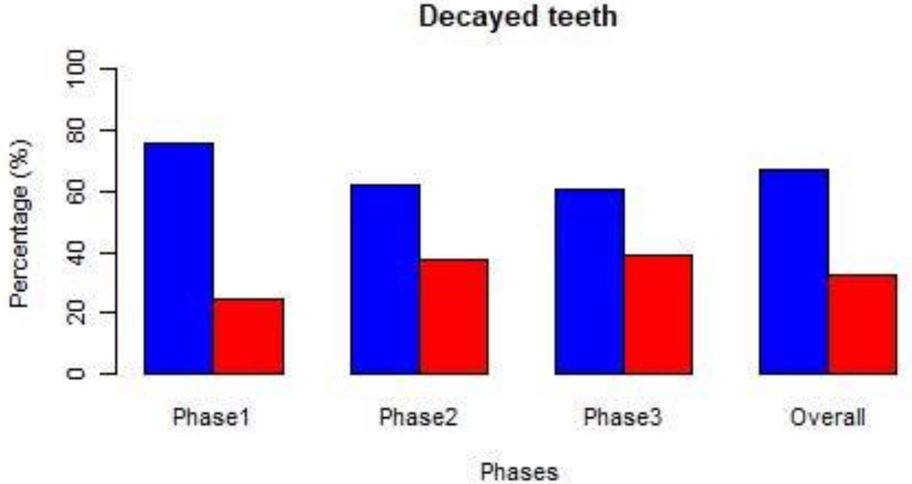
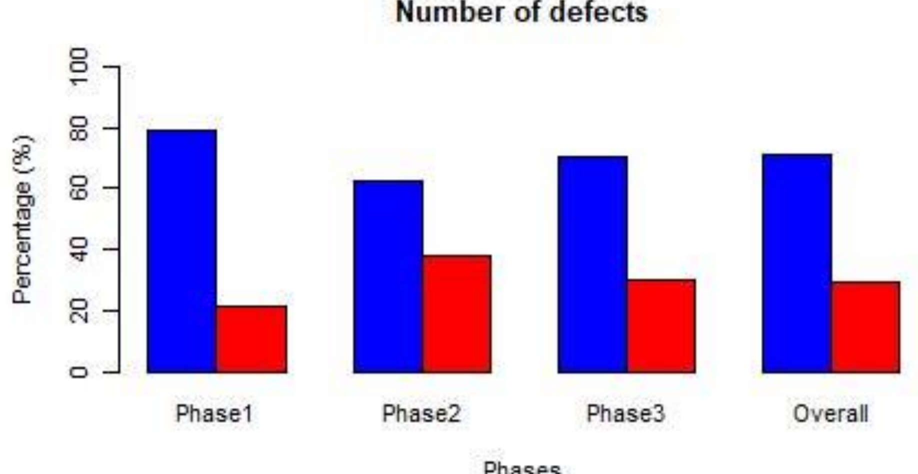


Figure 14 *The prevalence of each dichotomous dental outcome over all three study phases, shown as percentages for yes/no for the dental outcomes: all teeth present, missing teeth and plaque score*

Note: Yes = Red, No = Blue





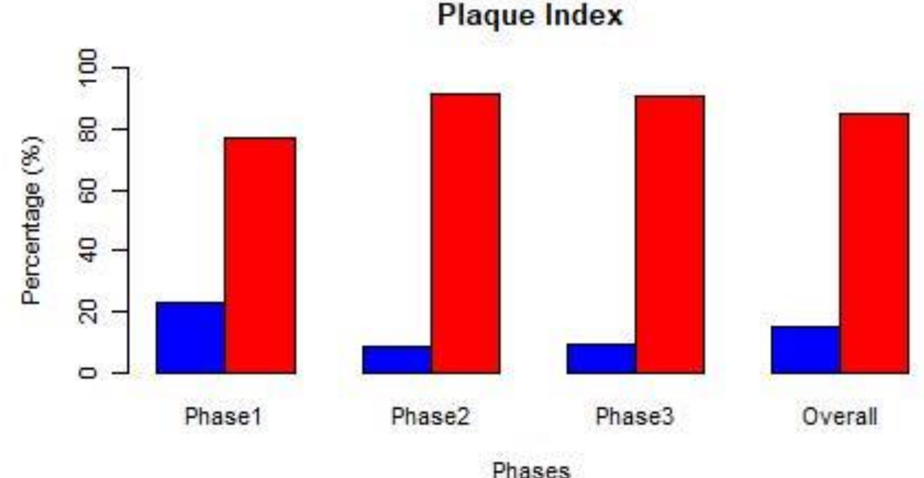


Figure 15 *The prevalence of each dichotomous dental outcome over all three study phases, shown as percentages for yes/no, for the dental outcomes: decayed teeth, number of defects and plaque index*

Note* Yes = Red, No = Blue

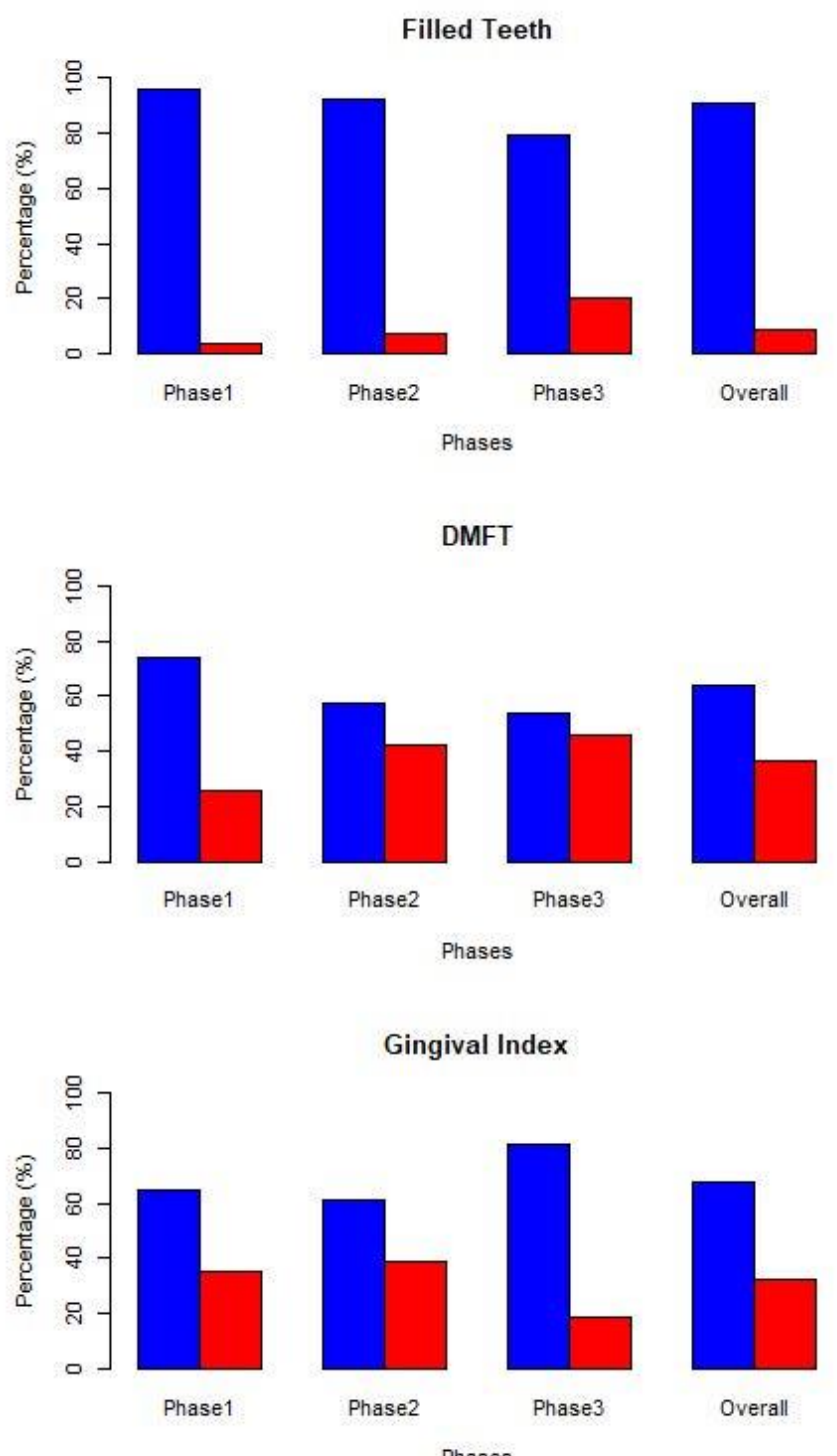


Figure 16 *The prevalence of each dichotomous dental outcome over all three study phases, shown as percentages for yes/no, for the dental outcomes: filled teeth, dmft and gingival index*

Note* Yes = Red, No = Blue

The results of the binary analyses for each dental outcome will be given in detail for each study phase, in the next section. Refer to **Table *27***, in which all the study results are detailed together, per each phase.

Table 27

Distributions of the nine OH outcomes by study phase (ages 2 year, 3 year, 4) and study group (placebo, lower dose vitamin D, higher dose vitamin D)

Oral health outcome		Study group				
		All children Median (25th-75th centile)	**Placebo Median (25th-75th centile)**	**Lower dose vitamin D Median (25th-75th centile)**	**Higher dose vitamin D Median (25th-75th centile)**	**p value**
Number of primary teeth	Phase 1	20 (20, 20)	20 (20, 20)	20 (20, 20)	20 (20, 20)	0.169
	Phase 2	20 (20, 20)	20 (20, 20)	20 (20, 20)	20 (20, 20)	0.984
	Phase 3	20 (20, 20)	20 (20, 20)	20 (20, 20)	20 (20, 20)	0.480
	Any phase					0.305
Number of decayed teeth	Phase 1	0 (1, 0)	0 (0, 1)	0 (0, 0)	0 (0, 1.5)	0.502
	Phase 2	0 (0, 2)	0 (0, 2)	0 (0, 2)	0 (0, 1)	0.298
	Phase 3	0 (0, 2)	0 (0, 2)	0 (0, 2.75)	0 (0, 1)	0.391
	Any phase					0.536
Number of filled teeth	Phase 1	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.383
	Phase 2	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.520
	Phase 3	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.438
	Any phase					0.696
Number of missing teeth	Phase 1	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.270
	Phase 2	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.801
	Phase 3	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.591
	Any phase					0.649

Oral health outcome		Study group				
		All children Median (25th-75th centile)	**Placebo Median (25th-75th centile)**	**Lower dose vitamin D Median (25th-75th centile)**	**Higher dose vitamin D Median (25th-75th centile)**	**p value**
Number of developmental dental defects	Phase 1	0 (0, 0)	0 (0, 0)	0 (0,0)	0 (0, 1)	0.577
	Phase 2	0 (0, 3)	0 (0, 2)	0 (0, 4)	0 (0, 2)	0.769
	Phase 3	0 (0, 1)	0 (0, 0)	0 (0, 1)	0 (0, 1)	0.304
	Any phase					0.737
Dmft Score <0	Phase 1	0 (0, 1)	0 (0, 2)	0 (0, 0)	0 (0, 0.5)	0.536
	Phase 2	0 (0, 2)	0 (0, 2)	0 (0, 2)	0 (0, 2)	0.854
	Phase 3	0 (0, 4)	0 (0, 4)	1.5 (0, 4.75)	0 (0, 1.5)	0.947
	Any phase					0.845
Plaque present	Phase 1	4 (2, 19.5)	5 (2, 17)	2 (1.5, 11)	4 (2, 20)	**0.009**
	Phase 2	10 (4, 20)	12 (4, 20)	10 (4, 20)	5 (3, 10)	0.951
	Phase 3	6 (4, 8)	6 (4, 8)	6 (4, 8)	6 (4, 8)	0.355
	Any phase					**0.034**
Plaque index score	Phase 1	1 (1, 2)	1 (1, 17)	1 (1, 2)	1 (1, 2)	0.149
	Phase 2	1 (1, 2)	1 (1, 2)	1 (1, 2)	1 (1, 1)	0.855
	Phase 3	1 (1, 1)	1 (1, 1)	1 (1, 1)	1 (0, 1)	0.882
	Any phase					0.380
Gingival index score	Phase 1	0 (0, 1)	0 (0, 17)	0 (0, 1)	0 (0, 1)	0.586
	Phase 2	0 (0, 1)	0 (0, 1)	0 (4, 20)	0 (0, 1)	0.933
	Phase 3	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 1)	0.578
	Any phase					0.935

Table 28

The relationship of study medicine group (vitamin D higher and lower dose) versus placebo supplementation on the nine child OH outcomes per phase

Dental Outcomes	Phase 1		Phase 2		Phase 3	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Presence of all 20 primary teeth						
Lower dose vitamin D vs. Placebo	1.58 (0.59-4.43)	0.370	0.83 (0.04-9.15)	0.884	1.63 (0.18-14.67)	0.644
Higher dose vitamin D vs. Placebo	3.16 (0.9-14.78)	***0.096***	0.75 (0.03-8.25)	0.818	0.58 (0.08-3.04)	0.538
Presence of any decayed teeth						
Lower dose vitamin D vs. Placebo	0.59 (0.22,1.52)	0.283	0.81 (0.3,2.21)	0.682	0.56 (0.16,1.85)	0.339
Higher dose vitamin D vs. Placebo	0.96 (0.35,2.56)	0.942	0.39 (0.13,1.11)	***0.081***	0.56 (0.16,1.85)	0.339
Presence of any missing teeth						
Lower dose vitamin D vs. Placebo	0.33 (0.02,2.71)	0.349	1.20 (0.11,26.63)	0.884	0.63 (0.02,16.63)	0.749
Higher dose vitamin D vs. Placebo	Did not converge		2.06 (0.25,42.95)	0.542	2.04 (0.24,43.17)	0.551
Presence of any filled teeth						
Lower dose vitamin D vs. Placebo	0.68 (0.09,4.29)	0.682	0.37 (0.05,2.37)	0.291	1.74 (0.33,13.21)	0.538
Higher dose vitamin D vs. Placebo	Did not converge		0.63 (0.11,3.66)	0.589	3.20 (0.68,23.22)	0.176
Presence of a dmft score > 0						
Lower dose vitamin D vs. Placebo	0.56 (0.22,1.38)	0.213	0.89 (0.33,2.43)	0.826	1.00 (0.3,3.3)	1.000
Higher dose vitamin D vs. Placebo	0.68 (0.24,1.8)	0.447	0.63 (0.22,1.76)	0.380	0.65 (0.19,2.14)	0.475
Presence of any developmental dental defects						
Lower dose vitamin D vs. Placebo	0.71 (0.25,1.94)	0.505	0.75 (0.27,2.12)	0.582	2.26 (0.62,9.62)	0.234
Higher dose vitamin D vs. Placebo	1.33 (0.47,3.69)	0.583	1.09 (0.39,3.1)	0.868	1.17 (0.29,5.15)	0.829

Dental Outcomes	Phase 1 OR (95% CI)	p value	Phase 2 OR (95% CI)	p value	Phase 3 OR (95% CI)	p value
Presence of any dental plaque						
Lower dose vitamin D vs. Placebo	1.27 (0.53,3.06)	0.594	0.40 (0.02,2.88)	0.419	Did not converge	
Higher dose vitamin D vs. Placebo	7.29 (1.87,48.52)	**0.012**	0.49 (0.02,4.06)	0.542		
Plaque index score > 0						
Lower dose vitamin D vs. Placebo	1.29 (0.53,3.18)	0.580	0.83 (0.11,4.59)	0.833	Did not converge	
Higher dose vitamin D vs. Placebo	4.20 (1.27,19.79)	**0.032**	1.01 (0.13,6.58)	0.988		
Gingival index score > 0						
Lower dose vitamin D vs. Placebo	0.88 (0.38,2.06)	0.772	1.21 (0.44,3.44)	0.716	0.43 (0.09,1.91)	0.267
Higher dose vitamin D vs. Placebo	1.43 (0.58,3.55)	0.438	1.04 (0.36,3.02)	0.946	0.57 (0.13,2.38)	0.429

5.4.1 Associations between the child dental outcomes (as binary) and time, over the three phases

5.4.1.1 All 20 primary teeth present

The measure for this dental outcome was the presence of all 20 primary teeth (yes/no). It was observed that children were **4.3 times** more likely to have all 20 primary teeth present in phase two (statistically significant, p = **0.004**), compared to phase one, although this declined by phase three (not statistically significant, p = 0.309). The decline in phase three is attributed to teeth affected by dental decay, losing primary teeth and teeth naturally exfoliating before permanent teeth erupt.

5.4.1.2 Decayed teeth present

The measure for this dental outcome was the presence of decayed teeth (yes/no). The prevalence of children with decayed teeth increased significantly over time (the number of participants with a dmft score >0). Children were **1.8 times** more likely to have decayed teeth in phase two (statistically significant, p = 0.027) compared to phase one, and **2 times** more likely to have decayed teeth in phase three (statistically significant, p = **0.028**) compared to phase one.

5.4.1.3 Filled teeth present

The measure for this dental outcome was the presence of filled teeth (yes/no). The proportion of children with filled teeth increased over time in this study. Children were **2 times** more likely to have filled teeth in phase two, compared to phase one (not statistically significant, p = 0.218), and **6.4 times** more likely to have filled teeth in phase three, compared to phase one (significantly higher, p = **0.001**).

5.4.1.4 Missing teeth present

The measure for this dental outcome was the presence of missing teeth (yes/no). The number of children with missing teeth in this study increased over time, however, the effects were not statistically significant. Children were **1.9 times** more likely to have missing teeth in phase two, compared to phase one (not statistically significant, p = 0.328), and **2.3 times** more likely to have missing teeth in phase three, compared to phase one (not statistically significant, p = 0.225).

5.4.1.5 Developmental dental defects

The measure for this dental outcome was the presence of developmental dental defects (yes/no). There was a significantly higher likelihood of dental defects in phase two, than in phase one, but not in phase three compared with phase one. Children were **2.2 times** more likely to have dental defects on teeth in phase two compared to one (statistically significant, p = **0.006**), and **1.6 times** more likely to have dental defects in phase three, compared to phase one (not statistically significant, p = 0.182).

5.4.1.6 Dmft Scores

The measure for this dental outcome was a positive dmft score (yes/no). A statistically significant increase in dmft scores were observed over the phases of this OH study. Higher dmft scores were present in phase two compared to phase one, a child was **2.1 times** more likely to have a positive dmft score compared with phase one (statistically significant, p **= 0.008**), and **2.4 times** more likely to have a positive dmft score (<0) in phase three, compared with phase one (statistically significant, p = **0.004**).

5.4.1.7 Plaque present on teeth

The measure for this dental outcome was the presence of dental plaque (yes/no). Significant increases in plaque presence were observed over the study phases, peaking during phase two. Children were **3.8 times** more likely to have plaque present on teeth in phase two, compared with phase one (statistically significant, p = **0.001**), and were **3.5 times** more likely to have plaque present on teeth in phase three, compared with phase one (statistically significant, p = **0.008**).

5.4.1.8 Plaque index scores

This dental outcome was related to the severity of plaque present on teeth, measured by the presence of a positive plaque index score <0 (yes/no). Plaque index scores indicated of the amount of plaque that was present on tooth surfaces, ranging from 0 (no plaque) to 3 (plaque covering 3 or more surfaces of a tooth), also mentioned in section 4.8.2 Data collection and bias. Plaque index scores increased significantly over time and peaked during phase two. Children were **3.2 times** more likely to have a higher plaque index scores in phase two, compared with phase one (statistically significant, p = **0.004**), and **2.8 times** more likely to have higher plaque index scores in phase three, compared with phase one (statistically significant, p = **0.020**).

5.4.1.9 Gingival index scores

This dental outcome was related to the severity of gingival inflammation present, measured by the presence of a positive gingival index score <0 (yes/no). There was a small, but non-significant increase in gingival index scores in phase two, where children were **1.2 times** more likely to have a positive gingival index score than in phase one (not statistically significant, p=0.571). However, in phase three, children were observed to have significantly lower gingival index scores (**0.43 times**), almost by half, compared with phase one (statistically significant, p = **0.016** (see **Table *29***).

Table 29

The effect of time on the nine OH outcomes

Variable	OR (95% CI)	Std. Error	p-value
All 20 primary teeth present	4.70 (3.05-7.55)	1.2582	**1.64E-11**
Phase 2 vs. 1	4.30 (1.70-13.19)	1.6694	**0.004**
Phase 3 vs. 1	1.54 (0.69-3.69)	1.5271	0.309
Any decayed teeth present			
Phase 2 vs. 1	1.87 (1.07-3.30)	1.3304	**0.028**
Phase 3 vs. 1	1.99 (1.08-3.70)	1.3677	**0.028**
Any missing teeth present			
Phase 2 vs. 1	1.90 (0.53-7.62)	1.9332	0.328
Phase 3 vs. 1	2.30 (0.59-9.56)	1.9883	0.225
Any filled teeth present			
Phase 2 vs. 1	2.05 (0.66-6.99)	1.7964	0.218
Phase 3 vs. 1	6.40 (2.36-20.56)	1.7159	**0.001**
Dmft score > 0			
Phase 2 vs. 1	2.10 (1.22-3.66)	1.3230	**0.008**
Phase 3 vs. 1	2.42 (1.33-4.45)	1.3591	**0.004**
Teeth with developmental dental defects present			
Phase 2 vs. 1	2.22 (1.26-3.98)	1.3398	**0.006**
Phase 3 vs. 1	1.56 (0.81-2.98)	1.3935	0.182
Dental plaque present on any teeth			
Phase 2 vs. 1	3.80 (1.73-9.24)	1.5239	**0.001**
Phase 3 vs. 1	3.51 (1.48-9.75)	1.6046	**0.008**
Plaque index score > 0			
Phase 2 vs. 1	3.20 (1.50-7.50)	1.5005	**0.004**
Phase 3 vs. 1	2.84 (1.24-7.40)	1.5657	**0.020**
Gingival index score > 0			
Phase 2 vs. 1	1.16 (0.70-1.98)	1.3109	0.571
Phase 3 vs. 1	0.4312 (0.21-0.84)	1.4172	**0.016**

Note: data as binary (yes/no)

5.4.2 The effects of vitamin D and placebo supplementation on the nine child dental health outcomes by phase

This section reports on the effects of vitamin D higher and lower dose versus placebo, on the nine child dental outcomes that were clinically measured and compared over the three study phases. The associations found for the nine child dental outcomes in relation to vitamin D supplementation versus placebo will be reported next. None of the findings were statistically significant (refer to **Table *30***).

1) The effects of vitamin D supplementation on all 20 primary teeth present

The effects of vitamin D versus placebo supplementation on all 20 primary teeth present, will be reported next, per phase (refer to **Table *28***).

Vitamin D supplementation and all 20 primary teeth present in phase 1

In phase one, there was no statistically significant difference for children having all 20 teeth present, when comparing the lower dose vitamin D group versus placebo (OR = 1.58, 95% CI = 0.59, 4,43, p = 0.370, or the higher dose vitamin D group versus placebo (OR = 3.16, 95% CI = 0.90, 14.78, p = **0.096**).

Vitamin D supplementation and all 20 primary teeth present in phase 2

In phase two, there was no statistically significant difference for children having all 20 teeth present, when comparing the lower dose vitamin D group versus placebo (OR = 0.83, 95% CI = 0.04, 9.15, p = 0.884), or the higher dose vitamin D group versus placebo (OR = 0.75, 95% CI = 0.03, 8.25, p = 0.818).

Vitamin D supplementation and all 20 primary teeth present in phase 3

In phase three, there was no statistically significant difference for children having all 20 teeth present, when comparing the lower dose vitamin D group versus placebo (OR = 1.63, 95% CI = 0.18, 14.67, p = 0.644), or the higher dose vitamin D group versus placebo (OR = 0.58, 95% CI = 0.08, 3.04, p = 0.538).

2) The effects of vitamin D supplementation on decayed primary teeth

The effects of vitamin D versus placebo supplementation on decayed primary teeth, will be reported next, per phase.

Vitamin D supplementation and any decayed primary teeth present in phase 1

In phase one, there was no statistically significant difference for children having decayed teeth, when comparing the lower dose vitamin D group versus placebo (OR = 0.59, 95% CI = 0.22, 1.52, p = 0.283) or the higher dose vitamin D group versus placebo (OR = 0.96, 95% CI = 0.35, 2.56, p = 0.942).

Vitamin D supplementation and any decayed primary teeth present in phase 2

In phase two, there was no statistically significant difference for children having decayed teeth, when comparing the lower dose vitamin D group versus placebo (OR = 0.81, 95% CI = 0.30, 2.21, p = 0.682).

The reduced odds of having any decayed teeth that was associated with being in the higher dose vitamin D group versus placebo group approached statistical significance (OR = 0.39, 95% CI = 0.13, 1.11, p = **0.081**), in phase two.

Vitamin D supplementation and decayed primary teeth present in phase 3

In phase three, there was no statistically significant difference for children having any decayed teeth, when comparing the lower dose vitamin D group versus placebo (OR = 0.56, 95% CI = 0.16, 1.85, p = 0.339), or the higher dose vitamin D group versus placebo (OR = 0.56, 95% CI = 0.16, 1.85, p = 0.339).

3) The effects of vitamin D supplementation on missing primary teeth present

Vitamin D supplementation and missing primary teeth in phase 1

In phase one, there was no statistically significant difference in the proportion of children having any missing teeth, when comparing the lower dose vitamin D group versus placebo (OR = 0.33, 95% CI = 0.02, 2.71, p = 0.349), and not enough data to compare the higher dose vitamin D group versus placebo (models did not converge).

Vitamin D supplementation and missing primary teeth in phase 2

In phase two, there was no statistically significant difference in the proportion of children having any missing teeth, when comparing the lower dose vitamin D group versus placebo (OR = 1.20, 95% CI = 0.11, 26.63, p = 0.884), or the higher dose vitamin D group versus placebo (OR = 2.06, 95% CI = 0.25, 42.95, p = 0.542).

Vitamin D supplementation and missing primary teeth in phase 3

In phase three, there was no statistically significant difference in the proportion of children having any missing teeth, when comparing the lower dose vitamin D group versus placebo (OR = -0.63, 95% CI = 0.02, 16.63, p = 0.749), or the higher dose vitamin D group versus placebo (OR = 2.04, 95% CI = 0.24, 43.17, p = 0.551).

4) The effects of vitamin D supplementation on filled primary teeth present

Vitamin D supplementation and filled primary teeth in phase 1

In phase one, there was no statistically significant difference in the proportion of children having filled teeth, when comparing the lower dose vitamin D group versus placebo (OR = 0.68, 95% CI = 0.09, 4.29, p = 0.682), or the higher dose vitamin D group versus placebo (models did not converge).

Vitamin D supplementation and filled primary teeth in phase 2

In phase two, there was no statistically significant difference in the proportion of children having any filled teeth, when comparing the lower dose vitamin D group versus placebo (OR = 0.37, 95% CI = 0.05, 2.37, p = 0.291), or the higher dose vitamin D group versus placebo (OR = 0.63, 95% CI = 0.11, 3.66, p = 0.590).

Vitamin D supplementation and filled primary teeth in phase 3

In phase three, there was no statistically significant difference in the proportion of children having any filled teeth, when comparing the lower dose vitamin D group versus placebo (OR = 1.74, 95% CI = 0.33, 13.21, p = 0.538), or the higher dose vitamin D group versus placebo (OR = 3.20, 95% CI = 0.68, 23.22, p = 0.176).

5) The effect of vitamin D supplementation on dmft scores

Vitamin D supplementation and positive dmft scores in phase 1

In phase one, there was no statistically significant difference in the proportion of children with a dmft score > 0, when comparing the lower dose vitamin D group versus placebo (OR = 0.56, 95% CI = 0.22, 1.38, p = 0.213), or the higher dose vitamin D group versus placebo (OR = 0.68, 95% CI = 0.24, 1.80, p = 0.447).

Vitamin D supplementation and positive dmft scores in phase 2

In phase two, there was no statistically significant difference in the proportion of children with a dmft score > 0, when comparing the lower dose vitamin D group versus

placebo (OR = 0.89, 95% CI = 0.33, 2.43, p = 0.826), or the higher dose vitamin D group versus placebo (OR = 0.63, 95% CI = 0.22, 1.76, p = 0.3800).

Vitamin D supplementation and positive dmft scores in phase 3

In phase three, there was no statistically significant difference in the proportion of children with a dmft score > 0, when comparing the lower dose vitamin D group versus placebo (OR = 1.00, 95% CI = 0.30, 3.30, p = 1.000), or the higher dose vitamin D group versus placebo (OR = 0.65, 95% CI = 0.19, 2.14, p = 0.475).

6) The effects of vitamin D supplementation on dental defects

Vitamin D supplementation and developmental dental defects in phase 1

In phase one, there was no statistically significant difference in the proportion of children having any developmental dental defects, when comparing the lower dose vitamin D group versus placebo (OR = 0.71, 95% CI = 0.25, 1.94, p = 0.505), or the higher dose vitamin D group versus placebo (OR = 1.33, 95% CI = 0.47, 3.69, p = 0.583).

Vitamin D supplementation and developmental dental defects in phase 2

In phase two, there was no statistically significant difference in the proportion of children having any developmental dental defects, when comparing the lower dose vitamin D group versus placebo (OR = 0.75, 95% CI = 0.27, 2.12, p = 0.582), or the higher dose vitamin D group versus placebo (OR = 1.09, 95% CI = 0.39, 3.1, p = 0.868).

Vitamin D supplementation and developmental dental defects in phase 3

In phase three, there were no statistically significant differences in the proportion of children having any dental defects, when comparing the lower dose vitamin D group versus placebo (OR = 2.26, 95% CI = 0.62, 9.62, p = 0.234), or the higher dose vitamin D group versus placebo (OR = 1.17, 95% CI = 0.29, 5.15p = 0.829).

7) The effects of vitamin D supplementation on dental plaque present

Vitamin D supplementation and the presence of dental plaque in phase 1

In phase one, there was no statistically significant difference in the proportion of children having any teeth with dental plaque present, when comparing the lower dose vitamin D group versus placebo (OR = 1.27, 95% CI =0.53, 3.06, p = 0.594), there were however, increased odds for children having plaque present on any teeth when the

higher dose vitamin D group was compared with placebo (OR = 7.29, 95% CI = 1.87, 48.52, p = **0.012**).

Vitamin D supplementation and the presence of dental plaque in phase 2

In phase two, there was no statistically significant difference in the proportion of children having any teeth with dental plaque present, when comparing the lower dose vitamin D group versus placebo (OR = 0.40, 95% CI =0.02, 2.88, p = 0.419), or the higher dose vitamin D group versus placebo (OR = 0.49, 95% CI = 0.02, 4.06, p = 0.542).

Vitamin D supplementation and the presence of dental plaque in phase 3

In phase three, when comparing the higher and lower dose vitamin group D versus placebo, the models could not converge for dental plaque, as there was not enough data for the models to run.

8) The effects of vitamin D supplementation on plaque index scores

Vitamin D supplementation and plaque index scores in phase 1

In phase one, there was no statistically significant difference in the proportion of children with a plaque index score > 0, when comparing the lower dose vitamin D group versus placebo (OR = 1.29, 95% CI = 0.53, 3.18, p = 0.580).

In phase one, there were statistically significant differences in the proportion of children with a plaque index score > 0, when comparing the higher dose vitamin D group versus placebo (OR = 4.20, 95% CI = 1.27, 19.79, p = **0.032**).
Children in the higher dose vitamin D supplementation group were at increased odds of having a plaque index score > 0, when compared to the children in the placebo group.

Vitamin D supplementation and plaque index scores in phase 2

In phase two, there were no statistically significant difference in the proportion of children with a plaque index score > 0, when comparing the lower dose vitamin D group versus placebo (OR = 0.83, 95% CI = 0.11, 4.59, p = 0.833).

In phase two, there were not statistically significant differences in the proportion of children with a plaque index score > 0, when comparing the higher dose vitamin D group versus placebo (OR = 1.01, 95% CI = 0.13, 6.58, p = 0.988).

Vitamin D supplementation and plaque index scores in phase 3

In phase three, when comparing the lower and higher dose vitamin D groups versus placebo for children's plaque index scores, there was not enough data available to investigate an association, therefore the models could not converge.

9) The effects of vitamin D supplementation on gingival index scores

Vitamin D supplementation and gingival index scores in phase 1

In phase one, there was no statistically significant difference in the proportion of children with a positive gingival index score, when comparing the lower dose vitamin D group versus placebo (OR = 0.88, 95% CI = 0.38, 2.06, p = 0.772).

In phase one, there was no statistically significant difference in the proportion of children with an elevated gingival index score when comparing the higher dose vitamin D group versus placebo (OR = 1.43, 95% CI = 0.58, 3.55, p = 0.438).

Vitamin D supplementation and gingival index scores in phase 2

In phase two, there was no statistically significant difference in the proportion of children with an elevated gingival index score, when comparing the lower dose vitamin D group versus placebo (OR = 1.21, 95% CI = 0.44, 3.44, p = 0.716).

In phase two, there was no statistically significant difference in the proportion of children with an elevated gingival index score, when comparing the higher dose vitamin D group versus placebo (OR = 1.04, 95% CI = 0.36, 3.02, p = 0.946).

Vitamin D supplementation and gingival index scores in phase 3

In phase three, there was no statistically significant difference in the proportion of children with an elevated gingival index score, when comparing the lower dose vitamin D group versus placebo (OR = 0.43, 95% CI =0.09, 1.91, p = 0.267).

In phase three, there was no statistically significant difference in the proportion of children with an elevated gingival index score, when comparing the higher dose vitamin D group versus placebo (OR = 0.57, 95% CI = 0.13, 2.38, p = 0.429).

The effects of vitamin D supplementation on the nine dental outcomes, were only statistically significant for dental plaque in phase one and not statistically significant ($p>0.05$) for the eight other dental outcomes measured.

5.5 Summary of the longitudinal analyses

The longitudinal models (analyses) did not identify any statistically significant effect of vitamin D dose (higher or lower) on seven of the nine child dental outcomes.

There there were however, two statistically significant results for:

- The presence of any dental plaque (higher dose vitamin D supplementation versus placebo) Log(OR) = 7.29, p = 0.012, and
- The plaque index score (higher dose vitamin D supplementation versus placebo) Log(OR) = 4.20, p = 0.032. The power was adequate as it was >99%.

5.5.1 The association between vitamin D (higher and lower dose combined) versus placebo and the nine dental outcomes, per phase

Results reported in section 5.4.2 showed the association between higher dose vitamin D versus placebo and lower dose vitamin D versus placebo. As mentioned above, no statistically significant results were observed. To improve the power to detect statistically significant effects in this sample, the vitamin D doses (higher and lower) were combined to compare the associations with the placebo group. Results are reported in this section here. A post hoc power calculuation has been provided in section 6.5.2 as this study was underpowered.

5.5.1.1 Vitamin D (higher and lower dose combined), versus placebo and the nine dental outcomes in phase 1

In phase one, when comparing vitamin D higher and lower dose versus the placebo group, there were no statistically significant associations found for the presence of all 20 primary teeth (OR = 2.01, 95% CI = 0.81, 5.06, p = 0.132), decayed teeth (OR = 0.73, 95% CI = 0.33, 1.67, p = 0.455), missing teeth (OR = 0.20, 95% CI = 0.01, 1.58, p = 0.163), filled teeth (OR = 0.40, 95% CI = 0.05, 2.48, p = 0.321), dmft scores (OR = 0.61, 95% CI = 0.27, 1.35, p = 0.217), developmental dental defects (OR = 0.94, 95% CI = 0.40, 2.27, p = 0.891), plaque presence (OR = 2.07, 95% CI = 0.91, 4.73, **p = 0.081**), plaque index scores (OR = 1.89, 95% CI = 0.82, 4.33, p = 0.132) or gingival index scores (OR = 1.08, 95% CI = 0.52, 2.30, p = 0.834). All associations for the dental outcomes with vitamin D (higher and lower dose) supplementation, were not statistically significant (see **Table *30***).

5.5.1.2 Vitamin D (higher and lower dose combined), versus placebo and the nine dental outcomes in phase 2

In phase two, when comparing vitamin D higher and lower doses versus placebo, there were no statistically significant associations found for the presence of all 20 teeth present (OR = 0.70, 95% CI = 0.04, 5.68, p = 0.838), decayed teeth (OR = 0.58, 95% CI = 0.23, 1.46, p = 0.245), missing teeth (OR = 1.60, 95% CI = 0.24, 31.46, p = 0.675), filled teeth (OR= 0.49, 95% CI = 0.11, 2.53, p = 0.352), dmft scores (OR = 0.76 95% CI = 0.31, 1.89, p = 0.552), developmental dental defects (OR = 0.90, 95% CI = 0.36, 2.31, p = 0.822), dental plaque (OR = 0.43, 95% CI = 0.02, 2.62, p = 0.4463), plaque index scores (OR = 0.91, 95% CI = 0.13, 4.07, p = 0.907), and gingival index scores (OR = 1.12, 95% CI = 0.45, 2.95, p = 0.805) (see **Table *30***).

5.5.1.3 Vitamin D (higher and lower dose combined), versus placebo and the nine dental outcomes in phase 3

In phase three, when comparing vitamin D (higher and lower) dose versus placebo, when comparing vitamin D higher and lower doses versus placebo, there were no statistically significant associations found for the presence of all 20 teeth present (OR = 0.88, 95% ci = 0.12, 4.08, p = 0.875), decayed teeth (OR = 0.56, 95% CI = 0.19, 1.64, p = 0.283), missing teeth (OR = 1.31, 95% CI = 0.18, 26.52, p = 0.816), filled teeth (OR = 2.42, 95% CI = 0.58, 16.57, p = 0.278), dmft scores (OR = 0.81, 95% CI = 0.27, 2.36, p = 0.692), dental defects (0R = 1.66, 95% CI = 0.51, 6.49, p = 0.426), and gingival index scores (OR = 0.50, 95% CI = 0.14, 1.85, p = 0.276).

The statistical models for investigating the association between vitamin D higher and lower dose against placebo for plaque and plaque index did not converge. This is because all the placebo children had no plaque present on their teeth in phase three, therefore there was no comparison data available for the model to run (see **Table *30***).

Table 30

The effects of vitamin D supplementation (higher and lower dose combined versus placebo) on the nine child OH outcomes in phases one, two and three

Variables	**Phase 1**			**Phase 2**			**Phase 3**		
	OR	**(95% CI)**	**p**	**OR**	**(95% CI)**	**p**	**OR**	**(95% CI)**	**p**
All 20 teeth present	2.01	(0.81,5.06)	0.132	0.79	(0.04,5.68)	0.838	0.88	(0.12,4.08)	0.875
Any decayed teeth	0.73	(0.33,1.67)	0.455	0.58	(0.23,1.46)	0.245	0.56	(0.19,1.64)	0.283
Any filled teeth	0.40	(0.05,2.48)	0.321	0.49	(0.11,2.53)	0.352	2.42	(0.58,16.57)	0.278
Any missing teeth	0.20	(0.01,1.58)	0.163	1.60	(0.24,31.46)	0.675	1.31	(0.18,26.52)	0.816
Any developmental dental defects	0.94	(0.4,2.27)	0.891	0.90	(0.36,2.31)	0.822	1.66	(0.51,6.49)	0.426
Dmft score > 0	0.61	(0.27,1.35)	0.217	0.76	(0.31,1.89)	0.552	0.81	(0.27,2.36)	0.692
Dental plaque present on teeth	2.07	(0.91,4.73)	***0.081***	0.43	(0.02,2.62)	0.446	Model did not converge		
Plaque Index Score > 0	1.89	(0.82,4.33)	0.132	0.91	(0.13,4.07)	0.907	Model did not converge		
Gingival Index Score > 0	1.08	(0.52,2.3)	0.834	1.12	(0.45,2.95)	0.805	0.50	(0.14,1.85)	0.276

5.6 Other variables impacting on childhood dental health

The other variables impacting on childhood dental health included the intake of sugar snacks per day, fluoride exposure, visits to a dental professional and time. The effects of the above variables were examined and looked at independently to check their impact on childhood dental health over and above vitamin D supplementation.

5.6.1 The effects of sugar snacks on the child dental outcomes (recoded as binary), over the three study phases

The following section reports the effects observed of sugar snacks per day on the following child dental health outcomes: gingival index score and dmft score.

5.6.1.1 The association between sugary snacks per day and gingival index scores

In phase one and two, there were no significant associations between child dental outcomes and the number of times a child ate sugar snacks per day. However, by phase three, it was found that a child was **4.8 times** more likely to have high gingival index gingival index score, if they had two sugar snacks per day (statistically significant, p = 0.037), and **5.8 times** more likely, if they had three or more sugar snacks per day (statistically significant, p = 0.040), compared to if they had one sugar snack per day (see **Table *31***).

5.6.1.2 The association between sugary snacks per day and dmft scores

In phase three, child who had three or more sugar snacks per day, were **4.1 times** more likely to have a higher dmft score (OR = 4.13, 95% CI = 1.04, 20.81, near statistically significant p = 0.057), compared to children having one sugar snack per day (see **Table *31***). Near statistically significant results were observed for the association between sugar snacks per day, with decayed teeth and higher dmft scores.

These analyses were unadjusted due to constraint on sample sizes, the statistical analyses have been detailed in the methods section 4.10.2.

Table 31
The effects of eating sugar snacks per day, on the childhood OH outcomes (recoded as binary) per phase, and calculated using a logistic regression model

Sugar snacks	**Phase 1**		**Phase 2**		**Phase 3**	
	OR (95% CI)	**P**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
All 20 teeth present						
Twice vs. Once	2.55 (0.85, 9.44)	0.119	1.50 (0.18, 31.08)	0.731	1.67 (0.33, 12.36)	0.562
Three or more vs. Once	1.75 (0.51, 8.11)	0.416	0.91 (0.11, 19.07)	0.937	0.76 (0.14, 5.85)	0.761
Any decayed teeth present						
Twice vs. Once	1.19 (0.47, 2.92)	0.714	0.81 (0.31, 2.07)	0.672	0.96 (0.32, 2.82)	0.943
Three or more vs. Once	1.38 (0.43, 4.02)	0.570	2.26 (0.78, 6.79)	0.135	3.85 (1.01, 16.75)	***0.055***
Any filled teeth present						
Twice vs. Once	0.60 (0.03, 4.84)	0.659	0.39 (0.02, 2.55)	0.395	0.75 (0.18, 2.72)	0.671
Three or more vs. Once	1.13 (0.05, 9.41)	0.916	1.35 (0.18, 6.94)	0.734	1.25 (0.24, 5.43)	0.774
Any missing teeth present						
Twice vs. Once	Did not converge		0.49 (0.02, 3.52)	0.533	Did not converge	
Three or more vs. Once			0.81 (0.04, 5.94)	0.854		
Any developmental dental defects present						
Twice vs. Once	1.29 (0.5, 3.20)	0.589	1.96 (0.79, 4.93)	0.146	1.68 (0.56, 5.09)	0.354
Three or more vs. Once	0.62 (0.13, 2.16)	0.490	1.34 (0.43, 3.97)	0.600	0.93 (0.18, 3.90)	0.928
Dmft score > 0						
Twice vs. Once	1.53 (0.63, 3.68)	0.345	0.77 (0.3, 1.91)	0.575	0.83 (0.28, 2.33)	0.718
Three or more vs. Once	1.38 (0.43, 4.02)	0.570	2.29 (0.79, 7.04)	0.133	4.13 (1.04, 20.81)	***0.057***
Any dental plaque present on teeth						
Twice vs. Once	0.68 (0.28, 1.70)	0.405	Did not converge		0.94 (0.15,7.59)	0.951
Three or more vs. Once	0.86 (0.28, 2.95)	0.794			0.94 (0.11,20.13)	0.961

*Note: Twice versus once and three or more versus once, refers to the number of sugar snacks per day.

5.6.2 The effects of fluoride on the child dental health outcomes, over all three study phases

The effects of fluoride on decayed teeth and dmft scores will be reported next, per study phase.

5.6.2.1 The association between fluoride and decayed teeth, filled teeth and dmft scores

In phase one, no statistically significant associations were observed for fluoride exposure and childhood dental outcomes.

In phase two, a child was **2.1 times** more likely to have decayed teeth (near statistically significant, OR = 2.09, 95% CI = 0.88, 5.00, p = 0.095), and **3.3 times** more likely to have a higher dmft score (OR = 3.30, 95% CI = 1.39, 8.10, statistically significant p = 0.008), if they had no exposure to fluoride.

In phase three, a child was **3.8 times** more likely to have decayed teeth (OR = 3.82, 95% CI = 1.22, 12.89, statistically significant p = 0.024), **3.1 times** more likely to have filled teeth (OR = 3.13, 95% CI = 0.88, 10.87, near statistically significant p = 0.071), and **5.4 times** more likely to have a higher dmft score (OR = 5.40, 95% CI = 1.64, 21.40, statistically significant, p = 0.008), if they had no exposure to fluoride.

The statistical analysis model for dental plaque present and plaque index scores, could not be run as there were no plaque variables to compare for the yes and no scenario (see **Table *32***).

Table 32

How the nine child dental health outcomes (as binary outcomes) differed by fluoride exposure over time, calculated using a logistic regression model

Fluoride No vs. Yes	**Phase 1**		**Phase 2**		**Phase 3**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
All 20 teeth present						
Fluoride No vs. Yes	1.14 (0.44,3.2)	0.788	0.27 (0.03,1.715)	0.164	0.52 (0.12,2.719)	0.397
Any decayed teeth						
Fluoride No vs. Yes	1.88 (0.82,4.27)	0.133	2.09 (0.88,5.00)	*0.095*	3.82 (1.22,12.89)	**0.024**
Any filled teeth	Did not converge		0.06 (0.02,0.14)		0.	
Fluoride No vs. Yes			2.48 (0.55,11.19)	0.221	3.13 (0.88,10.87)	*0.071*
Any missing teeth	Did not converge					
Fluoride No vs. Yes			2.43 (0.43,13.82)	0.295	2.52 (0.31,16.70)	0.335
Any developmental dental defects						
Fluoride No vs. Yes	0.96 (0.38,2.30)	0.931	1.09 (0.45,2.59)	0.841	2.13 (0.66,6.76)	0.199
Dmft Score > 0						
Fluoride No vs. Yes	1.64 (0.72,3.68)	0.230	3.30 (1.39,8.1)	**0.008**	5.40 (1.64,21.40)	**0.008**
Dental Plaque present					Did not converge	
Fluoride No vs. Yes	1.03 (0.44,2.52)	0.939	0.71 (0.16,3.63)	0.650		
Plaque Index Score > 0					Did not converge	
Fluoride No vs. Yes	0.80 (0.34,1.92)	0.610	0.51 (0.13,2.20)	0.344		

Fluoride No vs. Yes	Phase 1		Phase 2		Phase 3	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Gingival Index Score > 0						
Fluoride No vs. Yes	0.99 (0.45,2.10)	0.969	0.97 (0.4,2.29)	0.945	1.74 (0.42,6.408)	0.417

5.6.3 The effects of visiting a dentist on the 9 child dental outcomes (as a binary outcome)

The effects of visiting a dentist on the dental outcomes are presented below.

It was observed in phase one, that a child was less likely to have ‘all 20 primary teeth present’ if they had not visited a dentist by age two (OR = 0.28, 95% CI = 0.11, 0.72, statistically significant, p = 0.008).

There were no statistically significant associations found between visiting a dentist and childhood dental outcomes, in phases two and three (see **Table *33***).

Table 33

How the nine child dental health outcomes (as binary) differed by visiting a dentist over time, calculated using a logistic regression model

Visits to dentist	**Phase 1**		**Phase 2**		**Phase 3**	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Number of teeth present						
Dentist No vs. Yes	0.28 (0.11, 0.72)	**0.008**	Did not converge		0.68 (0.09, 13.85)	0.737
Any decayed teeth present						
Dentist No vs. Yes	0.99 (0.38, 2.41)	0.977	0.51 (0.14, 1.61)	0.2810	0.79 (0.1, 4.34)	0.792
Any filled teeth present						
Dentist No vs. Yes	Did not converge		Did not converge		2.08 (0.27, 11.91)	0.427
Any missing teeth present						
Dentist No vs. Yes	0.99 (0.05, 8.04)	0.993	Did not converge		3.15 (0.15, 27.26)	0.343
Any developmental dental defects present						
Dentist No vs. Yes	0.99 (0.36, 2.50)	0.979	1.39 (0.46, 4.07)	0.5535	2.53 (0.43, 14.73)	0.281
Dmft score > 0						
Dentist No vs. Yes	1.10 (0.43, 2.61)	0.842	0.41 (0.11, 1.27)	0.1456	1.23 (0.21, 7.09)	0.806
Dental plaque present on teeth						
Dentist No vs. Yes	2.02 (0.75, 6.44)	0.189	1.28 (0.21, 24.83)	0.8230	Did not converge	
Plaque index score > 0						
Dentist No vs. Yes	1.92 (0.71, 6.12)	0.226	1.48 (0.24, 28.51)	0.7202	Did not converge	
Gingival index score > 0						
Dentist No vs. Yes	1.08 (0.46, 2.42)	0.862	0.97 (0.31, 2.86)	0.9575	2.55 (0.32, 14.83)	0.313

5.6.4 The effects of dental plaque on gingival index score and dmft scores

Dental plaque was associated with higher gingival index and dmft scores in this study, which will be outlined next.

5.6.4.1 Gingival index scores associated with the presence of dental plaque

There was a clear relationship between gingival index scores and the presence of dental plaque over all three study phases. It was observed from the analyses, that a child with a gingival index score above zero (for instance, some plaque present) was **26.5 times** more likely to have dental plaque (OR = 95% CI = statistically significant, p = 0.001), compared to a child without a positive gingival index score. The model could only be run for the presence of dental plaque and not plaque index scores, as the plaque index variables had no comparable cases of no plaque, for the yes-gingival index score scenario.

5.6.4.2 Positive dmft scores were associated with the presence of dental plaque

There was a positive relationship between the presence of dental plaque and dmft severity scores. It was observed from the analyses, that a child who had dmft score above zero, was **3 times** more likely to have dental plaque present on teeth (statistically significant, p = 0.000), compared to a child without a positive dmft score.

A child with a positive dmft score (above zero) was **3.7 times** more likely to have a positive plaque index score (statistically significant p = 0.002), compared to a child without a positive dmft score.

In the next chapter, the findings from this study will be summarised and the strengths and weakness of this study will be outlined. Chapter 6, the discussion, will also compare the findings from this study with other published literature and convey why this topic of vitamin D supplementation and its effects on oral health, is important and requires further investigation in the future.

Chapter 6 Discussion

6.1 Outline

In this chapter, the study findings that were presented in chapter five will be discussed in relation to the study research questions (outlined in chapters one and four). Firstly, a brief overview of the study purpose is given, followed by reiterating the study hypothesis and research questions (mentioned in sections 1.5 and 4.1.4).

This discussion chapter begins with a statement of the principal findings, followed by a discussion of the strengths and weaknesses of this study, and then these strengths and weaknesses in relation to other published studies. Possible mechanisms explaining the study findings and the implications of this study for health practitioners and policy makers are then discussed.

The unanswered questions from this research and recommendations for future studies on oral health and early life vitamin D supplementation, will be provided at the end of this chapter.

6.2 Study purpose

The purpose of this study was to investigate the effects of pregnancy and early infancy vitamin D supplementation on the dental health of pre-school aged children in NZ.

6.2.1 Study hypothesis

The study hypothesis was vitamin D supplementation in pregnancy and early infancy protects the primary teeth from childhood dental caries and developmental dental defects.

6.2.2 Study research questions

The study research questions (mentioned in sections 1.5 and 4.5) have been summarised in **Table *34*** and aligned with the relevant study findings. These will be individually discussed further in the following sections, and it will be reflected upon whether pregnancy and infancy vitamin supplementation may protect the primary dentition from ECC and developmental dental defects, based on the findings from this study.

Table 34

Research questions and summarised study findings

Study research questions	Key study findings during phase 1 (2 years old), phase 2 (3 years old), and phase 3 (4 years old)
Research question one What were the effects of vitamin D supplementation during pregnancy and infancy on early childhood dental health?	**Dental caries:** • Higher or lower dose vitamin D supplementation does not appear to show any effect on dental caries in phases one and three (ages two and four). The only suggestion of a potential beneficial effect was that the smaller number of dental caries (in phase two - children aged three years), in the higher dose vitamin D supplementation group versus the placebo group approached statistically significant, p = 0.081). **Developmental dental defects:** • Vitamin D supplementation does not appear to show any effect on developmental dental defects at any phase in this study.
Secondary research questions a. What were the effects of the other potential risk factors on dental caries in this study?	• Dental plaque: There was a greater accumulation of dental plaque in phase one, in children who had received higher dose vitamin D supplementation versus placebo (p = 0.032). • Irregular visits to the dentist: Children who had irregular visits to the dentist, were less likely to have all 20 primary teeth present in phase one (p = 0.008). • Sugar snacks: Having two or more snacks per day containing sugar, was associated with an increased likelihood of dental caries in phase three (statistically significant, p = 0.040). • Fluoride exposure: Children who lived in households which did not have a fluoridated water supply, had an increased likelihood of dental caries in phase three (p = 0.024).
b. Were the mother's oral health statuses equal in all three study groups?	• Mothers self-reported dental health: The mother's oral health status, as reported in the OHIP questionnaires, was comparable in the three study groups (higher dose vitamin D, lower dose vitamin D, and placebo).

6.3 Statement of the principal study findings

This cross sectional, observational, clinical study on the effects of early life vitamin D supplementation and childhood dental health, indicates a possible relationship between higher dose vitamin D supplementation during pregnancy and infancy and lower rates of ECC, but only at age three years. The study results did not suggest any relationship of vitamin D supplementation during pregnancy and infancy with the presence of developmental dental defects.

6.3.1 Key study findings

Key observations in this study were that there was no conclusive evidence that vitamin D supplementation during pregnancy and infancy improves the health of primary dentition:

- With the exception of a non-significant difference in the proportion of children with any dental caries in the higher dose vitamin D supplementation versus placebo groups at age three years, there was no evidence that vitamin D supplementation during pregnancy and infancy prevented dental caries.
- There was no effect observed of vitamin D supplementation during pregnancy and infancy on developmental dental defects in the children's teeth across all phases of this study.

6.3.1.1 Additional measured impacting risk factors for ECC demonstrated that:

- At age two, children who had received higher dose vitamin D supplementation, had a greater accumulation of dental plaque ($p = 0.032$).
- At age four years, children who had regular dental visits were more likely to have all 20 primary teeth ($p = 0.008$).
- At age four, children who lived in households that did not have a fluoridated water supply had increased odds of teeth with dental caries (statistically significant, $p = 0.024$).
- At age four, children who had more than three sugar containing snacks per day, had increased odds of dental caries that approached statistical significance ($p = 0.055$).

These key findings will be critically discussed in more in depth in the following section (6.4).

6.4 Critical discussion of the key study findings

The key study findings will now be critically evaluated in the order of research questions that have been listed in **Table *34***.

6.4.1 Research question one

6.4.1.1 What were the effects of vitamin D supplementation during pregnancy and infancy on early childhood dental health?

With the exception of a non-significant difference in the number of children with any dental caries in the higher dose vitamin D supplementation group versus the placebo group at age three years only, there was no evidence that vitamin D supplementation during pregnancy and infancy prevented dental caries in preschool aged children. This finding differs from that reported in several other studies where vitamin D supplementation given to pregnant women and young children, was identified as having a positive effect on reducing dental caries in young children's teeth (Mellanby & Pattison, 1928; Day & Sedwick, 1934; McBeath, 1934; Schroth et al., 2014; Tanaka et al., 2015).

Several studies have investigated the relationship of maternal vitamin D status during pregnancy with ECC. These studies have shown that low maternal vitamin D statuses during pregnancy, is associated with children acquiring dental caries at a younger age (Schroth, 2010; Hujoel, 2013; Botelho et al., 2020). In addition, Schroth et al. (2012) reported that children aged three who had low vitamin D statuses, were at increased odds of developing S-ECC, compared with children who had adequate vitamin D status. The study by Brown et al. (2012) reported similar findings, whereby a high proportion of children under age five who had VDD (68.8%) experienced dental caries, compared with children who did not have VDD (7%). In a recent study in infants aged 12-35 months old, VDD (25(OH)D <30 nmol/L) at birth, measured by taking children's cord blood samples, was also associated with higher rates of ECC (Singleton et al., 2019).

In this OH study, no consistent significant protective effect of higher, or lower dose vitamin D supplementation during pregnancy and infancy was evident. Possible

reasons for this lack of a protective effect may be ascribed to several factors, these will be discussed next.

It is possible that other overriding risk factors for ECC, may have had a stronger influence on primary teeth, which could have masked the effects of vitamin D supplementation on developing dentition. One of these risk factors worth may have been a lack of tooth brushing or home care, as despite the vitamin D supplementation, there was a greater accumulation of dental plaque seen in phase one, in children who had received higher dose vitamin D supplementation ($p = 0.032$). Irregular dental visits also impacted on children not having all 20 primary teeth present in phase one ($p = 0.008$) and the effect of no fluoride exposure ($p = 0.008$ in phases two and three) on positive dmft scores, were also potential reasons for no effect being seen from the vitamin D supplementation.

Most published research on this topic shows positive associations between adequate levels of vitamin D during tooth development stages and reduced odds of dental caries in primary teeth (Hujoel, 2013). In the prior PIVID study by Grant et al. (2016) it was reported that infants, upon completion of the vitamin D intervention had mean serum 25(OH)D concentrations of 61 nmol/l at age 18 months old, and they did not differ between study groups ($p = 0.30$). In this OH study the effects of prental and early life vitamin D supplementation during tooth formation stages, were assessed on early childhood dental health. However, it is worth noting this study had limited power, with increasing loss to follow up at each study phase. Therefore, it is possible, that there was a difference between the study groups for the presence of dental caries, but the size of this study sample was too small to identify this.

6.4.2 Secondary research questions

In this next section the specific additional risk factors that were assessed for ECC in this study are critically discussed for how they impacted upon the primary findings.

6.4.2.1 What were the effects of the other potential risk factors on dental caries in this study?

In this OH study, a strong association was found with children who were ingesting more than two sugar containing snacks per day, had irregular dental visits and had no fluoride exposure, with a larger number of dental caries. Thereby, children who were

having more than two or more sugar snacks per day, had not visited a dentist regularly or lived-in households with a non-fluoridated water supply, had increased odds of dental caries. Therefore, it may have been difficult to identify any specific effect of vitamin D in the context of these other pervasive factors. Focusing on these specific findings, what the reasons may have been for them will be discussed next.

Children who received higher dose vitamin D supplementation had a greater accumulation of dental plaque on their teeth in phase one of this study ($p = 0.012$). However, there is no likely explanation for why vitamin D supplementation during pregnancy and infancy could result in a greater amount of dental plaque being present at age two years. Currently there is no literature which reports that vitamin D may promote plaque accumulation on teeth. On the contrary most published literature has shown that deficiency in vitamin D levels has been associated with a higher likelihood of periodontal disease (Botelho et al., 2020).

This therefore was an unexpected study finding, rather it was expected that the vitamin D supplementation may have reduced the production of dental plaque in these study children, as other studies have found (Khammissa et al., 2018). In Denmark for instance, it was found that in older adults who took a daily intake of >6.8 ug dietary vitamin D there was a significant correlation with lower plaque index scores ($p = 0.02$) (Adegboye et al., 2013). Another study in adults also found that vitamin D and calcium supplementation for 12 months, improved periodontal disease outcomes (Garcia et al., 2011). Therefore, it was unlikely in this study, that the higher dose vitamin D supplementation was responsible for a greater accumulation of plaque on the children's teeth at age two years. Other possible explanations for increased amounts of dental plaque on children's teeth (in the group who had received higher dose vitamin D supplementation), were it may have been related to their intake of sugar containing food or drinks, lack of oral hygiene practices, and/or lack of fluoride exposure. This study was not powered to account for these multiple confounders during the analyses.

It is acknowledged that the independent effect of vitamin D supplementation on dental caries may have been visible if the analysis was possible by controlling for the

confounding variables. However, detailed data were not collected on the additional risk factors for dental caries in this PhD, as it was outside the scope of this study.

Noting that despite the higher dose vitamin D supplementation children having a greater accumulation of plaque, they did not have an increase in dental caries, with the analysis of data from phase two suggesting that a smaller proportion of the higher dose vitamin D group had dental caries (non-significant trend with $0.10>P>0.05$). This finding also draws parallels to other studies, where vitamin D supplementation has been shown to reduce the odds of ECC in children's teeth (Botelho et al., 2020). Definitive reasons for this are not yet established, however, it has been suggested that this may be due to producing stronger teeth, which are more resistant to the cariogenic effects of bacterial plaque (Schroth, 2010; Gyll et al., 2018). It is possible that the higher dose vitamin D group may have coincidentally had a greater accumulation of dental plaque.

Study children who had irregular visits to the dentist were at increased odds of having fewer than 20 primary teeth in phase one (age two years), compared with children who had regular dental visits (statistically significant, $p = 0.0080$). A plausible explanation for children not having all 20 teeth present at age two years, could have been that their tooth eruption times varied, as some children have delayed or earlier eruption patterns. According to a study in NZ of 3,466 children aged five to 13, Pacific children have been reported for having earlier tooth eruption patterns compared with NZ European children (Kanagaratnam & Schluter, 2012) . Majority of the participants in this study were of Pacific ethnicity. Another reason for this finding could be that the study children may have received dental treatment outside of this study, and it is possible if teeth were extracted, the children would present with fewer teeth at the time of examination. Studies have shown that children visiting dental professionals earlier in life, have a higher retention rate of teeth, due to an earlier detection of caries and reducing their risk of ECC by application of preventative products such as fluoride varnish (Ramos-Gomez et al., 2007; Beil et al., 2014). This is why, in NZ it is recommended that infants have a dental exam as early as six months old, as teeth are at risk of developing caries from the time they appear in the mouth (MOH, 2008). This is an important message for parents and caregivers to reduce the odds of future caries in young children and have their children dentally examined early once teeth erupt.

Children who had more than one sugar containing snack per day were possibly at increased odds of having teeth with dental caries ($0.10 > p > 0.05$). The finding would be in keeping with previous evidence from several studies, affirming that sugar is a high-risk factor for dental caries especially in young children (Touger-Decker & Van Loveren, 2003; Punitha et al., 2015). This was not an unexpected finding; however, it did demonstrate this study population group were exposed to these pervasive risk factors, which influenced their caries rate significantly.

Children who lived in households which did not have fluoridated water were found to be at increased odds of dental caries (statistically significant in phase three, $p = 0.024$), higher dmft rates (statistically significant, $p = 0.009$) and possibly also more dental restorations ($p = 0.071$), in this study, compared with children who lived in households with a fluoridated water supply.

Comparable studies have reported that children living in areas with non-fluoridated water or using non-fluoride toothpaste tend to experience worse OH outcomes (higher dmft scores), compared to those who have had fluoride exposure (Dirks, 1974; Newbrun, 1989; Centers for Disease Control and Prevention, 2001; Lee & Dennison, 2004; Shanthi et al., 2014; Iheozor-Ejiofor, 2015). A recent meta-analysis by Moynihan et al. (2019) affirms that fluoride exposure in early life, via fluoridated water or toothpastes is a proven and effective way to prevent ECC in young children. The findings of this OH study were therefore similar, in keeping with previous studies that none or little fluoride exposure increases the risk of dental caries.

6.4.2.2 Were the mother's oral health statuses equal in all three study groups?

The mothers OH statuses were measured to ensure they were all comparable at baseline, by the Oral Health Impact Questionnaire (OHIP-14), as it was not possible to dentally examine the mothers in this study. It was found that there were no differences in the mothers OH at baseline, therefore they were not impacting upon the three child groups for dental outcomes. Mothers who had received, higher dose, lower dose or placebo supplementation all had self-reported oral health statuses which did not differ at baseline, upon commencement of this oral health study. This allowed all the study children to have equal distribution of the study participants in terms of the mother's oral health. It was important to factor this in, as it is known that poor

maternal OH is associated with increased odds of poor child OH (Shearer et al., 2011). Since there were no differences found between the mothers OH statuses for the 3 groups, we did not need to control for confounding variables, as they were not impacting upon the three child groups for dental outcomes.

6.5 The strengths and weaknesses of this study

6.5.1 Study strengths

The strengths of this study will be outlined first, beginning with this being the first prospective, double-blind study that assessed the effects of pregnancy and infancy vitamin D supplementation on early childhood dental health in children. An additional strength of this study were that it enrolled a population group at higher risk of VDD and ECC. This study was conducted on an ethnically diverse and lower socio-economic population who would most benefit from caries prevention.

Due to this being a double-blind study, the study design minimised and eliminated examiner and participant bias, therefore non-biased dental examination results could be collected at each study phase. Dental professionals collecting the data were also calibrated to a gold standard, ensuring examination procedures were carried out uniformly. Therefore, the same quality study data were collected at three different time points of this longitudinal study, when the children were two, three and four years old. This allowed a repeated measures study design, where the variables could be assessed overtime.

Self-reported study questionnaires were able to gather data on potential risk factors for ECC. These questionnaires were answered by the mother or caregiver who was predominately responsible for the child in this study. Administration of these questionnaires allowed investigation of the relationship of OH risk factors with the ECC, in addition to investigating the effect of vitamin D supplementation.

A strength of this study was that the vitamin D (intervention) supplementation was given to participants prior to tooth eruption, and whilst teeth were forming in utero. This meant that the supplemental vitamin D could influence the formation of teeth during early tooth development period. Such a study design has not been reported previously. To our knowledge, this is the only study of vitamin D supplementation

where two different dosing regimes were used and where vitamin D supplementation occurred both during pregnancy and infancy.

Another strength of this study is that it was conducted in NZ where there is no mandatory vitamin supplementation policy. Thus, this study sample had a high prevalence of VDD at the time of initial enrolment into the PIVID study. The current dietary recommendations are not sufficient to ensure adequate vitamin D status for most people in NZ. Non-mandatory fortification of foods with vitamin D and use of sunlight exposure alone are not proving sufficient to achieve adequate population vitamin D status, particularly in high-risk groups such as were enrolled into this study.

6.5.2 Study weaknesses

It is important to acknowledge that this study was a follow on from the PIVID study and thereby a pre-existing cohort of study children were recruited and examined. Therefore, the sample size for my study was limited to the number of children originally enrolled in the PIVID study, for whom consent was granted for ongoing contact, and who could then be contacted.

Attrition of participant numbers in this study was expected, like any longitudinal research study (Dumville et al., 2006). Albeit, regardless of attrition being within the expected range of over >50% at both follow up phases (80% in phase two and 70% in phase three), this OHS study was not a fully powered RCT hence did not have sufficient power to answer the research questions that are addressed in this thesis. This is noted as a limitation, however this study was undertaken as a pilot in order to collect information on the population of vulnerable children, where there currently exists no published data. A post hoc power analysis has been conducted for this research study, and it is estimated that for there to be statistically significant (p<=0.050) with power = 80%, assuming the placebo groups had a minimum of 50 participants, the study would require a minimum of 85 participants in the higher dose vitamin D supplementation group and the lower dose vitamin D supplementation group. This would be to achieve a reasonable medium Cohen's d of 0.50 difference (equivalent to Log (OR) = 0.91) between either dosage against the placebo group (total sample size = 220). To achieve power of 85% a sample of 127 participants would be required in both the higher and lower dose vitamin D supplementation groups (total sample size N = 304). Lastly, to

achieve power of 90% a sample of 264 participants would be required in both the higher dose and lower dose vitamin D supplementation groups (total sample size N = 578).

In the prior PIVID study by Grant et al. (2016), it was reported that at age 18 months, 12 months after completion of the intervention, the mean serum 25(OH)D (61 nmol/l) concentrations did not differ between study groups ($p = 0.30$). The children's vitamin D concentrations were not measured at the beginning of this PhD study, as the aim was to observe the effects of prenatal and early vitamin D on the children's primary teeth. Therefore, before being enrolled into this study, all of the children had the same mean 25(OH)D serum concentrations at baseline. This study population is also representative of people who have a darker skin colour, and it is known that VDD is more prevalent during pregnancy and infancy in these higher risk ethnic groups (Grant et al., 2009).

The severity of dental caries was not measured in this study, and developmental dental defects were also not categorised in this study, as with oral examinations of young children, there was a limited window of time to complete the examinations. Therefore, dental caries were not graded by scores or how progressed the disease was, the dmft index was applied as a simple way to capture this information, similar to other studies reported by the WHO (Petersen et al., 2013). Where ECC was noted present if there were cavitated, non-cavitated and WSLs present on teeth at the time of examination, as per the international caries detection and assessment system (ICDAS) (Young et al., 2015). Similarly, opacities/WSLs and EHP were all categorised as a developmental dental defect present or not. These study limitations prevented early lesions to be separated or be compared with the number of existing carious lesions or open cavities on the children's teeth, as opposed to a non-exposed layer of dental caries. Measuring both dental outcomes could be considered in future studies, but longer dental examinations would be required and older children may be more cooperative. For instance, where the dental examiner has the examination time to adequately blow air, use a magnification light, and spend time with dental instruments where developmental dental defects or ECC is suspected.

The fluoride exposure for children in this study was a variable that could not be controlled for; however, Auckland city and South Auckland is an area where water

fluoridation exists, therefore at baseline all children had equal exposure. An additional study weakness was, when recording fluoridation statuses of the participants, many of the mothers/caregivers were unaware of fluoride exposure for their child, for instance if the child was using a fluoridated toothpaste or not. Therefore, the study survey for this potential risk factor may not have been accurately answered. This would impact on the true effect of fluoride exposure on ECC in this study sample over the three study phases.

As for the other confounding variables for caries risk, there were other factors to consider and they could not be controlled for in this study. This was due to the study population group being transient as well as having other health and life priorities. In this study, the mother or parents reported in the study questionnaires on the child's exposure to fluoride, sugar intake and diet, toothbrushing or home oral care habits and visits to the dentist. The answers provided may not have always been accurate, as the mother/parent were not always the sole provide for the child and may not have known some of this information, for instance if grandparents or other family members were taking care of the child whilst the mother worked.

It would have been beneficial to collect more data on the additional risk factors (variables) for dental caries to control for them, versus vitamin D supplementation in the study analysis. This is a limitation of this study, and a wider and more detailed collection of information on the following the additional risk factors would be required, for: sugars intake (the amount), fluoride exposure, visits to the dentist and oral hygiene habits, in a future fully scaled trial.

In terms of controlling for sugar intake, one of the shortcomings of this study is that a validated questionnaire on diet and sugar intake was not used. For instance, a Food Frequency Questionnaire, for collecting comprehensive information on the amount and type of sugar containing snacks that were ingested during the study, over its three phases. The existing study questionnaires also did not identify the intake of sugar sweetened beverages separately and the frequency or amounts that were consumed. Therefore, the parents in this PhD study, answered self-reporting questionnaires on their child's intake of sugar snacks, without specifically disclosing information on any additional intake of sugar sweetened drinks. This was an omission in the data

collection of their overall dietary habits, as young children are at high risk of caries from the intake of sweetened drinks. For this reason, the total dietary sugars intake and feeding practices of children could not be compared with their total energy intake against their approximate basal metabolic rate (BMR), similar to other studies. The WHO's guidelines on total snacks or sugars per day, recommend that one should not exceed five to 10 teaspoons of sugar per day or over 50 grams per day (WHO, 2015). As detailed information on sugar intake was not collected, this confounding variable was not able to be assessed. Although, there are more detailed questionaires available, for this particular demographic, a simple questionnaire was developed to best suit the sample population (refer to Appendix F), which supports the extensive consultation and validation process this questionnaire went through.

In addition to the confounding variables that exist for dental caries in this study, the participants demographic characteristics also placed them at higher risk for ECC. This may have made it more difficult to observe the true effect of vitamin D supplementation on the dental outcomes that were assessed in this study. It is however noted that the dominant risk factors were no fluoride exposure, irregular dental visits, and a higher intake of sugar snacks, as reported in the results (section 5.6). It is unlikely that addressing only one of the several risk factors will result in a more modest improvement in dental status, as dental caries has a multifactorial aetiology.

The findings from this current study add to the current evidence available on vitamin D and dental caries prevention, but it does not give definitive answer for its effects. This relates back to the underpowering of this study as well as being unable to control for the confounding variables.

6.6 Other study factors to consider

A positive observation of this study was that despite the expected attrition of participants numbers in this study, many of the participants remained over two or three study consecutive phases. Reasons that this being a difficult population group to remain in contact with were: this was a transient population group, moving residences often, frequently with other more pressing priorities in life, lower socio-economic backgrounds, and dental unawareness, as well as barriers to accessing dental care.

Some of the underlying factors for non-attendance included mother's unawareness of free dental care in NZ for children under age 18 years, barriers to transport in accessing health care and taking time off or other duties to attend appointments. To facilitate a higher attendance of participants, weekend appointments were offered, and taxi vouchers were provided where necessary. Both weekend appointments and taxi vouchers proved to be successful for strategies for engaging with this cohort. Information on free dental care and enrolment information with the community dental services was also given to participants during this study.

6.7 Unanswered research questions

The prevention of childhood dental caries is the primary goal for dental health providers. In NZ, this is currently undertaken by modifying existing risk factors post tooth eruption. This OH study investigated the effects of pre- and post-natal vitamin D supplementation on early childhood dental health.

One of the prime unanswered question of this research remains:

- Does pregnancy and early infancy vitamin D supplementation protect the primary dentition from developmental dental defects in early childhood?

6.7.1 Existing studies and evidence on vitamin D and oral health

Despite an increasing number of studies on vitamin D supplementation and its role in dental health, there is an insufficient amount of strong evidence from randomised controlled trials to show whether vitamin D supplementation does protect the primary dentition from dental caries and dental defects.

The reasons for not having strong associations between vitamin D and oral health for existing studies are largely dependent on the study designs that have been carried out to date. Most studies for instance may have focused on select population groups by recruiting people of select numbers from particular origins, ethnicities, or age groups. These further limit participants being multicultural or study results being large enough or applicable to the general public. The duration of studies is also a factor in being able to identify whether vitamin D has a formative impact on dental defects or dental caries, as well as if it regulates their incidence or prevalence.

There are other additional factors that may have contributed to not being able to provide a definitive verdict on the effects of vitamin D and oral health. These include available funding, timelines, researcher interests, clinician expertise, or participant consent to examine teeth, as well as existing protective factors for dental caries, such as water fluoridation. There is therefore limited cumulative evidence on the relationship between the two.

6.7.2 Complexities in exploring the links between vitamin D and oral health

The effects of pregnancy and early infancy vitamin D supplementation on childhood dental health is a complex and multidimensional topic. This is due to the external and intrinsic factors which influence the dental disease of caries, as well as the presence of developmental dental defects. Some of these were discussed in section 6.5.2 (under study weaknesses), but to answer these questions in future these complexities must be determined and controlled for to be able to produce strong evidence-based research on vitamin D and dental health.

Future investigations on whether dental caries can be prevented by vitamin D supplementation, should design studies with detailed assessment of the other determinants for caries in alongside vitamin D. This may be in the form of measuring the amount of sugar intake, by quantifying the sugar intake with validated questionnaires, fluoride exposure externally (in the form of toothpaste or varnishes) as well as internal fluoride exposure (such as water fluoridation), and the percentage of dental plaque on teeth, which may be calculated by a O' Leary plaque index. If future studies were to be conducted on previously examined population groups, it may also be worthwhile retrieving the participants dental history, and gaining information on participants existing dental restorations, tooth extractions and dental visits. Often data on these additional factors can be difficult to quantify and assess. Therefore, by collecting comprehensive information on the influencing co-factors for caries, it help ascertain if vitamin D supplementation has a causal relationship with reducing dental caries.

There is clear need to explore this topic in future, to help uncover any new unestablished correlations for vitamin D supplementation and child oral health and add to the existing body of research.

6.8 Recommendations for future research

The study findings show some support for its hypothesis, and future studies on the effects of higher dose vitamin D supplementation on child dental health should be considered. Future exploration on this topic should, however, consider adjusting for confounding variables by collecting information on the total intake of dietary sugars (amount of sugar consumed), and measuring baseline vitamin D status, as well as the severity of dental caries.

This study first time study of its kind in NZ is a foundation for future studies to be developed. Larger randomised controlled clinical trials could be designed and implemented, to have adequate power, to establish if early life vitamin D supplementation can protect the primary dentition from dental disease. Further key questions for future research are listed below.

6.8.1 Questions for future research

Other areas of interest and investigation that this study was not able capture, could be looked at and explored by in future studies. Some of these key areas would be:

- Does vitamin D supplementation reduce dental caries?
- Does vitamin D supplementation in utero and early infancy, influence the permanent dentition?
- How did the other influencing factors on ECC impact upon the effect of vitamin D supplementation on early childhood dental health?

6.8.2 Investigating the effects of vitamin D on the permanent dentition in future studies

There is currently no research which has assessed the relationship between vitamin D and dental caries on the permanent dentition in NZ or internationally. This would be an important question to study, as the effects of vitamin D supplementation in utero during the tooth development period would have also influenced the formation of permanent tooth buds. The effects of vitamin D supplementation in pregnancy and infancy may thereby be more visible in the permanent dentition. It would therefore be worthwhile to design a similar OH study, with enrolment of an adequate sample to

investigate oral health outcomes and follow study children into older childhood and possibly adolescence.

6.8.2.1 Other influencing factors on ECC and how they impact upon the effect of vitamin D supplementation on early childhood dental health

Other influencing factors that were shown to be associated with ECC in phase three of this study were:

- A higher frequency of sugar snacks was associated with positive dmft scores that were marginally raised ($p = 0.057$), and less fluoride exposure was associated with a higher number of decayed teeth ($p = 0.024$) and higher dmft scores ($p = 0.008$).

The above confounding variables were considered in this study as they are known to influence the development and progression of ECC. The effects of the potential risk factors for ECC and how they may have impacted upon the effect of early life vitamin D supplementation on primary dentition were not adjusted for in this study. In future studies, it would be beneficial to assess whether the confounding variables were more prominent than the effect of vitamin D supplementation on the primary dentition, to establish if they may be overriding the effects of vitamin D supplementation on ECC.

If this study sample were to be re-examined to assess the permanent dentition, they may also exhibit similar traits in future towards the potential risk factors for ECC. Therefore, by collecting information via validated questionnaires on the potential confounding variables and adjusting for them in a future study, the effects of the other risk factors on ECC compared with the effect of vitamin D may be assessed.

It would be beneficial to do a future extended OH study examining the effects of vitamin D on dental caries and developmental dental defects in a larger sample population beyond the duration and scope of this study. It would also be useful to evaluate if other potential risk factors for ECC improve overtime, such as access to dental professionals, the frequency of sugar intake, fluoride exposure and oral hygiene practices.

Due to the study participants not having measured baseline vitamin D status, it remained unknown in this study whether the children had a VDD. In future studies it

would be highly recommended that researchers incorporate taking serum vitamin D concentration measures of participants to then be able to investigate the relationship of current vitamin status with dental caries in the study cohort. As noted previously, it would be important for such a study to collect detailed and high-quality information on intake of free sugars. Information should be collected on the frequency and type of intake of sugar food and drink intake, as well as the amount of sugar ingested per day.

The WHO guidelines advise to reduce individuals sugar intake to <10% of the total energy intake, or <5% of total energy intake from the diet, this includes sugars from food and drinks (WHO, 2015). This threshold can be applied when reviewing the total intake of sugar consumption per day.

Various methods exist for studies to examine sugar impact on caries, these include:

- A food frequency questionnaire (FFQ) in children, validated with a 24-hour dietary recall is a frequently applied method in other developed countries and allows for measuring a child's diet related activity by parental report (Parrish et al., 2003). Such an FFQ would capture information on how many times a week does the child have an intake of fruits, vegetables, sweets, and sugar containing drinks (Currie et al., 2014)
- Potential tools that could be used are:
    - The children's eating habits questionnaire (CEHQ-FFQ), is one that parents predominantly fill in at home, reporting on the child's intake of forty-three food groups, including food and drinks, with frequencies per week however no portion sizes are included (Bel-Serrat et al., 2014).
    - The energy children's questionnaire, which collects information in relation to children's energy related behaviours and assesses information across the following domains: demographic information, soft drinks and expenditure on them, fruit juice intake, breakfast, physical activity and screen viewing and dietary intake (Singh et al., 2011).
    - The Toy box children's questionnaire, this entails information on preschool children's intake of 37 food and drink items, with their frequencies and portion sizes reported per day, by their parents and caregivers (Mouratidou et al., 2019).

These are all validated and reliable dietary questionnaires, which parents/teachers and observers can answer and complete for their children, with the benefits of including sugar containing food and drinks. However, it would be necessary to also validate any chosen tool within the population within which it is going to be used.

In future studies, any of these dietary approaches should be considered so that these additional risk factors can be measured and considered in the analysis. It is important to note differences in these tools include the number and range of sugar sweetened beverages and portion sizes that are measured. However, by using a validated sugar survey or questionnaire on each child's daily intake in future, would enable controlling for this confounding risk factor (variable) that impacts upon dental caries. This may then enable the effect of vitamin D supplementation on ECC and developmental dental defects to be more easily identifiable.

6.9 The wider implications of this study

One of the most important wider implications from this study is how can dental caries be prevented in high-risk population groups within NZ, particularly in young children. Current approaches of oral health promotion, fluoridation, and giving advice on reducing the intake of sugars, simply does not seem to be eradicating or reducing the prevalence dental caries in young NZ children. As does not, training health care providers, on prevention methods for ECC, such as early assessment, fluoride varnish applications or annual dental examinations. Parents/caregivers and children do not seem to have easy access to the provision of free dental health checks (available in NZ for children under the age of 18) at the community dental clinics, for regularly assessing their child's teeth for dental decay. It was reported in 2020, that 2,500 children in the Auckland, NZ region had not visited the free school dental service since 2016, in a 12 month period (Quinn, 2020). In the years 2018 and 2019, the prevalence of NZ children aged one to 14 years who had not visited a dental service, was 82% and 67% in children aged one to five (MOH, 2021). This may be attributable the following reasons, of: families coming from low socioeconomic backgrounds, parents or caregivers having limited dental knowledge, and also child or parental dental phobias to the dental clinic, due to their experience of previous dental visits.

In addition to these, small children are irresponsible for brushing their own teeth (for dental plaque removal), and/or controlling for external risk factors such as the amount of sugar they intake, as their dietary behaviours are largely depending on parental influence (WHO, 2019a). For this reason, the current oral health disparities continue to exist, particularly in low SES groups and those from Māori or Pacific ethnic groups. Public health approaches in this instance, may be more effective in reducing inequalities by simple interventions such as introducing a vitamin D supplementation programme or a vitamin D food fortification policy in pregnancy and infancy, both of which may require little individual behavioural action. For example, making vitamin D supplementation free of charge for those who have high caries risk or are predisposed to VDD.

Another discussion may be around foods being fortified by vitamin D and having earlier intervention strategies for children and pregnant women to have a higher intake of these, to prevent VDD and possibly caries risk. In terms of dietary behaviours, there has been recent emphasis on introducing a tax on sugars in NZ to reduce public consumption of it, and therefore reduce adverse health outcomes including obesity and dental decay. However, these are yet to be implemented. Water fluoridation is also not available to all children, for instance those living in non-fluoridated areas or on tank water. It has been well established in many studies as well as this PhD study, that fluoride exposure and dental visits were associated with lower caries rates, however, for a successful preventative measure across all children vitamin D supplementation during tooth development periods, may be an additional strategy for the prevention of caries.

Dental health inequalities have been documented as being present between indigenous Māori children and NZ European children for decades and remain evident. If vitamin D supplementation can prevent ECC it has the potential to be more effect in Māori, in whom VDD is more prevalent compared with NZ European (Grant et al., 2009). Thus, I has the potential to reduce the dental inequities amongst young NZ children.

This OH study, conducted in this unique study sample, contributes to the understanding the effects of vitamin D supplementation on the primary dentition in NZ

children, as well as providing additional information on established risk factors for dental caries. Vitamin D supplementation could not be shown to prevent dental defects in this study; though, the results of this study do provide some preliminary evidence that higher dose vitamin D supplementation could be a preventative agent in protecting against ECC.

This topic warrants further investigation to determine if pregnancy and infancy vitamin D supplementation reduces the occurrence of ECC and developmental dental defects. Future studies in this area, would be particularly advantageous for high caries risk population groups and those who have a higher likelihood of VDD.

Prenatal and early life vitamin D supplementation is therefore a potentially viable and economical approach to achieve adequate vitamin D status during pregnancy and early infancy (Grant et al., 2014), in the aim of preventing dental diseases in childhood.

Chapter 7 Conclusion

This will be the first study to date to have assessed the effects of two different doses of early life vitamin D supplementation on childhood dental health in NZ. This unique double blind RCT study was undertaken to identify whether higher and lower dose vitamin D supplementation in pregnancy and infancy, could protect the primary dentition from dental caries and defects. Study results indicate that higher dose vitamin D supplementation may have the potential to minimise dental caries in childhood, as children who took high dose vitamin D in tooth development years showed reduced odds for dental caries at age three, compared to those who did not take it.

No apparent effects of vitamin D supplementation higher or lower dose were identified on developmental dental defects in this study. Developmental dental defects were not generally prevalent in this study sample of children either.

Potential risk factors that influence the development of ECC were briefly considered in this study, and their relationship with the dental outcomes were considered. The known independent risk factors for dental caries that showed significant association with ECC in this study were: the frequency of visits to a dentist, the intake of sugary snacks and fluoride exposure.. However, despite the effects of external and host risk factors of ECC, this study did show that vitamin D supplementation during pregnancy and infancy has the potential to have a beneficial effect on childhood dental caries. This was shown by lower rates of ECC in three-year-old children, who had received early life vitamin D supplementation. The impact of early life vitamin D supplementation on ECC, should therefore, be further explored in the future.

Current efforts to prevent ECC are not entirely successful as seen by the existing high rates of childhood dental caries that remain in in NZ and worldwide. The Findings of this study suggest that vitamin D supplementation may be able to improve childhood dental disparities in NZ children, by reducing ECC.

In future a larger, well designed RCT is warranted to determine if pregnancy and infancy vitamin D supplementation may be able to impede upon the development of

developmental dental defects and ECC to improve overall oral health outcomes in young children.

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Appendices

Appendix A: HDEC Ethical Approval

Health and Disability Ethics Committees
Ministry of Health
Freyberg Building
20 Aitken Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

27 February 2015

Associate Professor Cameron Grant
Paediatrics: Child & Youth Health
University of Auckland
Private Bag 92019 Wellesley Street, Auckland
Auckland 1142

Dear Associate Professor Grant

Re:	**Ethics ref:**	**NTX/09/11/101/AM07**
	Study title:	Randomised placebo controlled study of vitamin D during pregnancy and infancy

I am pleased to advise that this amendment has been *approved* by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

The main issues considered by the HDEC in giving approval were as follows.

- Questionnaires to be administered

Non-standard conditions:

1. Please provide us with a copy of the questionnaire that will be used.

Please submit your non-standard conditions by email HDECS@moh.govt.nz

Please note HDEC review is not required for non-standard conditions; however they must be completed prior to commencing your study. Do not submit non-standard conditions as a post approval form (PAF).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted and approved

Document	Version	Date
PIS/CF for persons interested in welfare of non-consenting participant: participant information sheet	version 8	08 October 2014
PIS/CF for persons interested in welfare of non-consenting participant: consent form	version 8	08 October 2014
Post Approval Form	07	10 February 2015

Appendix B
Statement of compliance and list of members

Statement of compliance

The Northern A Health and Disability Ethics Committee:

- is constituted in accordance with its Terms of Reference
- operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
- is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
- is registered (number 00008714) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Dr Brian Fergus	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Dr Karen Bartholomew	Non-lay (intervention studies)	01/07/2013	01/07/2016
Ms Susan Buckland	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Ms Shamim Chagani	Non-lay (health/disability service provision)	01/07/2012	01/07/2015
Dr Christine Crooks	Non-lay (intervention studies)	01/07/2013	01/07/2015
Mr Kerry Hiini	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Mr Mark Smith	Non-lay (intervention studies)	01/09/2014	01/09/2015
Ms Michele Stanton	Lay (the law)	01/07/2012	01/07/2015

http://www.ethics.health.govt.nz

Health and Disability Ethics Committees
Ministry of Health
Freyberg Building
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0800 4 ETHICS
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31 May 2016

Associate Professor Cameron Grant
Paediatrics: Child & Youth Health
University of Auckland
Private Bag 92019 Wellesley Street, Auckland
Auckland 1142

Dear Associate Professor Grant

Re:	**Ethics ref:**	**NTX/09/11/101/AM08**
	Study title:	Randomised placebo controlled study of vitamin D during pregnancy and infancy

I am pleased to advise that this amendment has been *approved* by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

The main issues considered by the HDEC in giving approval were as follows.

— The Committee queried how contact will be made, by phone or letter.

Non-standard conditions:

1. If contact is being made by letter, please provide a copy to HDEC for our records. This can be done by email and please include the HDEC reference number noted above.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by HDEC before commencing your study.

If you would like an acknowledgement of completion of your non-standard conditions letter you may submit a post approval form amendment. Please clearly identify in the amendment that the changes relate to non-standard conditions and ensure that supporting documents (if requested) are tracked/highlighted with changes.

For information on non-standard conditions please see section 128 and 129 of the Standard Operating Procedures at http://ethics.health.govt.nz/home.

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted and approved

Document	Version	Date
Post Approval Form	08	-

Appendix B
Statement of compliance and list of members

<u>Statement of compliance</u>

The Northern A Health and Disability Ethics Committee:

— is constituted in accordance with its Terms of Reference
— operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
— is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
— is registered (number 00008714) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

<u>List of members</u>

Name	*Category*	*Appointed*	*Term Expires*
Dr Brian Fergus	Lay (consumer/community perspectives)	11/11/2015	11/11/2018
Ms Rosemary Abbott	Lay (the law)	15/03/2016	15/03/2019
Dr Karen Bartholomew	Non-lay (intervention studies)	13/05/2016	13/05/2019
Dr Charis Brown	Non-lay (intervention studies)	11/11/2015	11/11/2018
Ms Susan Buckland	Lay (consumer/community perspectives)	11/11/2015	11/11/2016
Ms Shamim Chagani	Non-lay (health/disability service provision)	11/11/2015	11/11/2016
Dr Christine Crooks	Non-lay (intervention studies)	11/11/2015	11/11/2018
Dr Kate Parker	Non-lay (observational studies)	11/11/2015	11/11/2018

Unless members resign, vacate or are removed from their office, every member of HDEC shall continue in office until their successor comes into office (HDEC Terms of Reference)

http://www.ethics.health.govt.nz

Health and Disability Ethics Committees
Ministry of Health
C/- MEDSAFE, Level 6, Deloitte House
10 Brandon Street
PO Box 5013
Wellington
6011

0800 4 ETHICS
hdecs@moh.govt.nz

08 August 2013

Associate Professor Cameron Grant
Paediatrics: Child & Youth Health
University of Auckland
Private Bag 92019 Wellesley Street, Auckland
Auckland 1142

Dear Associate Professor Grant

Re:	**Ethics ref:**	**NTX/09/11/101/AM03**
	Study title:	Randomised placebo controlled study of vitamin D during pregnancy and infancy

I am pleased to advise that this amendment has been *approved* by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

Non-standard conditions:

1. Please delete the compensation clause in the CF as it is not relevant for this stage.

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

BJ Fergus

Dr Brian Fergus
Chairperson
Northern A Health and Disability Ethics Committee

Encl: appendix A: documents submitted
appendix B: statement of compliance and list of members

Appendix A
Documents submitted

Document	Version	Date
PIS/CF: NTX 09_11_01 oral health amendment subject information sheet	Version 8	24 July 2013
PIS/CF: NTX 09_11_01 oral health amendment consent form	8	24 July 2013
Post Approval Form		24 July 2013

Appendix B
Statement of compliance and list of members

Statement of compliance

The Northern A Health and Disability Ethics Committee:

— is constituted in accordance with its Terms of Reference
— operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
— is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
— is registered (number 00008714) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

Name	*Category*	*Appointed*	*Term Expires*
Dr Brian Fergus	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Ms Susan Buckland	Lay (consumer/community perspectives)	01/07/2012	01/07/2015
Ms Shamim Chagani	Non-lay (health/disability service provision)	01/07/2012	01/07/2014
Dr Christine Crooks	Non-lay (intervention studies)	01/07/2013	01/07/2015
Mr Kerry Hiini	Lay (consumer/community perspectives)	01/07/2012	01/07/2014
Dr Etuate Saafi	Non-lay (intervention studies)	01/07/2012	01/07/2014
Ms Michele Stanton	Lay (the law)	01/07/2012	01/07/2014

Appendix B: Site Locality Approval by AUTEC

2 October 2013

Anita Nolan
Faculty of Health and Environmental Sciences

Dear Anita

Re: **13/287 Randomised placebo controlled study of vitamin D during pregnancy and infancy,**

Thank you for submitting your application for ethical review. I am pleased to confirm that the Chair of the Auckland University of Technology Ethics Committee (AUTEC) and I have approved your ethics application for three years until 2 October 2016.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/researchethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 2 October 2016;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/researchethics. This report is to be submitted either when the approval expires on 2 October 2016 or on completion of the project;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply within their.

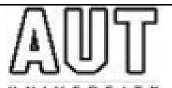
To enable us to provide you with efficient service, we ask that you use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

Kate O'Connor
Executive Secretary
Auckland University of Technology Ethics Committee

Auckland University of Technology Ethics Committee
WA507 Level 5 WA Building City Campus
Private Bag 92006 Auckland 1142 Ph: +64-9-921-9999 ext 8316 email ethics@aut.ac.nz

Appendix C: Participant study information sheet

THE UNIVERSITY OF AUCKLAND FACULTY OF MEDICAL AND HEALTH SCIENCES	**Participant Information Sheet: Does vitamin D status during pregnancy and infancy affect dental health?** Dr. Cameron Grant, Associate Professor Department of Paediatrics: Child and Youth Health University of Auckland, Private Bag 92019, Wellesley Street Auckland Phone 923 6192 Dr. Anita Nolan, Professor of Oral Medicine, Oral Health, AUT University, North Shore Campus, Private Bag 92006, Auckland 1142, Telephone: 09 921 9999 ext 7759	

An investigation into the effects of vitamin D supplementation during pregnancy and infancy on dental health in childhood.

We invite you to continue to take part in this study which follows on from the Pregnancy and Infancy Vitamin D (PIVID) study. You do not have to take part if you do not want to. If you do not want to take part, it will not affect your future treatment by your doctor.

The study is being carried out by medical and dental researchers at the University of Auckland; and AUT University, Auckland along with medical researchers from the University of Otago and Harvard University.

Aim of the Study

Normal vitamin D levels are necessary for good health. Research suggests that vitamin D may improve the quality of children's teeth and result in less dental disease. The aims of this research are:

1. To look at the effects of vitamin D on the numbers of defective, decayed, missing and filled teeth in children who have been part of the Pregnancy and Infancy Vitamin D (PIVID) study.
2. To find out about the use of Oral Health services and habits of mothers and children enrolled in this study, as such factors could also impact on dental health of their children.

You are invited to take part in the study because you and your child have been enrolled in the vitamin D during pregnancy and infancy study (PIVID study).

What's involved

You shall be asked to complete a questionnaire on your own oral health, dental hygiene habits and the use of oral health services

You will be asked about your child's dental hygiene, visits to the dental surgery and about the types of food and drinks your child consumes.

Your child will then have a dental examination undertaken, but no treatment will be done.

When the clinical examination is completed, you will be given a brief verbal report of your child's dental health and you will be given oral health advice. If your child requires dental treatment, you will be given full advice on how to obtain this.

The questionnaires and the child's dental examination will establish your child's risk of dental disease. You will be told about your child's dental health at the time the examination takes place and you will be guided to dental services if your child is not already under dental care. At the end of the study we will inform you of the study results.

We would like your permission to:

- Inform your family doctor / dental therapist that you and your child are participating in the study.
- Access your child's records of consultations with your family doctor, accident and medical centres, and any dental or hospital records.

All identifiable information collected in all parts of the study is **confidential** and will not be available to anyone other than the small team of researchers from the University of Auckland, AUT University, University of Otago and Harvard University.

Study benefits

At the completion of the study you will be informed about your child's dental health. Additionally, it will familiarize your child with dental surgery and help them feel relaxed about future dental visits. The findings from the study may benefit others in the future by showing if dental disease can be reduced by giving vitamin D to women during their pregnancy and to children during infancy.

Risks of the study

There are no risks and there is no discomfort as no treatment will be undertaken.

Compensation

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.

The study is not part of your routine care and is for research purposes only. You are free to withdraw from the study at any time without having to explain why. If you choose not to participate, it will not affect any treatment that you currently receive.

We will contact you in a few days time to see if you wish to take part in the study. If so, we will make a time to interview you, when we will get your written consent for participating in the study.

A small koha is offered to acknowledge the time spent in being part of this study.

If you would like to know more about the study, or wish to ask any questions, please contact:

- Dr Cameron Grant (Auckland): phone 09-923 6192 ext 86192
- Dr. Anita Nolan (Auckland) 09 921 9999 ext 7759

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact an independent Health and Disability Advocate:

Telephone: 0800 555 050
Free Fax: 800 2787 7678 (0800 2 SUPPORT) Email: advocacy@hdc.org.nz

The study has received ethical approval from the Northern X Regional Ethics Committee, Auckland.

Appendix D: Study participation consent form

Study ID No ☐☐☐☐

Title of project:

An investigation into the effects of vitamin D supplementation during pregnancy and infancy on dental health in childhood.

Full name of parent/caregiver:

....
(first) (middle) (last)

Address: ....

....

Name of child: ....
(first) (middle) (last)

Consent Form

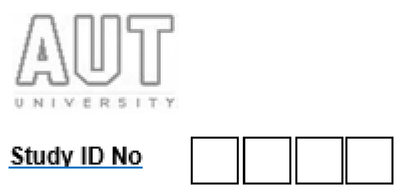
Interpreter box			
DEAF	I wish to have a NZ sign language interpreter	Yes	No
ENGLISH	I wish to have an interpreter	Yes	No
MAORI	E hiahia ana ahau ki tangata hei korero ki ahau	Ae	Kao
SAMOAN	Oute mana'o e iai se fa'amatala upu	Ioe	Leai
TONGAN	'Oku ke fiema'u ha fakatonulea	Io	Ikai
COOK ISLAND	Ka inangaro au I tetai tangata uri reo	Ae	Kare
NIUEAN	Fia manako au ke fakaaoga e tagata fakahokohoke	E	Nakai

Principal Investigators:

Dr Cameron Grant, Department of Paediatrics, University of Auckland (Grafton Campus), Phone 3737 599, ext 86192

Anita Nolan, Professor of Oral Medicine, Oral Health, AUT University, North Shore Campus, Private Bag 92006, Auckland 1142. Phone: 921 9999 ext 7759

- I have read and understand the information sheet. I have had an opportunity to ask questions and to have them answered.
- I understand that I may withdraw my child from the project at any time and that, if I do, the medical care for me and my child will not be affected in any way. I know who to contact if I have any questions about the study in general. I understand the compensation provisions for this study.
- I understand that my child's participation in this study is confidential and that no material that could identify my child will be used in any reports on this study.
- I have had time to consider if my child should take part in the study. I have had the opportunity to use whānau support or a friend to help me ask questions and understand the study



Study ID No ☐☐☐☐

I consent for my child to take part in the study.	YES / NO
I consent to my child's medical records being reviewed and used in the event that required them	YES / NO
I wish to receive a copy of the study results	YES / NO
I agree to my child's GP or other current health care provider being informed of my child's participation in this study/the results of my child's participation in this study	YES / NO

I ………………………………………………………………………………………… (*full name*)

hereby consent to my child taking part in the continuation of the Vitamin D study

Date:	
Signature:	
Full names of researchers:	
Contact phone number for researchers:	
Project explained by:	
Project role:	
Signature:	
Date:	

Appendix E: Mother's self-reporting questionnaire on child's dental health habits

Study ID		**Date**	

Your Child's details
Please circle the answer that best fits

Male/Female		Date of birth		Current age (years and months)	

1. How many times a day do you brush your child's teeth?					
More than once a day	Once a day	Not every day	Less than once/week	Don't know	
2. Does someone help your child to brush their teeth?					
Yes	No	Sometimes	Refused	Don't know	
3. Do you use a fluoride containing toothpaste for your child?					
Yes	No	Sometimes	Refused	Don't know	
4. Does your child use regular (adult) toothpaste or one specially for kids					
Adult	Child's	Refused	Don't know		
5. Has your child ever visited a dentist?					
Yes	No	Refused	Don't know		
6. If yes to question 5 how frequently have they been?					
Once	Twice	Three	More than three times		
7. Does the child use a pacifier?					
Yes	No	Refused	Don't know		
8. If Yes to question 7, is it cleaned by boiling, rinsing in tap water, or by the parents sucking on it?					
Boiling	Rinsing in water	Parent sucking	Other		
9. Who else shares the child's toothbrush?					
Mother	Father	Sibling	Grandparent	Other	Nobody

Appendix F: Mother's self-reporting questionnaire on child's feeding habits

I.D. Number

Feeding Practices For each of the following questions, please circle the answer which best applies to you.

Question				
1. Have you ever added a sweetener/sugar or Milo to your Child's milk?	YES	NO	REFUSED	DON'T KNOW
2. Does your child usually have a drink (other than water) or snack before going to bed?	YES	NO	REFUSED	DON'T KNOW
3. Are your child's teeth brushed after the snack/drink before they go to bed?	YES	NO	REFUSED	DON'T KNOW
4. Does your child sleep with a bottle?	YES	NO	REFUSED	DON'T KNOW
5. Have you ever added a sweetener/sugar to their drinks?	YES	NO	REFUSED	DON'T KNOW
6. Does your child eat sugary snacks	YES	NO	REFUSED	DON'T KNOW
7. How many times a day does your child eat sugary snacks?	ONE	TWO	THREE	MORE THAN THREE

Appendix G: Mother's self-reporting questionnaire on their own dental habits

1

Mother Trial ID: **Date**

Please circle the answer that is correct for you

1. **When do you usually brush your teeth?**

More than once a day	Once a day	Not every day	Less than once/week	Never

2. **How would you describe the health of your teeth or mouth?**

Excellent	Very good	Good	Fair	Poor

3. **When did you last see a dentist?**

In the last year?	1-2 years ago	2 or more years ago	Never been

4. **What is your usual reason for seeing a dentist?**

Check-up	Problem	Never been

5. **What was the reason for your last dental visit?**

Check-up	Problem	Never been

6. **All things considered, would you say that, over the past year, the health of your mouth has**

Improved	Stayed the same	Got worse

7. **In general, compared to <u>other</u> persons your age, would you say your <u>dental health</u> is:**

Excellent	Better than average	Below average	Among the worst

Appendix H: Mothers self-reporting OHIP 14 questionnaire

For each of the following questions, please circle the answer which best applies to you during the last 4 weeks.

Because of trouble with your teeth, mouth or dentures:

1.	Have you had trouble pronouncing any words?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
2.	Have you felt that your sense of taste has worsened?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
3.	Have you had painful aching in your mouth?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
4.	Have you found it uncomfortable to eat any foods?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
5.	Have you been self-conscious?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
6.	Have you felt tense?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
7.	Has your diet been unsatisfactory?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
8.	Have you had to interrupt meals?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
9.	Have you found it difficult to relax?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
10.	Have you been a bit embarrassed?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
11.	Have you been a bit irritable with other people?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)

Please turn over

12.	Have you had difficulty doing your usual jobs?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
13.	Have you felt that life in general was less satisfying?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)
14	Have you been totally unable to function?	NEVER (0)	HARDLY EVER (1)	OCCAS-IONALLY (2)	FAIRLY OFTEN (3)	VERY OFTEN (4)

Appendix I: Study data collection on child's dental health information

Examination Data

Date:

Patient ID Number:

Checked that there is no contraindication to probing for e.g. heart defect Y N

1. Number of primary teeth	
2. Number of decayed primary teeth	
3. Number of filled primary teeth due to decay	
4. Number of primary teeth missing due to decay	
5. Number of teeth with enamel defects (for example enamel hypoplasia)	
6. Severity of dental decay in primary teeth: (dmft score)	
7. Plaque accumulation on teeth?	
8. If yes, on how many teeth is plaque accumulation	
9. Plaque index score	
10. Gingival index score	