Influence of Maternal Prenatal Vitamin D Status on Infant Oral Health

Robert J Schroth, DMD, MSc

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirement for the degree:

DOCTOR OF PHILOSOPHY (PhD)

Department of Community Health Sciences
Faculty of Medicine
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
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Abstract

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Objectives: Inadequate maternal vitamin D (25(OH)D) levels during pregnancy may affect primary tooth calcification predisposing enamel hypoplasia, a risk factor for Early Childhood Caries (ECC). The purpose of the study was to determine the 25(OH)D status of expectant mothers, the incidence of enamel hypoplasia and ECC among their infants and the relationship between prenatal 25(OH)D concentrations and both enamel hypoplasia and ECC.

Methods: This prospective study recruited participants during pregnancy. A prenatal questionnaire was completed and serum sample drawn for a 25(OH)D assay. Infant dental exams were completed at follow-up appointments; enamel hypoplasia and ECC were recorded while the parent/caregiver completed a questionnaire. The examiner was blinded to each mother's prenatal vitamin D status. EH and ECC were defined by established indices. A p value of ≤ 0.05 denoted significance.

Results: 207 women were enrolled during their second trimester with a mean age of 19.0 ± 4.7 years. A total of 89.8% declared themselves to be of Aboriginal heritage. The mean serum 25(OH)D was 48.1 ± 24.4 nmol/L (median 43.0 nmol/L). 35.0% had levels ≤ 35 nmol/L, a formerly-defined threshold of deficiency. Only 10% of women had concentrations ≥ 80 nmol/L, denoting adequacy. Multiple regression analyses revealed that vitamin D concentrations were significantly associated with the frequency of milk consumption (p=.000), use of vitamins (p=.0062), education level (p=.017), ethnicity (p<.001), and season (p=.000). 135 infants (55.6% male) were examined at 16.1 ± 7.4 months of age. Enamel hypoplasia was identified in 21.6% of infants while 23.0% had

ECC and 36.3% had ECC when white spot lesions were included. Mothers of children with enamel hypoplasia had lower, but not significantly different mean serum 25(OH)D concentrations during pregnancy than those of children without enamel hypoplasia (43.2 ± 21.1 vs. 51.4 ± 27.4 nmol/L, p=.072). However, mothers of children with ECC had significantly lower 25(OH)D levels than those whose children were caries-free (41.4 ± 20.4 vs. 52.4 ± 27.4 nmol/L, p=.045). Univariate Poisson regression for the rate of untreated decay (dt score) revealed a significant inverse relationship with maternal vitamin D concentrations (p=.0002). Infants with enamel hypoplasia were significantly more likely to have ECC (p<.001). Logistic regression identified low maternal calcium levels (p=.034), not having heard of vitamin D (p=.036), and not using margarine daily (p=.024) as being significantly associated with hypoplasia in the primary dentition of infants. Backwards logistic regression revealed that enamel hypoplasia (p<.001), infant age (p=.002), and lower 25(OH)D levels during pregnancy (p=.019) were significantly associated with ECC.

Conclusions: This study shows for the first time that maternal vitamin-D levels may have an influence on the primary dentition and the development of ECC.

Funded by: Manitoba Medical Service Foundation, Manitoba-Institute-of-Child-Health, Dentistry-Canada-Fund, and the University of Manitoba.

Acknowledgements

This longitudinal study was made possible by operating grant support from the Manitoba Medical Service Foundation, the Manitoba Institute of Child Health and the Children's Hospital Foundation of Manitoba Inc., Dentistry Canada Fund, and the Faculty of Dentistry, University of Manitoba. Research Fellowship support was provided by the Children's Hospital Foundation of Manitoba Inc., the Manitoba Institute of Child Health, and a CIHR Strategic Training Fellowship in the Canadian Child Health Clinician Scientist Program (CCHCSP).

I would like to recognize the staff of the Outpatient Department at Women's Hospital, Health Sciences Centre (HSC), Health Action Centre, and Mount Carmel Clinic for their assistance. Special thanks are extended to Donna Duncan, Cheryl Fainman, and Dr. Maggie Morris (Women's Hospital, HSC), Sheelagh Smith and Kathi Christenson (Health Action Centre), and Linda Urich and Lori Black (Mount Carmel Clinic).

Heartfelt thanks are extended to my PhD thesis advisory committee (Dr. Michael Moffatt, Dr. Christopher Lavelle, Dr. Robert Tate, and Dr. Sharon Bruce) for their support, guidance, and constructive feedback. I am so thankful that each one of you took the time from your busy schedules to serve as my committee. I would particularly like to express my appreciation to Dr. Michael Moffatt for peaking my interest in vitamin D, children's oral health, and Aboriginal health several years ago. Despite your busy schedule I want to thank you for giving me a chance to be your student, for encouraging me in my research endeavors, and for providing me numerous opportunities to partner and develop networks with the broader child health community and health decision-

makers. You have been extremely supportive throughout this entire process and have provided me with valuable feedback and mentorship.

I would like to thank Dr. Chris Lavelle for his constant enthusiasm, gentle prodding, and insight. Had it not been for your encouragement to pursue graduate studies, I would not have given research much thought. I hope this former dental student of yours has made you proud. Further thanks are extended to Dr. Tate for his help and guidance with the advanced statistical analyses and methodology and for his one-on-one tutorial sessions. I also need to thank Dr. Bruce for her encouragement and practical advice. Additional words of appreciation are extended to my external examiner Dr. Ronald Billings from the University of Rochester Medical Centre.

Lastly, this would not have been possible without the support and encouragement of my family.

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Chapter 1 – Introduction & Review of the Literature

Section 1 – Introduction

Prevalence and Consequences of Early Childhood Caries

Primary tooth decay during the preschool years is called Early Childhood Caries (ECC). While caries among the general population is on the decline, the same is not true among certain groups of Canadians, including young Aboriginal children. Evidence from the United States indicates that caries among preschool children is on the rise.[1] In fact, caries is the most common chronic disease of childhood.[2]

Early childhood is a crucial time in a child's development as it sets the foundation for overall long-term health, including oral health. Infant and preschool children are particularly vulnerable for developing caries in their primary dentition as they are entirely dependent on parents and caregivers for activities of daily living, including nutrition and feeding behaviours, oral hygiene, dental prevention, and access to oral health care.

While not obvious to most, ECC is in fact a major public health concern. If left untreated, dental caries can progress to infections and pain and ultimately affect a child's quality of life.[3] Further, children afflicted with ECC are significantly more likely to suffer additional dental caries in both primary and permanent dentitions throughout the continuum of childhood and may also suffer from future orthodontic malocclusions.[4-6]

Like many other chronic diseases and pediatric health conditions in Canada, ECC disproportionately affects North American Aboriginal children. For example, Indigenous children are known to have higher rates of obesity and type 2 diabetes, asthma and respiratory tract infections, otitis media, and face more nutritional challenges.[7-10] There are three distinct groups of Aboriginal people in Canada, First Nations people, both

Status and Non-Status Indians, the Inuit, and the Métis. Several Canadian studies have revealed that the prevalence of decay among Aboriginal children and other children living in the north is high, compared with the general population, ranging from 50% to as high as 98%.[11-18] Table 1.1-1 reports published prevalence and caries rates for Canadian preschool children over the past two decades.[11,12,15-17,19-26] These studies indicate that First Nations and other Aboriginal children suffer disproportionately from ECC than other groups. Reasons for such discrepancies are generally attributed to the lower SES of these groups and their limited access to dental care.[11,12,18] Chronic diseases, like dental caries, are strongly influenced by the social determinants of health. The reality is that only Status Indians and the Inuit are recipients of dental benefits through the Non-Insured Health Benefits (NIHB) program of First Nations and Inuit Health (FNIH), Health Canada. The federal government is charged with the responsibility of providing dental care to registered First Nations and Inuit people in Canada, but regular access to dental care in rural and remote northern communities is often limited. Even Aboriginal peoples residing in urban centres face challenges in accessing ideal dental care.

In the past, decay among preschool children has often been referred to as "baby-bottle tooth decay" and "nursing caries". These terms have now been replaced by the term ECC, in an attempt to raise awareness of the multiple factors contributing to this disease, rather than continuing to attribute causation solely to inappropriate feeding practices.[27-31] Preventive efforts to curb improper feeding methods alone have had limited or modest success in reducing ECC.[32-35] As associations between ECC and improper feeding practices are inconsistent it is no longer considered the principle etiology.[12,27,30,31,36-38] A listing of antecedent terms appears in Table 1.1-2.[39]

A current definition of ECC, adopted by the American Academy of Pediatric Dentistry (AAPD), is the presence of at least one primary tooth affected by caries in children under six years of age (< 72 months).[27,40] Others have classified ECC according to three specific presentations: isolated decay of primary incisors or molars, decay of primary incisors with or without molar decay, and decay exhibited throughout most of the primary dentition.[41] Whether these three patterns are discrete or represent sequential stages of a single disease process remains obscure.

Table 1.1-1 – Recent studies reporting the prevalence and severity of caries among Canadian preschool children (Modified from Schroth & Moffatt 2005)[20] (SEE COPYRIGHT)

Study	Region of Canada	Population	Age	Prevalence of ECC*	Mean deft ± S.D. (range)
Schroth & Cheba 2007[26]	Winnipeg, MB	Community Dental Clinic	< 72 months	71%	3.7 ± 3.9
Harrison et al 2006[19]	Hartley Bay (Gitga'at) First Nation, BC	First Nation	3.7 ± 1.2 years	31%	9.9 ± 12.1
Schroth et al 2005[12]	Northern First Nation, MB	First Nation	2.9 ± 1.8 years	58.6%	4.5 ± 4.9 (0-17)
	Thompson, MB	Urban	2.8 ± 1.7 years	51.4%	4.3 ± 5.2 (0-17)
	Winnipeg, MB	Urban	3.0 ± 1.7 years	43.3%	3.1 ± 4.4 (0-16)
	Roseau River First Nation, MB	First Nation	2.9 ± 1.8 years	56.5%	4.4 ± 5.2 (0-16)
Schroth & Moffatt 2005[20]	Carman, MB	Rural Caucasian	3 year olds	44.3%	2.0 ± 3.3
Schroth et al 2005[11]	Garden Hill First Nation, MB	First Nation	3-5 year olds	98%	13.7 ± 3.2.
Lawrence et al 2004[17]	Souix Lookout Zone, ON	First Nation			
		High Intervention Community	2 year olds	91.5%	10.2†
			3 year olds	85.2%	10.2†
			4 year olds	79.4%	10.0†
		Low Intervention Community	2 year olds	86.5%	8.0†
			3 year olds	90.5%	12.2†
			4 year olds	88.1%	10.9†
Peressini et al 2004[16]	District of Manitoulin, ON	First Nation	3 year olds	67%	3.5 ± 4.0
			5 year olds	78%	4.8 ± 4.1
Harrison & Wong 2003[21]	Vancouver, BC (1996 Follow-up)	Urban Vietnamese			
		Intervention Group	22.1 ± 5.0 months	6.2%	
		Control Group	22.7 ± 5.8 months	57.1%	

Study	Region of Canada	Population	Age	Prevalence of ECC*	Mean deft ± S.D. (range)
Harrison et al 1997[22]	Vancouver, BC	Urban Vietnamese	3-74 months < 18 months ≥ 18 months	 0% 79.5%	 0.0 8.4 ± 4.6
Weinstein et al 1996[23]	Edmonton, AB	Urban Caucasian and Diverse Ethnic	Mean age 19 months	4.6%	0.14
Young et al 1995[15]	Keewatin Region, NWT	Inuit	0-2 years 3-5 years	50% to 100% Depending on Community	≈1.8 ≈8.0
Harrison & Davis 1993[24]	BC (1988 Survey)	First Nation	5 year olds	87.5%	7.5 ± 4.9
Williams & Hargreaves 1990[25]	Edmonton, AB	Urban Asians	2 year olds 3-4 year olds 5 year olds	0% 28% 62%	0.0 1.2 ± 2.2 3.2 ± 3.7

MB=Manitoba
BC=British Columbia
ON=Ontario
AB=Alberta
NWT=Northwest Territories
*ECC is defined as ≥ 1 primary tooth affected by decay in children < 6 years of age (based upon deft ≥ 1).[27,40]
deft is the cumulative score of all decayed, extracted, and filled primary teeth.
†Excluding stainless steel crowns.

Table 1.1-2 – Previous used terms for ECC among infants and preschoolers (Modified from Schroth et al 2007)[39] (SEE COPYRIGHT)

Baby-bottle tooth decay[36,42-44]
Baby bottle syndrome[45]
Labial caries[46]
Circular caries[47]
Nursing bottle mouth[48]
Milk bottle caries[49]
Nursing caries[14,50-52]
Nursing bottle caries[33,45]
Nursing bottle syndrome[53-55]
Bottle propping caries[56]
Bottle baby syndrome and bottle mouth caries[57]
Rampant caries[58]
Melanodontie infantile/"les dents noire de tout-petits"[59,60]
Sucking cup caries[61]
Sugared-tea caries[62]
Sweet-tea caries[63]
Sugar nursing bottle syndrome[64]
Polycaries[65]

Contributing Factors towards Early Childhood Caries

The traditional etiological triad model for dental caries includes the susceptibility of the host (tooth enamel), diet (fermentable sugars), and cariogenic bacteria over time.[66,67] However, it is overly simplistic to believe that these are the only determinants for ECC. ECC, like other complex diseases, is multifactorial in origin, including these same factors of sugar consumption, enamel integrity, cariogenic microorganisms and time.[68] However, additional factors have also been associated with ECC, including socioeconomic status (SES), psychosocial issues, child rearing practices, and the social determinants of health.[38,69-73]

Significant attention has been devoted to investigating the relationship between infant feeding practices and ECC, yet some suggest that the duration or method of

feeding, either bottle or breast, has little influence on ECC.[74-77] Bottle-use for nutritive purposes must be distinguished from pacification, where inappropriate bottle use increases the risk for caries.[12,37,38,78] Other evidence concludes that "at-will breastfeeding" and enamel hypoplasia may predispose a child to ECC.[79] Further, a recent systematic review suggests that there is also insufficient scientific evidence that a strong association between breastfeeding and ECC exists.[80] Promising prevention efforts include the use of fluoride varnishes, chemotherapeutics, and motivational interviewing techniques to assist parents in adopting healthy early childhood oral health practices.[21,81-83]

ECC is an important and often overlooked pediatric health issue. Caries prevention is crucial for this age group as preschool children who exhibit primary tooth decay at young ages, are more likely to have an increased caries burden along the continuum of childhood[4-6,84,85] Therefore, efforts to reduce caries among the very young may have a broader impact. Treatment alone is not the appropriate solution for this devastating pediatric dental syndrome as the risk of recurrent caries following complex restorative care is considerable.[86-88] Rather, more attention needs to be spent on effective preventive strategies during key periods of early childhood development such as bolstering prenatal nutrition, reducing maternal levels of *streptococcus mutans*, reducing the vertical transmission of cariogenic microorganisms from mothers to infants, early screening of infants and toddlers, promoting regular infant oral hygiene, and applying fluoride varnish or other chemotherapeutic agents (i.e. silver fluoride, chlorhexidine, betadine).[12]

The Link Between Enamel Hypoplasia and Early Childhood Caries

The integrity of the primary tooth enamel (host resistance) is an important aspect in ECC development. Enamel hypoplasia results from defective amelogenesis, and is clinically identified by absences of, pitting, grooves, or other irregularities of enamel.[89-93] These structural defects may place an infant's primary teeth at greater risk for bacterial colonization, specifically, by *Mutans streptococci*, resulting in dental caries.[94,95] These and other cariogenic oral bacteria convert dietary carbohydrates into acids, which can lead to the demineralization of tooth enamel and subsequent decay. Therefore, the calcification process of primary teeth, when enamel formation is occurring is crucial to understanding the significance of enamel hypoplasia. The primary maxillary anterior teeth begin to calcify during the second trimester, specifically between weeks 13 and 17 in utero, and this process does not end until 3 months postnatal.[96,97] Therefore, it is important to investigate the possible etiologies in utero that can disrupt normal enamel formation, as enamel hypoplasia is consistent with the period of amelogenesis, documented to originate in utero and end soon after birth.

Causes of Enamel Hypoplasia and the Potential Link with Vitamin D

Primary tooth enamel defects have been correlated with several factors ranging from genetic disorders to difficulties arising during prenatal and early postnatal periods.[66] Such disorders include low birth weight, malnourishment, prematurity, and metabolic difficulties.[66] Deficiencies of vitamin D in utero are also believed to be associated with enamel hypoplasia, because of metabolic insult to ameloblasts[98-103], a theory first proposed by Lady May Mellanby in the 1920s.[104,105]

The general prevalence of hypoplastic defects in primary teeth ranges from 13-39% and can approximate 62% among premature infants.[66] Enamel hypoplasia has also been found to be more prevalent among children of low SES[66,96,106-109] and those with a history of premature birth[100,110,111].

Controversy with the prenatal nutritional hypothesis primarily relates to the lack of demonstrated association between nutritional deficiencies in utero and enamel hypoplasia and ECC[97], although children with neonatal tetany resulting from maternal deficiencies demonstrate enamel defects[98], a key risk factor for ECC. Another dilemma involves the possible confounding effects of other variables, such as low birth weight and prematurity when attempting to investigate the associations between enamel hypoplasia, malnutrition and ECC.[112,113] Regardless, what we know about the effect of factors during the prenatal period on ECC has generally come from retrospective investigations.

Evidence suggests that the prevalence of hypoplasia in the adult upper incisors may be reduced through vitamin supplementation both pre and post-natally, over several years.[106,114,115] Likewise, children whose mothers received 400 IU of vitamin D beginning in the 12th week of pregnancy, in a placebo-controlled trial, had a lower prevalence of hypoplastic defects in primary teeth.[116] Therefore, vitamin supplementation may help reduce the incidence of these defects. However, only 63 children of a total of 627 children in this cohort underwent the dental examination between two and three years of age.[116] The small sample size is a limitation along with the fact that identifying true enamel hypoplasia in the primary anterior teeth is difficult at this age, as some defects may have been masked by subsequent caries.

If enamel hypoplasia results from nutritional inadequacies and if it provides refuge for cariogenic bacteria[94,117,118] that can increase the risk of caries, then an association between enamel hypoplasia and caries is reasonable.[112,119-121] As mentioned, hypoplasia is associated with caries and dramatically increases the tooth's susceptibility to caries.[89,92,120,122-129] However, more information on the association of ECC and enamel hypoplasia is imperative.[93]

Although nutritional supplementation can lead to a decrease in the incidence of enamel hypoplasia, assessments of this have been primarily conducted in permanent dentitions of adolescents[112] with the exception of one prenatal supplementation study that included hypoplasia as an outcome.[116] To date, direct evidence that nutritional inadequacies place a child at increased risk for both enamel hypoplasia and dental decay[127] is ambiguous. New research must determine whether nutritional deficiencies of 25-hydroxyvitamin D (25(OH)D) in utero play a role in ECC. Therefore, attention must focus on the role of inadequate nutrition during dental development in enamel hypoplasia and ECC.[107] Current literature is skewed to those factors influencing decay on erupted teeth rather than those factors that act before eruption.

Vitamin D plays an important role in calcium and phosphorus homeostasis, controlling intestinal calcium and phosphorus absorption.[130] The main source of 25(OH)D is from endogenous, UV-dependent synthesis. Serum 25(OH)D concentrations remain relatively consistent during pregnancy for those not receiving supplements.[131] Reports indicate that the biological half-life of 25(OH)D may last up to 5 weeks.[132,133] For many residing in northern regions, exposure to adequate ultraviolet (UV) irradiation is often insufficient, necessitating reliance on exogenous sources.[134-136] Furthermore, food

sources containing vitamin D including fish, fortified milk and soy products, eggs and liver[135] may be too expensive for those of low SES. Thus the use of vitamin D supplements may be essential to ensuring 25(OH)D sufficiency.

Research must determine the role of poor prenatal care and nutrition as it can result in low birth weight and impact the development of the primary dentition.[137] For example, low SES influences food security, which in turn affects the nutritional status of expectant mothers during periods of critical fetal tooth formation.[138] Young children who have suffered deficiencies of essential nutrients, such as calcium and vitamin D, are believed to have a higher prevalence of decay than those with adequate nutrition during craniofacial development.[127,138-141] Episodes of newborn malnutrition, during tooth formation, increase the risk of caries in the primary dentition.[127]

Brief episodes of nutritional insufficiency of calcium can increase the likelihood of enamel hypoplasia, although the impact of prenatal vitamin D deficiencies on the incidence of enamel hypoplasia has yet to be evaluated prospectively.[142] Both vitamin D and vitamin A deficiencies are primary systemic factors associated with enamel hypoplasia.[93] It is an important public health concern as it is remarkably prevalent among poorly nourished children, in which afflicted teeth have increased vulnerability to caries attack.[119,143-145]

Much of the initial focus on the role of vitamin D in enamel hypoplasia and caries occurred during the 1910s, 1920s, and 1930s, which has generally been forgotten by the dental profession. During this period, research examining the relationship between the fat-soluble A accessory (now vitamin D) demonstrated that animal diets deficient in this metabolite produced hypoplastic defects in enamel, delayed loss of deciduous teeth and

malocclusions.[145] Additional human investigations demonstrated that the use of vitamin D resulted in significant reduction of caries in children who received irradiated ergosterol.[146,147] Historical evidence documents the beneficial effects of vitamin D supplementation in reducing dental caries among children.[146-149]

Further, the pioneering efforts of Mellanby gave credence to the belief that the critical period to influence the development of the primary dentition lay in utero.[105,150] Maternal prenatal dietary interventions were shown to improve dental mineralization and increase host resistance.[105,150]

Since the duration of primary tooth calcification is short and begins during the second trimester of pregnancy, prenatal nutrition has a tremendous influence on the formation of dental tissues.[139] Prenatal and postnatal nutrition may emerge as a focus for new primary prevention efforts against enamel hypoplasia and ECC[151] since deficiencies during these stages are believed to place the newborn at risk for an assortment of diseases, including caries.[152]

Adequate serum concentrations of 25(OH)D during pregnancy are essential for the calcification of body structures. Considering Manitoba's geographic location which limits endogenous synthesis, many residents are believed to have insufficient 25(OH)D concentrations.[153-155] Thus, dietary intakes are essential in maintaining 25(OH)D adequacy for northern populations and especially expectant mothers.[156] However, many Aboriginal expectant women are at risk for not attaining adequate nutrition.[142] Lactose intolerance among Aboriginals is also believed to be a significant influence on the consumption of vitamin D fortified dairy products.

It is well known that nutritional deficiencies exist among Canada's Aboriginals.[142] Recent research places the prevalence of vitamin D deficiency during pregnancy in three northern communities in Manitoba in excess of 80%.[157] Reasons for these nutritional insufficiencies are generally ascribed to a lack of purchasing ability, cost, availability and access, along with inadequate dietary education in the community.[142] For example, recent attempts have been made to draw attention to the cost of milk in northern Manitoba First Nations communities, which hovers around $12.00 Canadian for 4 litres.[158,159] Such prices obviously deter many families from purchasing, and consuming milk. However, this is not as large a problem among urban Aboriginals.[134]

Such nutritional stressors during prenatal development may explain the high prevalence of ECC in northern Manitoba,[67,137,157] since enamel hypoplasia is a risk factor in ECC.[41,66,106,160] Episodes of malnutrition or deficiencies during enamel formation can predispose teeth to enamel hypoplasia.[97,145,157] Expectant mothers of low SES, especially those from northern First Nations communities, are at considerable disadvantage since increasing nutritional needs often cannot be secured with scarce finances. Therefore, attempts to investigate the relationship between prenatal nutrition and ECC should be encouraged. Like ECC, enamel hypoplasia has also been found to be more prevalent among children from lower socio-economic populations.[66,96,106,107]

New evidence is now uncovering the role of vitamin D in human immunology, indicating that deficiency states may reduce host immunologic responses towards microbial infections.[161] Therefore, it is also possible that deficiencies of vitamin D may also reduce host resistance to cariogenic bacteria.

Daily intakes of vitamin D during pregnancy have been recommended to achieve 25(OH)D sufficiency and reduce the development of hypoplastic lesions of enamel.[98] Current discussions have also raised the issue of whether biological identifiers of ECC in the pre-clinical state can be evaluated in the infant population under 12 months of age.[27] Perhaps both supplementation and early dental screenings may serve as effective preventive strategies to reduce both enamel hypoplasia and ECC. Preventive efforts during early periods of childhood development are needed.

As assays of 25(OH)D are expensive, many investigations have considered nutritional intakes as an alternative means to profile an individual's vitamin D status. However, dietary recalls and assessments can be problematic and difficult to achieve.[162,163] The use of multivitamins is often not sufficient to sustain improved 25(OH)D concentrations in the desired range.[164-168] Daily intakes of multivitamin preparations may also pose another issue, compliance. Thus it is necessary to consider alternatives, including the use of greater amounts of fortified dairy products or the use of high dose vitamin D preparations, or modified Stosstherapy (high dose vitamin D supplementation) as a means of achieving satisfactory levels of 25(OH)D.[11,157]

One frequently discounted risk factor for ECC and dental caries in general are developmental defects of enamel (DDE), specifically enamel hypoplasia. This thesis explores the relationship between prenatal nutrition and both enamel hypoplasia and ECC. Of prime interest is whether maternal 25(OH)D concentrations during pregnancy is associated with both of these states. The next section is an informal review of risk factors for DDE, including enamel hypoplasia.

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Section 2 – Risk Factors for Enamel Hypoplasia in Young Children

The previous section identified enamel hypoplasia as a key risk factor in the development of dental caries, including Early Childhood Caries (ECC). The intent of this chapter is to review our understanding of factors contributing to these enamel defects in children.

Dental enamel is a unique hard tissue of ectoderm rather than connective tissue origin.[1] Ameloblasts, the cells that form enamel first secrete an enamel matrix, which is both inorganic and organic in composition.[1] Secretion and limited mineralization of the matrix continues until the enamel has reached its intended thickness. Following this process, the matrix undergoes a maturation process that results in the loss of protein and water from the matrix and the addition of minerals leading to mature enamel.[1]

The primary dentition begins to form at week 6 in utero and amelogenisis, the process of enamel formation generally commences during the 18th week.[1] The formation of hard tissues of the primary maxillary and mandibular incisors begins between 4 and 4.5 months in utero.[2] While the bulk of enamel deposition occurs during prenatal life the remainder is formed during infancy.[3] Disturbances to the developing enamel matrix (ameloblasts) are irreversible and serve as a permanent record of the insult to enamel formation in the primary and permanent dentitions. Thus, it is possible to estimate when the disturbance to developing enamel might have occurred (Figure 1.2-1).

Developmental defects of enamel (DDE) serve as records of such disturbances to the amelogenesis process. DDE have been implicated as risk factors for ECC.[4,5] Specifically, these sites are preferentially colonized by cariogenic microorganisms including, *Mutans streptococci*.[6-10]

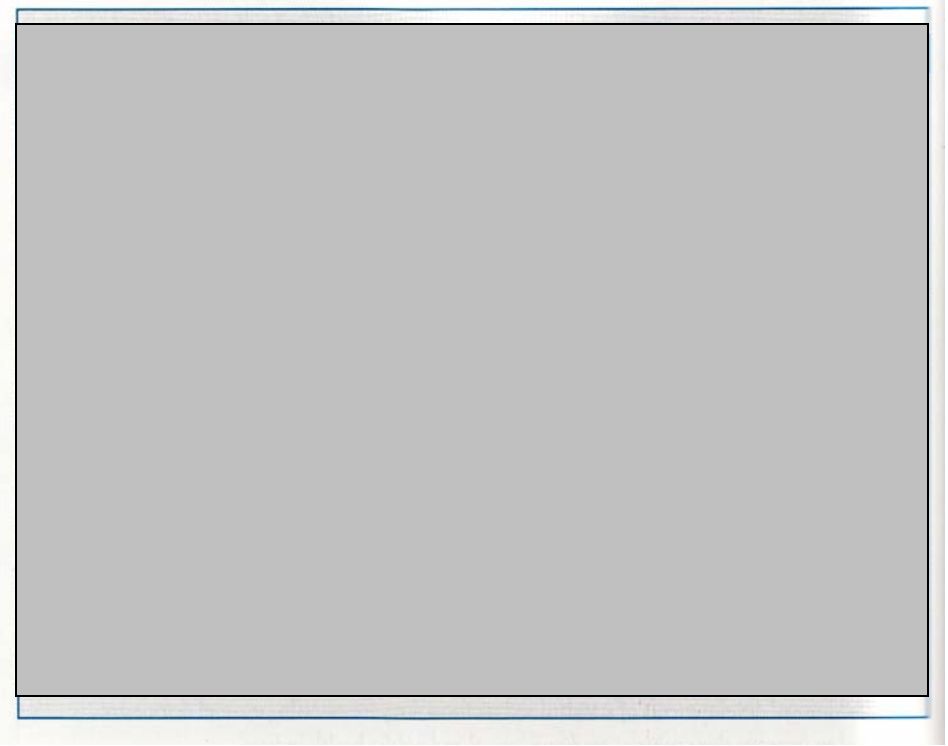

Figure 1.2-1 – Pathology of Developmental Defects of Enamel (DDE) from Cutress[11]

DDE are generally classified into three distinct categories, namely diffuse opacities, demarcated opacities, and hypoplastic defects.[12,13] Naturally, these diffuse opacities, demarcated opacities and enamel hypoplasia are key elements of indices used to record enamel defects for epidemiological investigations.[12] The average dental practitioner may not have a full understanding of the nuances of these classifications systems as reviewing the existing indices for DDE is not part of the undergraduate dental curriculum. While enamel hypoplasia is likely the most recognized defect of enamel in both the primary and permanent dentitions it should not overshadow the significance of opacities. All three development defects of enamel signify some sort of influence on the developing ameloblast or matrix at the time of enamel development. Thus, hypoplastic enamel and enamel opacities can shed light on their possible etiologies as they

correspond to the timing of tooth development. This may assist in narrowing down the potential contributing agents or conditions that may predispose such enamel defects.

Electron microscopy of exfoliated teeth may serve as a unique method to detect DDE at the microscopic level.[14] Current epidemiological survey techniques do not allow for further microscopic examination of the enamel surface of primary teeth. Therefore, minute areas of enamel hypoplasia may not be visible without magnification.

The introduction of several indices for DDE has helped propel this area forward to the stage where established criteria are used for research purposes. Having standardized indices improves the reliability of studies and assists in the comparison of prevalence and risk factors for DDE among published reports.

By definition, demarcated opacities are alterations in the normal translucency of enamel, but the thickness of enamel is not altered.[12,13,15] The defect has clear margins and may be white, cream, yellow, or brown in colour. Diffuse opacities are also changes in the translucency of enamel but these defects lack clear boundaries and can be patchy, confluent, or even linear.[12,13,15] As dental fluorosis primarily manisfests as diffuse opacities, it too is recognized to be an enamel defect. Separate indices for fluorosis exist, but their discussion is beyond the scope of this review.

While opacities are qualitative changes, enamel hypoplasia involves quantitative changes to enamel.[12,13,15,16] Hypoplastic lesions may typically appear as pits, grooves, or missing enamel of varying size.[12,17] Table 1.2-1 highlights the classifications of DDE according to the modified DDE Index.[12] In some instances, both enamel hypoplasia and opacities can exist simultaneously and current DDE indices account for this possibility.[12]

Table 1.2-1 – Modified DDE Index for use in epidemiological studies [12]

	Code
Normal	0
Demarcated opacities:	
White/cream	1
Yellow/brown	2
Diffuse opacities:	
Lines	3
Patchy	4
Confluent	5
Confluent/patchy + staining + loss of enamel	6
Hypoplasia:	
Pits	7
Missing Enamel	8
Any other defect	9
Combinations:	
Demarcated and Diffuse	A
Demarcated and hypoplasia	B
Diffuse and hypoplasia	C
All 3 defects	D

Several indices have been proposed for standardizing DDE, including enamel hypoplasia.[12,13,15,16,18] Currently, one index is preferred for recording and reporting enamel opacities and hypoplasia for epidemiological purposes although several others have also been proposed.[12] Clarkson and O'Mullane proposed two versions of their index, a simplified one for screening purposes and a more comprehensive tool for dental surveys.[16] Likewise, the World Health Organization also has an index for epidemiological surveys.[15] Unfortunately, it only records enamel defects for specific teeth in the mouth[15] and thereby underestimates the true prevalence of DDE. Finally, another proposed index for DDE does not account for qualitative changes in the translucency of enamel (i.e. opacities), and instead only focuses on enamel hypoplasia.[18]

The literature contains numerous studies reporting the prevalence and risk factors for enamel hypoplasia. Expert reviews on the subject of enamel hypoplasia have also

been published.[3,4,17,19] The intent of this chapter is not to undertake an exhaustive systematic review of the literature, but rather provide some insight into those risk factors which have been identified as contributing to enamel hypoplasia and opacities. It is also important to distinguish between enamel hypoplasia and opacities in the primary and permanent dentitions. Both dentitions begin forming during the early childhood period and genetic conditions and stressors in utero and during periods of early childhood development can negatively alter the quality and structure of enamel. Therefore, not only will information be presented specific to enamel and other DDE in deciduous teeth but data will also be presented for factors influencing the adult dentition.

Etiological risk factors for enamel hypoplasia can be ascribed to three main groupings.[17] Apart from altered enamel formation as a result of genetic conditions affecting only the teeth (e.g. amelogenesis imperfecta), inherited syndromes (e.g. ectodermal dyspalasia, ectodermal-mesodermal disorders) and inherited metabolic disorders (e.g. calcium metabolic disorders, parathyroid disease, vitamin D dependent rickets, hypophosphatasia), enamel hypoplasia may also arise from both systemic or localized factors.[17]

Systemic factors include infections experienced by the mother or infant, metabolic disease, nutritional disorders and deficiencies, premature birth and low birth weight, and ingestion or exposure to chemical compounds.[17,19] In contrast, localized factors include cleft lip and palate, trauma to teeth during amelogenesis, and primary tooth infections that may harm the developing successor.[17,19]

Table 1.2-2 provides some evidence for the association between some genetic, systemic and local factors that have been associated with DDE. As this paper primarily

focuses on quantitative alterations in enamel (i.e. hypoplasia), opacities are not differentiated.

Table 1.2-2 – Risk factors for DDE in primary and permanent teeth based on human studies, case reports, and reviews

Implicated Risk Factor	**Developmental Defects of Enamel (DDE)**	
	Primary Dentition	**Permanent Dentition**
Genetic conditions:		
Amelogenesis imperfecta and hereditary disorders	Opacity (hypocalcification)[20] Enamel hypoplasia[17,19,20]	Opacity (hypocalcification)[20] Enamel hypoplasia[17,19,20]
Incontinentia pigmenti (ectodermal dysplasia)		Opacity (hypocalcification)[21] Enamel hypoplasia[21]
Tuberous sclerosis		Enamel hypoplasia[19,22]
Inherited disorders of calcium metabolism:		
Hypocalcaemia and deranged calcium metabolism (hereditary vitamin D-dependent rickets, hypoparathyroidism/ psuedohypopara-thyroidism)	Enamel hypoplasia[23-25]	Enamel hypoplasia[25]
Vitamin D-dependent rickets type 1		Enamel hypoplasia[26]
Hypophosphatemic vitamin D-resistant rickets		Enamel hypoplasia[27]
Pseudo-hypoparathyroidism		Enamel hypoplasia[28]
Deficiencies, metabolic abnormalities/ health disorders:		
Vitamin D deficiency	Enamel hypoplasia[3,17,29,30]	
Neonatal rickets	Opacity[31] Enamel hypoplasia[31]	
Rickets	Enamel hypoplasia[32,33]	Enamel hypoplasia[32,33]
Neonatal tetany seconday to maternal vitamin D deficiency	Enamel hypoplasia[34]	
No vitamin D supplementation during pregnancy	Enamel hypoplasia[35]	

Implicated Risk Factor	Developmental Defects of Enamel (DDE)	
	Primary Dentition	**Permanent Dentition**
Deficiencies, metabolic abnormalities/ health disorders (continued):		
Hypervitaminosis D		Enamel hypoplasia[36]
No supplementation (including 20,000 IU vitamin D) during pregnancy, lactation or infancy		Enamel hypoplasia[37]
No vitamin D prophylaxis (viosterol) during early childhood	Enamel hypoplasia[32]	Enamel hypoplasia[32]
No nutritional supplementation during tooth formation among malnourished children		Enamel hypoplasia[38]
Hyperbilirubinemia	Opacity[39] Enamel hypoplasia[39]	
Hypovitaminosis A	Enamel hypoplasia[40]	
Galactosemia		Enamel hypoplasia[41]
Nephrotic syndrome		Opacity[42] Enamel hypoplasia[42]
Intestinal lymphangiectasia	Demarcated opacitiy[43] Enamel hypoplasia[43]	Enamel hypoplasia[44,45]
Coeliac disease	Opacity[46] Enamel hypoplasia[46]	Opacity[46] Enamel hypoplasia[46]
Congenital cardiac disease	Opacity[47] Enamel hypoplasia[47]	
Infections:		
Congenital cytomegalovirus	Opacity[48] Enamel hypoplasia[48]	
Infant infection during first 35 days	Enamel hypoplasia[40]	
Syphilis		Enamel hypoplasia[2]
Maternal rubella viral infection	Enamel hypoplasia[49,50]	
Postnatal measles	Enamel hypoplasia[30]	

Implicated Risk Factor	Developmental Defects of Enamel (DDE)	
	Primary Dentition	**Permanent Dentition**
Preterm Low Birth Weight (PLBW):		
Premature birth	Opacity[39,51] Enamel hypoplasia[10,14,30,39,51-56]	Opacity[51] Enamel hypoplasia[51]
Low birth weight (LBW)	Enamel hypoplasia[53,55,57]	
Low birth weight and premature	Enamel hypoplasia[58]	Opacity[58]
Low birth weight and premature with neonatal rickets	Opacity[31] Enamel hypoplasia[31]	
Mineral deficiency/cortical bone in very LBW (VLBW)	Enamel hypoplasia[53]	
Malnutrition:		
Malnutrition (protein malnutrition)	Enamel hypoplasia[59,60]	
Malnutrition (third degree)	Enamel hypoplasia[61]	
Childhood malnutrition	Opacity[7] Enamel hypoplasia[7,62]	Enamel hypoplasia[37,38]
Child's stature	Enamel hypoplasia[63]	
Maternal diabetes:		
Maternal diabetes	Enamel hypoplasia[64,65]	
Toxic exposure or ingestion of substances:		
Amoxicillin	Diffuse opacity consistent with fluorosis[66]	Diffuse opacity consistent with fluorosis[67]
Tetracycline	Opacity[2,68] Enamel hypoplasia[1]	Opacity[2] Enamel hypoplasia[1]
Fluoride	Diffuse opacity consistent with fluorosis[66]	Diffuse opacity consistent with fluorosis[67,69,70]
Thalidomide embryopathy	Opacity[71] Enamel hypoplasia[71]	
Cancer and anti-neoplastic therapy		Opacity[72]
IV alimentation	Enamel hypoplasia[55]	
Lead poisoning	Enamel hypoplasia[29]	Enamel hypoplasia[73]

Implicated Risk Factor	Developmental Defects of Enamel (DDE)	
	Primary Dentition	**Permanent Dentition**
Respiratory problems:		
Cystic fibrosis		Opacity[74]
Respiratory distress syndrome in infancy	Enamel hypoplasia[75]	
Neonatal hypoxia/asphyxia	Opacity[39] Enamel hypoplasia[39]	
Neurologic and sensory disorders:		
Cerebral palsy	Enamel hypoplasia[19,29]	
Retardation and hearing defects	Enamel hypoplasia[19]	Enamel hypoplasia[19]
Trauma to tooth bud and abscess of primary teeth:		
Primary tooth abscess		Enamel hypoplasia[17]
Intubation	Opacity[31] Enamel hypoplasia[53,76]	
Trauma – laryngoscopy and endotracheal intubation, neonatal ventilation	Opacity[76] Enamel hypoplasia[3,51,53,55,76]	
Cleft lip and palate:		
Cleft lip/cleft palate	Opacity[77] Enamel hypoplasia[77]	Opacity[77] Enamel hypoplasia[77]
Other:		
Rural vs. urban residence	Enamel hypoplasia[63]	
Delayed first prenatal visit (after 1st trimester)	Enamel hypoplasia[30]	
Prenatal tea consumption (≥ 3 cups/day)	Enamel hypoplasia[30]	
Prenatal acetaminophen use	Enamel hypoplasia[30]	
Increased pregnancy weight	Enamel hypoplasia[30]	
Young mothers	Opacity[78]	
Month of birth (amount of sunshine)	Opacity[79] Enamel hypoplasia[79]	

Implicated Risk Factor	Developmental Defects of Enamel (DDE)	
	Primary Dentition	**Permanent Dentition**
Other (continued):		
Factors associated with low socio-economic status (SES)	Enamel hypoplasia[80]	

The following section informally assesses the strength of the evidence of risk factors with DDE according to the Canadian Guide to Clinical Preventive Health Care from the Canadian Task Force on the Periodic Health Examination (Table 1.2-3).

Table 1.2-3 – Levels of Evidence according to the Canadian Guide to Clinical Preventive Health Care[81]

I	Evidence from at least one properly randomized controlled trial
II-1	Evidence from well-designed controlled trials without randomization
II-2	Evidence from well-designed cohort or case-control studies
II-3	Evidence from comparisons between times or places with or without intervention
III	Opinions of respected authorities, based on clinical experience, descriptive studies or reports of expert committees

Genetic Conditions

Genetic conditions are known to predispose both primary and permanent teeth to exhibit enamel defects.[17,19] This includes amelogenesis imperfecta[17,19,20], inherited conditions whose manifestations include dental defects (e.g. ectodermal dysplasia[21]), and tuberous sclerosis[22].

The quality of evidence (Table 1.2-3)[81] implicating genetic conditions with both enamel opacities and hypoplasia would at best be categorized as level III, that being the lowest quality of evidence. However, this should not minimize the relationship.

Systemic Factors

- **Inherited Disorders of Calcium Metabolism**

There are several inherited disorders that affect overall calcium metabolism, which undoubtedly affect enamel formation of both primary and permanent teeth. Enamel defects are known to arise due to enzyme disorders like vitamin D dependent rickets, or parathyroid diseases such as hypoparathyroidism and pseudohypoparathyroidism.[17,23-28]

Overall, the quality of evidence for the relationship between inherited disorders of calcium metabolism would be categorized as level III.

- **Preterm Low Birth Weight (PLBW)**

There is no doubt that premature birth and a low birth weight are associated with the presence of enamel defects in the primary detition.[10,14,30,31,39,51-55,57,58] The main defect appears to be hypoplasia of enamel although opacities may also result from these disturbances to the developing ameloblasts. Such systemic insults can also affect the permanent dentition.[51] A recent study also concluded that preterm children, in addition to having a higher prevalence of enamel defects, also had thinner enamel on the primary central incisors than children born at term.[14] Potential explanations for the effect of preterm birth on the development of dental enamel include altered metabolism, including calcium, and trauma from intubation and ventilation of premature infants.

Only one study could be classified as level II-1[51], while the majority were either categorized as level II-2 or level III.

- **Metabolic Abnormalities and Health Disorders**

Children with hyperbilirubinemia, galactosemia, nephrotic syndrome, intestinal lymphangiectasia, celiac disease, and congenital cardiac disease may also display a greater propensity for enamel hypoplasia and enamel opacities.[39,41-47]

Some of the studies reporting a relationship between DDE and metabolic abnormalities and health disorders were well-designed cohort or case-control studies (level II-2), while many others were categorized as level III. Overall, much of this literature would fall between these two groupings.

- **Nutritional Deficiencies**

Brief episodes of nutritional insufficiency of calcium can increase the likelihood of enamel hypoplasia. Deficiencies of vitamin D (i.e. hypovitaminosis D, vitamin D deficiency), a theory first proposed by the work of Lady May Mellanby, and vitamin A (i.e. hypovitaminosis A) are primary systemic factors associated with enamel hypoplasia.[17] Further, both vitamin D deficiencies and instances of vitamin D excess are associated with enamel defects including enamel hypoplasia.[36] While some have tried to discount the connection of vitamin D to enamel defects, which can predispose children to ECC, research shows that the ameloblast and odontoblast cells, responsible for enamel and dentin formation are target cells for 1,25-dihydroxyvitamin D.[82] Evidence indicates that such deficiencies of vitamin D in utero can lead to enamel hypoplasia, because of metabolic insult to ameloblasts.[24,25,31,34,83-85]

Most of the above noted literature involving the relationship between nutritional deficiencies and DDE would be considered to be of low quality, level III, although two

studies that refer to the role of vitamin D supplementation are of somewhat superior quality (level II-1)[35,51].

- **Infections**

Maternal and infant infections are known to affect enamel development and may leave permanent markers. Some of the reported infections that can result in defective enamel in primary teeth include cytomegalovirus[48], maternal rubella[49,50], and measles during infancy[30]. Even unspecified infant infections during the first 35 days of life are known to cause enamel hypoplasia.[40] Meanwhile, syphilis can lead to hypoplasia in the permanent dentition.[2]

Two of the above referenced studies can be grouped into the level II-2 evidence category[40,50] while the others belong in the level III category.

- **Malnutrition**

Episodes of malnutrition during infancy and early childhood do result in enamel disturbances. The literature indicates that childhood malnutrition, including severe forms and protein malnutrition predispose children to enamel hypoplasia.[7,37,38,59-61,63]

Only one article describing the relationship between DDE and malnutrition was of average quality[61] (level II-2). The remaining studies were of a lower quality.

- **Toxic Exposure or Ingestion of Substances**

While amoxicillin use during infancy has recently been found to be associated with diffuse opacities consistent with dental fluorosis, but not enamel hypoplasia in both primary and permanent dentitions, such associations have only been reported at the bivariate level.[66,67] Logistic regression from these reports reveal that enamel opacities in the permanent central incisors and molars are associated with amoxicillin use in the first

12 months of life. However, such associations reported in the primary dentition have not held up to this rigorous statistical testing.[66,67] Both amoxicillin and fluoride appear to have separate influences on the formation of the enamel organ.[66] Other reports of the influence of amoxicillin on enamel have not been well disseminated and may not hold up to scientific scrutiny.[86]

Excess fluoride ingestion from a variety of sources including water, toothpastes, and supplements can result in diffuse opacities in both the deciduous and adult dentitions. Severe mottling of enamel may even lead to quantitative changes in the enamel.

Other known chemical agents that have been known to affect the primary and permanent dentition include thalidomide[71], anti-cancer agents[72], IV administration of agents[55], and lead poisoning of mothers and children[29,87].

Overall, the majority of studies can be categorized as being of lower quality (level III), while the two studies dealing with the influence of antibiotic use and enamel defects are of a higher quality, level II-2[66,67].

- **Respiratory Problems**

Neonatal hypoxia and severe respiratory stress during infancy have been found to be associated with DDE in the primary dentition[39,75], while children with cystic fibrosis may display enamel opacities in their permanent teeth[74].

One of the above noted studies can be classified as level II-2[75] while the other two studies are of a lower quality level (level III)[39,74].

- **Neurologic and Sensory Disorders**

Children with cerebral palsy, cognitive impairments and hearing deficits are reported to have an increased prevalence of enamel hypoplasia in the primary dentition.[19,29]

This evidence is considered to be of lower quality, level III.

Local Factors

- **Trauma to Tooth Bud**

Intubation, laryngoscopy, endotracheal intubation, and neonatal ventilation are known to disturb enamel formation in primary teeth, which leads to both qualitative enamel defects but more notably, enamel hypoplasia.[3,31,51,76,88] The forces from these procedures on the edentulous ridge of infants are sufficient to disturb enamel formation.

With the exception of one study, the majority of studies reporting a relationship between trauma to the developing tooth bud and enamel defects can be classified as level III evidence.

- **Primary Tooth Abscess**

Dental abscesses from primary teeth may provide enough of an insult to negatively impact the completion of enamel formation of permanent successors, often referred to as Turner's hypoplasia.[2,17]

Overall, the evidence for this is of low quality (level III).

- **Cleft Lip and Palate**

Children born with cleft lip and cleft palate may show a higher prevalence of enamel defects, both opacities and hypoplastic enamel, in the primary and permanent dentitions.[77] Surgical correction of these facial deformities may result in trauma to ameloblasts of maturing teeth.[2]

This evidence is considered to be of low quality, level III.

Other Factors

Some uncommon risk factors for enamel hypoplasia were discovered during the course of this informal review. It is likely that these factors have some systemic influence on the developing dentition, but they are difficult to categorize. Most of these factors were associated with the presence of DDE on bivariate statistical analyses. Children from rural locales appear to demonstrate an increased prevalence in enamel hypoplasia.[63] This finding may be attributed to a lower SES than an urban environment and may also be related to nutritional differences between urban and rural children. Another report also implicated lower SES as a risk factor for enamel defects in the primary dentition.[80]

There is some evidence that the month of a child's birth may also be a predictor of DDE, both opacities and hypoplasia.[79] This may be related to the amount of sunshine mothers received during their pregnancy[79], which would obviously suggest some relationship with vitamin D and altered calcium regulation.

Offspring of young mothers[78] and those whose mothers first sought prenatal care after the first trimester[30] may be more likely to display enamel hypoplasia in baby teeth. One might suppose that young mothers may face certain challenges during pregnancy that may result in systemic influences on the dentitions of their children. Similarly, a delayed first obstetrical visit might suggest limited access to care resulting from socioeconomic inequities.

Two other risk factors were discovered in one report and were related to consumption behaviours. Prenatal use of acetaminophen was found to be significantly associated with enamel hypoplasia in offspring as was daily intake of three or more cups of tea during pregnancy.[30] This study also reported an association between increased

weight during pregnancy and an increased prevalence of enamel defects, which may be suggestive of gestational diabetes.

The majority of these other identified factors come from studies that would be classified as belonging to the lowest category of evidence, level III, although the study that reported a relationship between month of birth and DDE would fall into the category II-2[79].

Enamel hypoplasia results from defective amelogenesis and is clinically identified by absences of pitting, grooves, or other irregularities of enamel.[8,12,78] These structural defects may place an infant's primary teeth at greater risk for the colonization of bacteria, specifically, *Mutans streptococci* resulting in dental caries.[4] Therefore, the calcification process of primary teeth, when enamel formation is occurring is crucial to understanding the significance of enamel hypoplasia. The primary maxillary anterior teeth begin to calcify during the second trimester, and this process does not end until three months postnatal.[30,55,89] Therefore, it is important to investigate the possible etiologies in utero that can disrupt normal enamel formation, as enamel hypoplasia is consistent with the period of amelogenesis, documented to originate in utero and end soon after birth.[89]

Primary tooth enamel defects have been correlated with several factors ranging from genetic disorders to difficulties arising during prenatal and early postnatal periods.[4] Such prenatal and perinatal disorders include low birth weight, malnourishment, prematurity, and metabolic difficulties.[4] Enamel hypoplasia has also been found to be more prevalent among children of low SES.[4,7,30,61,90,91]

A better awareness of these factors that increase the likelihood of enamel hypoplasia may provide additional complimentary strategies for combating the

devastating problem of ECC. Improving the integrity of primary tooth enamel would ultimately improve host resistance to developing ECC. Vitamin D supplementation during pregnancy is known to be associated with a reduced incidence of enamel hypoplasia in the primary dentition[35], yet such supplementation for preterm infants has been found to have no effect on DDE in both primary and permanent teeth.[51] A recent Canadian study was unable to demonstrate a reduction in the prevalence of enamel hypoplasia as a result of high dose vitamin D supplementation during pregnancy or infancy.[92] However, there is ample evidence that episodes of malnutrition are associated with primary tooth decay as such events predispose enamel hypoplastic defects and also reduce salivary gland function.[60]

Obviously, stressors experienced in utero and during periods of early childhood development can negatively impact the primary and permanent dentitions of children. While not all DDE involve quantitative changes in enamel, those that involve structural changes increase the child's susceptibility of developing dental decay. The presence of DDE during infancy should be of concern as this likely contributes to the epidemic proportions of ECC experienced by residual populations. This thesis study will help to provide better quality of evidence of the relationship between prenatal risk factors, particularly vitamin D deficiency, and both DDE and ECC.

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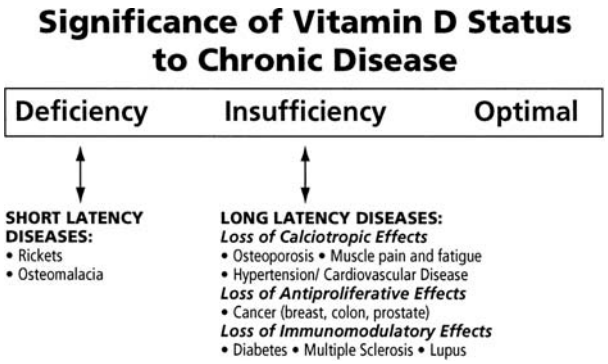
Section 3 – Influence of Vitamin D on Oral Health

Over the last decade, there has been a tremendous increase in the interest in vitamin D, particularly how it relates to overall health and well-being. In fact, vitamin D levels have now been linked to many chronic health conditions and diseases including osteoporosis, bone fractures, muscle weakness, cardiovascular disease, well-being and depression, cancer, and diabetes.[1] Additional health conditions associated with vitamin D status include asthma, multiple sclerosis, obesity, schizophrenia and reduced immunity (see Figure 1.3-1).[2]

Figure 1.3-1 – Significance of serum 25(OH)D to chronic disease (Whiting, S. J. and Calvo, M.S., J. Nutr. 2005;135:304-309)[2]



While so much attention has been given to the relationship between vitamin D status and these specific health conditions, there is also ample evidence supporting its association with oral health conditions. The intent of this chapter is to provide a brief overview of vitamin D and to present data from published studies on the relationship between vitamin D and oral health conditions including, but not limited to, periodontal disease, tooth loss, and oral bone loss, enamel hypoplasia, and caries.

Vitamin D may be defined as a vitamin and a hormone, in that it can be obtained exogenously as a nutrient or synthesized endogenously in the skin on exposure to ultraviolet (UV) radiation of the appropriate wavelength.[3,4] As much as 90% of the total vitamin D requirement for humans comes from endogenous synthesis.[5] The serum half life of 25(OH)D is reported to be between 12 to 19 days.[6] Although 1,25-dihydroxyvitamin D is the most active form of vitamin D[3,4], 25(OH)D is the main circulating form. Vitamin D is involved in mineral homeostasis ensuring that serum calcium and phosphorus levels are sufficient for the mineralization of bones and other calcified tissues, including teeth, in addition to neuromuscular functions.[4,5,7]

Several physiologic, environmental and cultural factors affect vitamin D availability and thus vitamin D status in the population.[8] Due to our northern latitude, endogenous synthesis of vitamin D in Manitoba, Canada is negligible from October to March. Further, vitamin D production might be diminished due to increasing calls to limit summer sun exposure in order to reduce the of risk of skin cancer. This has led to greater usage of sunscreen and protective clothing, and perhaps a reduction of outdoor summertime activities.

Dietary Sources of Vitamin D

Apart from endogenous production of 25(OH)D, the other ways humans acquire vitamin D comes from exogenous sources, either from diet or dietary supplements.[3,9] Apart from dietary supplements, foods known to contain vitamin D include fatty fish and fish oils[4], liver, eggs[10], vitamin D fortified milk and dairy products[11,12] (e.g. fortified milk products and margarines), in addition to some cereals[4,13,14]. Exogenous vitamin D can be derived

from two forms: 1) animals (D_3 or cholecalciferol from endogenous origin) frequently contained in fatty fish and fish oils, or 2) from plants (D_2 or ergocalciferol).[4,9,15] Vitamin D supplements were usually in the form of ergocalciferol (D_2), but now most supplements contain cholecalciferol (D_3) as it is more effective in bolstering serum concentrations.[6,16]

Evidence now indicates that our present intakes of vitamin D fall short of recommendations.[1,17,18] Insufficient data exist for an established recommended dietary allowance (RDA) for vitamin D. Therefore, an adequate intake (AI) has been adopted, as it represents the level of intake sufficient to maintain healthy blood levels of an active form of vitamin D. However, emerging research would suggest that current adequate intakes (AI) for vitamin D are set too low for many groups as they were originally set at or above 25 – 30 nmol/L (concentrations known to prevent rickets and osteomalacia).[2,17-20] For instance, the AI for children and adolescents is only 200 IU, even though children undergo considerable growth and development during these stages of life.[21] The Canadian Paediatric Society has recently recommended higher AIs for infants, expectant and lactating mothers, and children and have stated that research on the vitamin D requirements for toddlers and older children should be encouraged (recommendation grade A).[22]

Assessment of Vitamin D Status

25(OH)D is the primary form of vitamin D in circulation and is regarded to be the best indicator of vitamin D status as it is a good measure of total vitamin D received from both endogenous and exogenous sources.[3,4,6,9,14,15,23-25] Different investigative methods

have been used to profile vitamin D status including assaying serum concentrations of 25(OH)D, taking dietary histories and food frequency questionnaires (FFQ), and assessments of intake of vitamin supplements. Others recommend concomitant measurement of other serum metabolites to assist in profiling vitamin D adequacy, including parathyroid hormone (PTH). The inverse relationship between PTH and 25(OH)D[9,26] also indicates that PTH may be an appropriate indicator of vitamin D sufficiency.

Nonetheless, the most reliable method to determine vitamin D status is still the simple serum assay. Although there is variation in laboratory methodologies such as radioimmunoassay (RIA) or high-performance liquid chromatography (HPLC) for measuring and reporting 25(OH)D, such assays are accepted for investigative studies.[16,19] The lower limit of normal for serum 25(OH)D is controversial, with suggested values in the literature ranging from 15 to 40 nmol/L.[6,24,27-33] 25(OH)D concentrations <40 nomol/L have been equated with insufficiency[27] and deficiency[29,31,32], while levels <25 nmol/L are believed to be associated with rickets or osteomalacia[29,31]. Some researchers are now adopting 80 nmol/L as the new cutoff threshold to differentiate vitamin D deficiency from adequacy, but this value is not universally adopted.[1,6,17,18,22,27,34] Figures 1.3-2 and 1.3-3 highlight the recent advancements in our understanding of vitamin D adequacy. Previously, a threshold of 35 nmol/L was used as the cutoff for vitamin D deficiency. However, between 1997 and 2005 this threshold has continued to rise and now is set at 80 nmol/L (as seen in Figure 1.3-2).[35] Similarly, recommendations for vitamin D daily intakes also increased over this same period.[35] This is essentially due to the fact that this is the level of 25(OH)D where PTH is known to plateau.

Figure 1.3-2 – Schematic representation of three dramatic shifts that have taken place in vitamin D nutrition (from Whiting, S. J. et al. J. Nutr. 2006;136:1114-1116)[35]

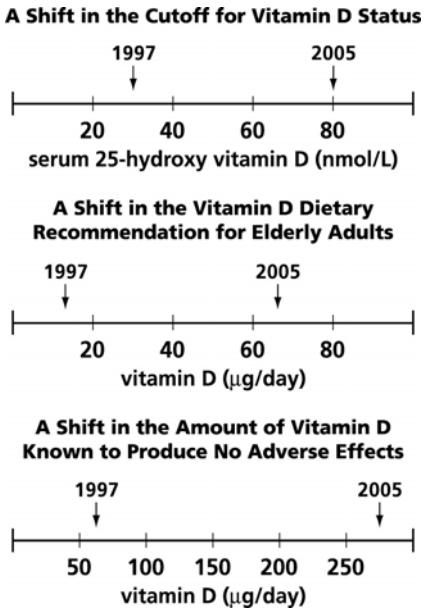
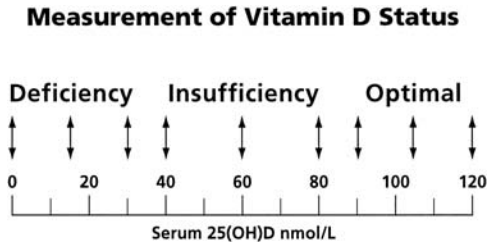


Figure 1.3-3 – Relationship between serum 25(OH)D levels and vitamin D status (Whiting, S. J. et al. J. Nutr. 2005;135:304-309)[2]



The remainder of this chapter examines the published literature on the relationship between vitamin D and oral health in humans. While it is not an exhaustive systematic review it presents information to substantiate the role of vitamin D in the maintenance of good oral health in children and adults. Literature was identified through a search of the Medline electronic database using key terms of 'vitamin D' and 'oral

health'. In addition, articles were also identified by examining reference lists of several publications.

In 1934, May Mellanby theorized that immunity to caries might also be due to the vitamin D attainment, from both endogenous and exogenous sources.[36] Her work led to the theory that vitamin D was not only essential for the normal development and calcification of enamel and dentin, but was also responsible for the accretion of secondary dentin after tooth eruption.[37] The vast majority of the literature on the influence of vitamin D on oral health has remained relatively ignored as much of it dates to the first half of the twentieth century.

This review examines the literature relating to several oral health conditions and vitamin D including periodontal disease, tooth loss, and alveolar bone health although these additional oral health conditions are unrelated to this thesis. The remainder of this chapter focuses on vitamin D and its influence in dental development, enamel hypoplasia, and caries.

Periodontal Diseases: Gingivitis and Periodontitis

Several publications have reported on the relationship between vitamin D and periodontal health. For instance, case report evidence suggests that individuals with vitamin D dependent rickets (VDDR) may suffer from generalized gingivitis and chronic periodontal disease.[38] In addition, Hildebolt's (2005) recent review on the effect of vitamin D and calcium on periodontitis highlights many important clinical studies in this field.[39] Overall, it is believed that vitamin D reduces the risk for periodontitis through the stimulation of LL-37, cathelicidin, by $1,25(OH)_2D$, which enhances immunity and has an antimicrobial effect.[40,41]

A 1971 publication presented results of a small randomized, double-blinded, placebo-controlled trial testing the effects of a calcium carbonate supplement on periodontal disease.[42] The supplement being administered over the one year study period was OS-CAL®, which contained 750mg of calcium and 375 IU of vitamin D.[42] While exact periodontal measures were not presented, nine of 15 individuals (60%) in the experimental group demonstrated significant improvement in their periodontal status compared to only three of 18 (16.6%) taking placebo.[42]

Cross-sectional evidence from 6,700 youth and adults who participated in the third National Health and Nutrition Examination Survey (NHANES III) revealed that those in the highest 25(OH)D quintile group (median level > 80 nmol/L) had significantly less bleeding on probing than those in the lowest quintile group.[43] Likewise, significant associations between 25(OH)D levels and the proportion of calculus, mean probing pocket depth, and mean gingival attachment loss were identified. Overall, there was less calculus, lower mean probing pocket depths, and less mean attachment loss associated with higher 25(OH)D quintiles.[43] Regression modeling controlling for many study variables also revealed less gingival bleeding on probing with higher 25(OH)D levels.[43] Further analysis of data from NHANES III relating to 25(OH)D serum concentrations and periodontal disease revealed significantly greater periodontal attachment loss (AL) among older adult males and females (≥ 50 years of age) with lower 25(OH)D levels, even after linear regression analyses.[44] Overall, it appeared that higher 25(OH)D concentrations were significantly associated with less periodontal attachment loss.[44]

A randomized controlled trial evaluating the benefits of calcium and vitamin D supplementation among the elderly included periodontal examinations two years after the

36 month trial had ended.[45] The number of teeth with periodontal pockets ≥ 3.5 mm were contrasted between those originally in the treatment arm and those in the control group, but no significant differences between the groups existed (p=.92).[45] Likewise, there was no significant difference between the groups relating to a history of treatment for periodontal disease (p=.38).[45]

A 2009 publication also reported results of a study involving patients with periodontal disease and healthy controls with respect to circulating concentrations of vitamin D.[46] A total of 66 Chinese individuals with generalized aggressive periodontitis, 52 with chronic periodontitis, and 60 healthy controls participated. A significant difference in 25(OH)D levels was observed between those with aggressive periodontitis and healthy controls. However, it was those with aggressive periodontitis who had the higher vitamin D levels.[46] A positive correlation was reported for bleeding index and 25(OH)D, but only in those meeting the criteria for aggressive periodontitis.[46]

However, new research is revealing that vitamin D receptor (VDR) and VDR gene polymorphisms may play a role in the development of periodontal disease. VDR plays an important role in mineral metabolism and genetic defects in the VDR can actually result in hereditary vitamin D resistance. Case-control evidence reveals that there was a significant over-representation of the FcyRIIIb-NA2 allelle among those with generalized early-onset periodontitis.[47] Another earlier study of subjects with early-onset periodontitis and healthy controls did not produce any significant differences in the VDR genotype or allele frequencies, but did identify significant differences when early-onset periodontitis was limited to those with localized early-onset periodontitis.[48]

Among Chinese adults, the VDR Taq I Tt genotype and t allele were significantly associated with early-onset periodontitis while among Japanese participants, the Taq I TT genotype was significantly associated with adult periodontitis.[49] Similarly, Sun and colleagues reported that significantly more individuals with early-onset periodontitis had the Taq I Tt genotype than healthy controls (24.3% vs. 5.1%) and that this genotype was significantly more frequent in those with early-onset periodontitis than adult periodontitis (24.3% vs. 4.2%).[50]

A more recent publication assessed both the Apa I and Taq I VDR gene polymorphisms and their relationship with periodontal disease (e.g. probing depth, bleeding on probing, alveolar bone loss, and clinical attachment loss) in the VA Dental Longitudinal Study.[51] The presence of the AA VDR genotype was significantly associated with greater alveolar bone loss and clinical attachment loss while there was significantly less bleeding on probing among those with the aa genotype.[51] No significant differences were identified between Taq I VDR genotypes and periodontal outcomes.[51]

VDR gene polymorphsisms are also associated with chronic periodontitis.[52] Results of multiple regression analysis from a Japanese case-controlled study revealed that the Taq I TT genotype is a significant risk factor for chronic periodontitis.[52] Similar work with a Brazilian adult population examined the relationship between the Taq I and Bsm I VDR polymorphisms in a group of healthy individuals with a cohort with chronic periodontitis.[53] Those with the Tt and tt Taq I genotypes were more likely to have periodontal disease than those with the TT genotype; overall, the TB/tb haplotype was frequent in those with chronic periodontitis.[53]

Recently, a cross-sectional study of Japanese males concluded that there were no significant associations between the genotypes of the Apa I, Bsm I and Fok I VDR polymorphsism and periodontal measures like the number of teeth present, the percentage of teeth with bleeding on probing, and average probing depth.[54] However, those with the Abf haplotype were significantly more likely to have severe chronic periodontitis, even after adjusting for age, diabetes, smoking status, and the number of teeth present.[54]

A new article reporting results from both cross-sectional and longitudinal investigations also examined the association between gene polymorphism and periodontal disease.[55] Logistic regression revealed an interaction between smoking status and VDR genotypes with the presence of periodontal disease.[55] This study provides evidence that VDR gene polymorphisms among smokers may be associated with periodontal disease.

A case-control study of Turkish adults reported no statistically significant differences in the genotype frequencies for Bsm I, Apa I, or Taq I between those with generalized chronic periodontitis and healthy controls.[56] Similarly, Li and colleagues reported no significant relationship between genotype and allele frequencies of the Bsm I, Apa I, and Taq I VDR polymorphisms and generalized aggressive periodontitis.[57] However, there was a notable difference between the groups relating to the Fok I polymorphism as those with the FF genotype and F allele were more likely to be present in those with generalized aggressive periodontitis.[57]

Tooth Loss and Alveolar Bone Loss

Investigators using data from NHANES III found that there was a significant relationship between 25(OH)D levels of participants and the mean number of missing teeth.[43] Those in the highest quintile of 25(OH)D, with a median exceeding 80 nmol/L, had significantly fewer missing teeth than those individuals in lower groups.[43]

A three year randomized, placebo-controlled trial of older adults receiving calcium (500mg/day) and vitamin D supplementation of 700 IU/day to study their effects on bone loss from the hip also included secondary outcome measures relating to oral health status of the participants.[45] Participants were then followed for another two years after the trial ended. During the trial period, logistic regression analyses concluded that those receiving supplementation had significantly lower odds of tooth loss compared with adults in the control group.[45] However, at the conclusion of the two year follow-up phase, no significant relationship was found between vitamin D intake and tooth loss.[45] Overall, this study provided strong evidence that vitamin D and calcium supplementation can significantly improve tooth retention among the elderly.[45]

Finally, a longitudinal study of American veterans reveals that the adjusted mean number of teeth lost was significantly associated with the presence of the Apa I VDR AA genotype ($p=.04$), which is considerable since there were no significant differences in the number of teeth present at baseline.[51]

In addition to tooth loss it appears that vitamin D status plays a fundamental role in the maintenance of oral bone. A cross-sectional study of healthy postmenopausal women with edentulous mandibular arches with severe residual ridge resorption were found to have low circulating concentrations of 25(OH)D.[58] Meanwhile, a one year

randomized, placebo-controlled study of adults who received multiple extractions and the placement of immediate dentures compared the mean amount of bone resorption between a group who received daily supplementation with calcium (750 mg) and vitamin D (375 IU) compared to a group receiving placebo.[59] There was significantly less total oral (36%), maxillary arch (34%) and mandibular arch (39%) bone loss among those benefiting from vitamin D and calcium during the one year follow-up period.[59] This study provides strong evidence of the protective effects of vitamin D and calcium supplementation against undesirable alveolar ridge resorption.

Another randomized, placebo-controlled study evaluated the benefits of calcitriol (1,25-dihydroxycholecalciferol) on mandibular bone mass in a sample of postmenopausal women.[60] Women were randomized to receive either calcitriol or placebo daily over two years. For those in the calcitriol arm, the mean dosage was 0.43 ± .03 ug/day.[60] In addition, women took a multivitamin supplement containing 400 IU of vitamin and attained 1000 mg of calcium daily.[60] At the end of the study period, there was no significant difference in mandibular bone mass between the groups indicating that 1,25-dihydroxycholecalciferol had little influence on maintaining bone. However, 83% increased or maintained bone mass in the mandible.[60]

More recently, a double-blinded, randomized, placebo-controlled trial of hormone replacement therapy (E/HRT) examined the influence on postcranial bone density and oral bone mass among postmenopausal women.[61] In addition to receiving either E/HRT or placebo, all women took calcium (1000 mg) and vitamin D_3 (800 IU) supplements on a daily basis during the 3 year study.[61] Although not designed to determine the influence of vitamin D on bone density and mass, this study concluded that there was a significant

increase in alveolar bone mass among the E/HRT group over controls.[61] However, even in the placebo group, where 800 IU of vitamin D_3 was administered daily, there were statistically significant increases in alveolar crestal height (3.5%) and alveolar bone mass (0.9%) over time.[61] A follow-up study invited all participants to continue participating for an additional two years.[62] Those women in the E/HRT arm of the original randomized study continued with the E/HRT regimen with calcium and vitamin D, while the control group who initially were only receiving vitamin D and calcium in the first three years took E/HRT.[62] At the completion of these two additional years of investigation, 26 women remained in the original E/HRT group while 21 were in the original control group that initially only benefited from vitamin D and calcium.[62] While logistic regression revealed that there were significant mandibular bone mass increases in both groups over the five year time span, there were no significant increases over these additional two study years.[62]

Tooth Development

Dental morphogenesis involves the development of the outer enamel structure that arises from enamel producing cells termed ameloblasts. Meanwhile, dentin tissue is produced by odontoblasts. Evidence of the role of vitamin D on the development of enamel and dentin is known as both ameloblasts and odontoblasts are target cells for 1,25-dihydroxyvitamin D_3.[63] The presence of VDR and calbindin-D28k in odontoblasts and ameloblasts implicates 1,25-dihydroxyvitamin D_2 as a regulator of dentin and enamel formation.[64] In addition, the presence of VDR at early stages of dental development also suggests that 1,25-dihydroxyvitamin D_3 may exert control on tooth crown development.[64]

Further, amelogenin and enamelin, proteins found in dental matrices are vitamin D-dependent.[65] In addition to enamel and dentin formation, VDR is strongly expressed in osteoblasts responsible for alveolar bone formation.[65]

Studies involving human embryonic and fetal tissue to demonstrate the role of $1,25(OH)_2D_3$ on enamel and dentin formation reveal that 1,25-dihydroxyvitamin D_3 membrane-associated rapid-response steroid binding protein is expressed during human tooth mineralization in ameloblasts and odontoblasts.[66]

In addition to its role in the initial production of dentin, Calbindin D-28k, the vitamin D-dependent calcium-binding protein, is important in the synthesis of tertiary dentin under caries lesions.[67]

Enamel Hypoplasia

Deficiencies of vitamin D during periods of tooth development are believed to result in developmental defects of enamel (DDE), which includes both enamel opacities and enamel hypoplasia.[68] A 1973 report in the Lancet reported that a considerable number of infants with neonatal tetany, resulting from maternal vitamin D deficiency, had noticeable enamel hypoplasia in the primary teeth when examined at a mean of 42.4 months (range 24 – 80 months) of age.[69] Overall, 56% had pronounced enamel hypoplasia in their primary teeth.[69] Histological examination of a subset number of exfoliated primary incisors revealed that much of the enamel hypoplasia coincided with enamel formation during the third trimester of pregnancy.[69]

There are also documented case reports of children who developed enamel hypoplasia as a result of inadequate vitamin D during periods of dental development.

Long-standing, vitamin D deficiency beginning during childhood has been found to contribute to generalized enamel hypoplasia in the permanent dentition and poorly developed enamel of permanent third molars.[70]

Interestingly, while low vitamin D results in increased risk for enamel hypoplasia, it also appears that hypervitaminosis D may result in dental hard tissue abnormalities.[71] A case report of extreme hypervitaminosis D during infancy, resulting from the over-fortification of milk from the local dairy, was responsible for enamel defects exhibited in permanent maxillary and mandibular central incisors and permanent mandibular first molars when examined at seven years of age.[71]

Rickets

There is considerable literature documenting the relationship between rickets and DDE, specifically enamel hypoplasia. In fact, enamel hypoplasia in both primary and permanent dentitions is the classic dental phenotype of rickets. However, not every child with a history of rickets displays enamel hypoplasia.[72] Some of the earliest published evidence originated with Lady May Mellanby who stated that the majority of her contemporary peers were fixated with risk factors for caries, but not the integrity of the tooth itself.[36,73,74] Her earlier work investigated the role of nutrition on the structure of teeth in animal models building upon her husband Edward's discovery of vitamin D in the treatment of rickets.[74-76] Her work would eventually prove that GV Black, the pioneer of North American dentistry, was wrong when he emphatically stated that the integrity of teeth played no role in caries susceptibility and was only a result of external environmental factors.[36]

A study of Australian infants who were premature and diagnosed, both clinically and radiographically, with rickets in the neonatal period reported that all children exhibited developmental defects of enamel in the primary dentition, with 47% having enamel opacities and hypoplasia, 20% with only opacities, and the remainder with enamel hypoplasia (33%).[77] Meanwhile, an early case-controlled study provided evidence that children with rickets were more likely to have enamel hypoplasia in both primary and permanent dentitions than children without rickets who received vitamin D prophylaxis (viosterol) during the early childhood period.[78] Specifically, those with rickets were more likely to have both marked hypoplasia and slight hypoplasia than ricket-free controls who received vitamin D prophylaxis in the form of viosterol.[78] There were further case reports of children with acute rickets having enamel hypoplasia in their dentitions.[79]

There is long-standing longitudinal evidence too, showing that children who experienced rickets during the early childhood period were more likely to have enamel defects in their permanent dentitions.[80,81] In fact, the more severe the case of rickets, the greater the prevalence and the more severe the enamel defects.[80,81] Another study contrasted dental findings of children with rickets and controls.[82] This case-control study revealed that children with rickets were nine times more likely to have hypoplasia in the permanent dentition than healthy controls.[82]

There are several classifications of rickets, including dietary, transport, matrix defect and metabolic.[77] One type of metabolic rickets is VDDR, where the synthesis of $1,25(OH)_2D$ is impaired.[83] Case study reports have confirmed that children with VDDR are highly prone to enamel hypoplasia.[38,83,84] Even those who were diagnosed with

vitamin D resistant rickets (VDRR) and received vitamin D_3 and phosphate therapy before 12 months of age displayed some manifestations of isolated enamel hypoplasia in the permanent dentition.[85] Those diagnosed and treated by 18 months of age also demonstrated isolated enamel hyoplastic lesions in the permanent dentition.[85]

Supplementation & Dietary Interventions

Knowing that vitamin D deficiencies and altered vitamin D metabolism can result in enamel hypoplasia, it is plausible that vitamin D supplementation during periods of enamel formation can reduce the incidence of hypoplastic defects.

Much of Mellanby's work on the effect of diet on dental structure and disease in man is summarized in her final report to the Medical Research Council published in 1934.[36] While most of this report focused on the relationship between diet and caries, she did conclude that vitamin D in the prenatal diet and during periods of dental development after birth results in a reduction of enamel hypoplastic defects.[36] Another of her reports on the influence of diet on caries in children's teeth would also support the theory that the addition of vitamin D to the diet during enamel development improves the structure of permanent first molars. Mellanby's early observations were that normal enamel and dentin structure were more frequent among children benefiting from cod liver oil in their diets compared to children whose diets contained little vitamin D.[86]

Supplementing infants with rickets with cod liver oil has been documented to reduce the incidence of enamel hypoplasia.[81] Further, this antirachitic therapy, which included cod liver oil and sunbathing during infancy and early childhood has also shown to be effective in decreasing the incidence of enamel hypoplasia in the permanent dentition.[80]

Both dietary interventions and supplementation during pregnancy and infancy can improve the integrity of enamel of primary teeth. A longitudinal cohort that provided expectant women with 400 IU of vitamin D beginning in the 12th week of pregnancy reported that infants developed significantly less enamel hypoplasia in the primary dentition compared with controls taking placebos.[87] However, this only involved a small sample of the total cohort. Meanwhile, a longitudinal investigation studied the influence of vitamin D supplementation (500 IU or 1000 IU/day) for six months in preterm children on the development of enamel hypoplasia in the primary and permanent dentitions, up to 11 years of age.[88] Results from this study indicated that there was no significant difference in the prevalence of enamel defects in primary or permanent teeth between those receiving 500 IU vitamin D daily or those receiving twice the dose.[88] In essence, there was no additional benefit found by doubling the dosage of vitamin D. Likewise, the benefits of vitamin D supplementation during the prenatal period and infancy do not continue beyond this point to aid in the development of enamel in the permanent dentition. A recent report was unable to demonstrate that such supplementation was associated with a decreased prevalence of molar incisor hypomineralization among children at 12 years of age.[89] On the other hand, a longitudinal study of children in Mexico investigated the influence of supplementation during pregnancy, infancy, and childhood with vitamins and protein, including 20,000 IU vitamin D.[90] A significant difference in the prevalence of linear enamel hypoplasia was found with children in the control group having nearly twice as much enamel hypoplasia as those who benefited from this high dose supplementation.[90]

A 1958 review on the topic of caries prophylaxis even concluded that there is sufficient work to prove that the quality of enamel structure is determined by vitamin D and mineral salts.[91]

Caries

Rickets

As previously mentioned, enamel hypoplasia is the phenotypic presentation of children with rickets. Likewise, caries is also common in these children. The early twentieth century caries literature reported clear associations between rickets and dental decay. For instance, longitudinal observations of children diagnosed with rickets during early life revealed that they were more likely to suffer from a higher prevalence of caries in their primary and permanent dentitions than those without rickets.[80,92] Similarly, Lady Mellanby concluded that caries was more prevalent in those with a history of severe manifestations of rickets than healthy children of the same socioeconomic class.[36] In fact, those with rickets as a result of vitamin D deficiency had more extensive forms of decay.[36]

Likewise, Hess and Abramson provided observational evidence of a connection between rickets and dental caries in the primary dentition.[93] Specifically, dental caries was more prevalent among those with rickets than children protected from rickets with cod liver oil during infancy.[93] However, a case control investigation only reported a slightly higher prevalence of caries in the primary and permanent dentitions of school-aged children with rickets compared to controls without the disease.[82] Similarly, Shelling and Anderson were unable to demonstrate that rickets predisposed children to a greater

incidence of caries in the primary dentition than children who benefited from additional vitamin D as a prophylactic approach for rickets.[78]

A few studies have attempted to curb the onset of caries in patients with rickets. Providing vitamin D in the form of cod liver oil during infancy has been shown to lower the incidence of caries.[81] In addition, the combination of both cod liver oil and sunbathing during infancy and early childhood also appeared to lessen the extent of decay.[80]

Observations of a group of children with rickets in India were not able to corroborate the findings of these other investigations as those with vitamin D deficient diets did not appear to have a greater risk for dental caries. However, considering the number of children was very small, the findings are not definitive.[72]

Another study examined the influence of a combined caries and rickets prophylaxis approach including both fluoride and vitamin D tablets among children six to 48 months of age.[94] Children who received the fluoride and vitamin D tablets for more than 12 months and who were breastfed for ≤ 3 months were more likely to be free from caries in primary dentition.[94] However, the reduction in caries exhibited in this trial was probably due to fluoride instead of the addition of vitamin D.

The introduction of vitamin D Stosstherapy as a rickets prevention strategy did not appear to have a profound effect on caries rates.[95] Results suggested that there was no significant difference in caries rates or prevalence between young children receiving vitamin D Stosstherapy three times during the first two years of life, Stosstherapy once before the first birthday and one or two additional times in the second year, and those who did not receive such vitamin D treatment.[95]

Gedicke's paper examined the influence of a public health approach to eradicate rickets through vitamin D supplementation on the oral health of children three to 18 years of age between the years 1952 and 1957.[96] This article provided evidence of the possibility of preventing caries and improvements in dental health through vitamin D preparations intended to prevent rickets.[96] A similar approach was undertaken as a means to prevent rickets in a Northern Manitoba community where a modified form of Stosstherapy was administered during pregnancy or infancy.[97] Unfortunately, no significant differences in caries rates were found between children who benefited from additional vitamin D and those who did not.[97] The findings are confounded by the fact that the majority of children examined had undergone aggressive rehabilitative dental surgery.

While children with VDRR have an increased prevalence of enamel hypoplasia, they are also prone to dental caries. The introduction of 1α-hydroxyvitamin D_3 during infancy and childhood to manage VDRR has lead to improvements in oral health.[98] Those who received vitamin D and continued to receive supplementation at the time of dental examination had lower caries rates in the permanent dentition than adults who either did not receive or who only received supplementation until adulthood (DMFT = 2.4 ± 2.4 vs. 20.4 ± 6.4).[98]

Supplementation and Dietary Interventions

There are several studies that point to the potential influence of vitamin D on the development and progression of dental caries. Unfortunately, much of this evidence has been overlooked by the dental community. The majority of these studies have examined the benefit of fortifying the diet with vitamin D or foods and compounds containing this

important nutrient. However, many of these studies have limitations as they did not report whether they controlled for other factors related to caries such as oral hygiene practices and bacterial levels. Further, most were not randomized trials and dental assessments were made by non-blinded individuals.

In 1931, an interim report by the Committee Upon Dental Disease provided initial experimental evidence that children benefiting from a daily intake of cod liver oil had significantly lower incidence and extent of caries after two years than children in groups receiving treacle or olive oil.[99] Children in the cod liver oil group had significantly lower increase in the incidence of caries involving primary teeth than those in the treacle and olive oil groups.[99] Likewise, the same findings were also found in relation to permanent teeth among children in the cod liver oil group.[99] In addition, this report provided early evidence that children receiving radiostol (vitamin D) had significantly lower incidence of caries after two years than a control group of children who only received olive oil.[99] Similarly, children receiving radiostol had a significantly lower incidence and extent of caries in the permanent dentition than controls after two years.[99]

An early study by the Committee on Dental Disease for the British Medical Research Council was undertaken to determine whether nutrition, particularly vitamin D, had a predominant impact on the structure of teeth and their resistance to caries.[100] Results from this group's work indicated that there was a lower incidence of dental decay among those receiving vitamin D in the form of cod liver oil.[100] Adding vitamin D to the diets during periods of tooth development prior to the complete eruption of the permanent teeth reduced the incidence of dental caries.[100] Likewise, the addition of vitamin D during the development of primary teeth also lowered the risk for caries and hindered the

progression of untreated decay.[100] The final conclusions drawn from this series of investigations was that vitamin D rich diets could diminish the risk of caries if delivered during periods of tooth development, even at later stages, and that even when given after teeth have erupted, it could also retard the spread of caries.[100]

Early work by Mellanby and Pattison tested different diets with and without cod liver oil, on a group of seven year old children with equivalent caries experience who were institutionalized and living under similar conditions. After nearly 8 months, those who benefited from diets containing cod liver oil had fewer teeth with new or increased caries activity compared with controls on a regular diet.[73] Further, children on this diet also had more hardening of caries lesions than controls.[73]

Other work by Mellanby and Pattison examined the influence of diet on the progression of caries in children of a mean age of 5 years.[37] A cohort of 21 children received radiostol (radiated ergosterol) for 28 weeks and were examined for the onset of new caries lesions, the progression of existing decay, and the arrest of caries lesions.[37] They concluded that vitamin D supplementation had a pronounced effect in the prevention of new caries, limiting the spread of existing lesions, and arresting the caries process for many teeth.[37] In fact, they also stated that vitamin D appeared to be one of the most dominant factors in the inhibition of caries.[37] Earlier, these same investigators conducted a clinical study involving children who were hospitalized with tuberculosis examining the benefits of diets with different amounts of vitamin D, in the form of cod liver oil, eggs and milk, on the dentition.[101] Those receiving higher amounts of vitamin D showed fewer new areas of caries, less spread of caries, less increase in the degree of

caries, and more hardening of existing lesions than children in other groups not benefiting from the same amount of vitamin D.[101]

Some years later, Mellanby and Pattison would again publish findings on their observations of children of a mean age of 5.5 years with enamel hypoplasia and caries present at the time of enrolment, who were exposed to cereal-free diets that were rich in vitamin D and calcium for a period of 6 months.[102] This dietary intervention resulted in the arrest of active caries while the initiation and spread of caries was almost eliminated.[102]

Some of the earliest work in this area, sponsored by the British Medical Research Council involved the addition of vitamin D to the diet, either as irradiated ergosterol or cod liver oil, as a means to reduce the incidence of caries.[100] Evidence from this large-scale and prolonged investigation of children demonstrated that a high vitamin D content in the diet can reduce the incidence of caries when administered during periods of dental development, including late dental development compared with children in control groups not benefiting from this fortification[100]. Further, the addition of vitamin D was shown to delay and retard the onset and spread of caries in erupted teeth.[100]

A follow-up report by Lady Mellanby provided additional data to support the connection between vitamin D and caries in children from several studies.[36] Several diets were contrasted including a diet that did not contain additional vitamins A or D, a diet that contained both, and a third diet that was rich in both vitamins A and D. It revealed that the vitamin D rich diet, primarily cod liver oil, inhibited the incidence and rates of caries, arrested caries, while also promoting the healing of caries lesions through the production of secondary dentin.[36] In fact, it was concluded that vitamin D and cod liver

oil retards the progression of caries in children and the administration of two cc of vitamin D daily helped prevent caries in children's teeth[36]

In this same era, other researchers would examine the relationship between vitamin D and caries. While unable to emphatically identify vitamin D as a key factor in the arrest of active caries lesions, Boyd and colleagues demonstrated that a controlled good diet that included a teaspoon of cod liver oil daily appeared to arrest active decay in children.[103] A trial of comparing the benefits of vitamin D supplementation in the amount of 10,000 U/day among children from New Zealand, under controlled conditions, demonstrated a disappearance of defective calcification.[104] Those in the vitamin D treatment group had fewer caries lesions progress to the state of requiring restoration and fewer incipient caries lesions than children in the control group not benefiting from additional vitamin D supplementation.[104]

Preliminary results from an early clinical study of dietary interventions involving orphaned children revealed that those receiving an experimental diet higher in vitamin D and cod liver oil had a smaller incidence of caries than controls.[105] This same team would later conduct a series of studies with American children in hospital investigating the effects of both dietary fortification and light radiation.[106] Evidence from the first study revealed that those receiving a diet high in vitamin D including three teaspoons of cod liver oil each day had a significantly lower prevalence of caries than children who did not benefit from the addition of vitamin D.[106] Afterwards, a cross-over study was undertaken with similar findings.[106] Meanwhile, a group of children exposed to light radiation and a group that received 15 drops of viosterol daily were found to have lower caries rates than

controls.[106] Interestingly, children who received quartz mercury light radiation appeared to have greater protection against caries than viosterol.[106]

Another study involving American children, ranging in age from four to 16 years assigned individuals to seven distinct dietary groups, including good and poor diets, with and without vitamin D drops or irradiation.[107] The addition of viosterol or irradiation to a well balance diet did not have further benefit in lowering the incidence of caries, yet those on a poor diet who received viosterol drops had significantly fewer new lesions than children who had a poor diet and no supplementation.[107] The influence of irradiation on a poor diet, while beneficial, was not as profound as the addition of viosterol.[107]

Many more dental researchers also considered fortifying foods with vitamin D as a potential vehicle to prevent caries. For instance, the fortification of reconstituted milk with daily vitamin D in doses of 100 units, 150 units, and 300 units provided evidence of a distinct correlation between increasing dosages of vitamin D in milk and a reduction in percentage of carious tooth surfaces,[108] supporting the important influence of vitamin D in dental decay.

A controlled clinical trial involving the administration of vitamin D drops to Canadian children ranging in age from two to 16 years was also conducted to substantiate the emerging evidence of the association between vitamin D and caries.[109] All children received good diets and spent considerable time outside daily. After one year, children were examined both clinically and radiographically with key findings that children who received the equivalent of eight drops of vitamin D (viosterol) daily, added to a cookie, had less progression of established caries lesions in primary teeth and fewer new caries lesions than unsupplemented controls.[109] Children in the vitamin D group also had fewer

new cavities in the permanent dentition and there were fewer lesions among newly erupted teeth in the group of children receiving additional vitamin D.[109] Overall, there were significantly lower mean number of new caries per child and significantly lower prevalence of new caries among children three to 10 years of age in the vitamin D group.[109] Likewise, there was a significantly lower mean number of new caries per tooth in both the primary and permanent dentitions among three to 10 year old children in the vitamin D group.[109]

A small group of children in a hospital metabolism ward were exposed to several dietary regimens over seven months, some of which included vitamin D supplementation in the form of fortified milk or cod liver oil.[110] The addition of cod liver oil to the diets of children appeared to contribute to the arrest of dental caries in those with decay at the start of the observation period and appeared to be the predominant factor in the diet influencing caries.[110]

A series of investigations on the administration of vitamin D to children six to 14 years of age were conducted over a span of four years.[111] In the first year, those benefiting from the addition of cod liver oil (800 USP units vitamin D) had a lower incidence of caries.[111] During the second year, twice weekly skin exposure to ultraviolet light from a quartz mercury lamp appeared to have a similar effect as cod liver oil on reducing the number of new decayed surfaces.[111] The third study tested a larger dose of viosterol and ultraviolet lamp exposure, but found that they were not superior to cod liver oil in preventing new decay.[111] The final study examined the benefits of adding vitamin D from animal source in doses of 250 U, 400 U, and 800 U, to milk and concluded that increasing concentrations were correlated with a progressive reduction in caries rates.[111]

A few years later, additional research would produce more data on the effect of differing doses and forms of vitamin D on the dentition of school-aged children. Children receiving daily supplementation of vitamin D from cod liver oil (either 400 units or 800 units) had significantly fewer new caries surfaces than unsupplemented controls (1.65 surfaces (800 units) and 2.48 surfaces (400 units) vs. 4.5 surfaces (controls)).[112] Similarly, the intake of viosterol (800 units or 3200 units) or vitamin D from cod liver oil (400 units or 800 units) significantly reduced the mean number of new decayed surfaces per child compared to unsupplemented controls.[112] However, 800 units and 3200 units of viosterol were not superior at reducing new caries lesions than 400 units of vitamin D derived from cod liver oil in these same children.[112]

In the 1940s, government measures were introduced to increase vitamin D intakes during pregnancy and early childhood, particularly cod liver oil and the fortification of margarine.[113] This provided evidence from a natural experiment as caries was assessed in five year old school children in London over three intervals, 1943, 1945 and 1947.[113]All children examined benefited from these vitamin D and calcium policies throughout antenatal and postnatal life.[113] Coincidentally, over those years, there was a marked decline in the number of children with caries in the primary dentition.[113]

By 1975 the results of an epidemiological investigation on vitamin D prophylaxis contrasted the caries rates of a cohort of preschool children in Hungary with previous data on children of the same age that was collected in 1955.[95,114] Overall, vitamin D_3 injections to prevent rickets during the first year of life, referred to as Stosstherapy, appeared to offer little protective effects against caries rates in primary dentition and was possibly insufficient to counteract consumption of high-caries-risk carbohydrates by

Hungarian preschool children as no logistic regression modeling was used to control for confounding variables.[114]

Recent research indicates that there was no significant difference in the number of teeth with untreated decay in elderly individuals originally enrolled into a randomized, placebo-controlled trial involving vitamin D and calcium supplementation.[45] However, longitudinal evidence from the Iowa Fluoride Study cohort revealed that lower vitamin D intake at three years of age is significantly associated with increased caries in the primary dentition.[115] The relationship was very strong as inadequate intake of vitamin D was associated with increased caries experience on logistic regression.[115]

Vitamin D Receptor Polymorphisms

Newer evidence has begun to uncover possible associations between dental caries and VDR gene polymorphisms. A longitudinal cohort of veterans that looked at the VDR genotype found significantly more permanent teeth with caries at baseline among those with the Apaq I VDR aa genotype compared to those with the Aa genotype (5 ± 6 vs. 3 ± 3, $p=.04$).[51]

Meanwhile, a published abstract in 2003 provides cross-sectional evidence of a strong association between VDR Taq 1 genotype polymorphisms and adult dental caries rates.[116] Allele distribution revealed those with the presence of the t allele were nearly three times more likely to develop high DMFT rates. In addition, those with the tt genotype were more than four times more likely to develop caries than those with the TT or Tt genotype.[116]

Delayed Tooth Eruption

Vitamin D may also have an influence on the eruption of the dentition. Specifically, low levels of vitamin D may result in delayed eruption. For instance, a cross-sectional study involving preschool Aboriginal children from a Northern Manitoba First Nations community reported that there was evidence of delayed eruption of primary teeth if mothers during pregnancy or infants were not receiving high-dose vitamin D supplementation.[97] Meanwhile, case report evidence suggests that long-standing, privational, vitamin D deficiency can result in many unerupted permanent teeth, including the maxillary canines, and molars.[70]

Other Clinical Manifestations

In addition to the previously discussed dental conditions and their associations with vitamin D, there are several other unique manifestations that require mentioning. These findings are summarized in Table 1.3-1.

Table 1.3-1 – Other dental conditions related to vitamin D status or rickets

Description of Population Studied	Clinical Findings
Seven year old who experienced hypervitaminosis D during infancy resulting from over-fortification of milk	• Abnormal pulp horns in permanent central incisors at 7 years of age[71] • Pulp horns partially filled in[71]
19 year old with long-standing, privational, vitamin D deficiency	• Abnormal pulp chambers and root canals in permanent teeth[70]
Children with acute rickets	• Presence of interglobular spaces in dentin[79]
Children with rickets	• Minimal or no crowding in the permanent dentition[82] • Higher incidence of malocclusion compared to controls receiving vitamin D prophylaxis (viosterol) during early childhood[78]
Children with vitamin D dependent rickets (VDDR)	• Dental malocclusion, large pulp chambers and short roots[38] • Interglobular spaces in the dentin, large pulp chambers and prominent pulp horns, loss of lamina dura, and early exfoliation of primary teeth[83,84] • Disturbed normal dentinogenesis (dentin formation) in primary teeth[117] resulting in the presence of abundant interglobular dentin, lack of predentinal layer, thin dentin and large pulp chambers[117] • Deficient mineralization of dentin, exhibited as globular dentin of varying degrees[118]
Children with VDDR receiving treatment with vitamin D_3 and phosphate	• Improved tooth mineralization[118]
Children with vitamin D resistant rickets (VDRR) (X-linked hypophosphatemic rickets)	• Globular dentin[118,119] • Large pulp chambers and frequent dental absecesses[119,120] • Increased prevalence of taurodontism and ectopic eruption of permanent canines[121]
Children with VDRR receiving 1α-hydroxyvitamin D_3	• Fewer endodontically treated teeth[98] • Fewer dental abscesses[98] • Aids in root canal and pulpal development[117] • Normal enamel mineralization if receiving 1α-hydroxyvitamin

Description of Population Studied	Clinical Findings
Children with VDRR receiving 1α-hydroxyvitamin D_3 (continued)	(1α-$(OH)D_3$) since infancy [98] • Improved dentin development and mineralization, resulting in improved dental status, less caries and less pulpal necrosis[122]
Children with VDRR receiving vitamin D_3 and phosphate at early stages (before 2 years of age)	• Lower severity of dental manifestations including enlarged pulp chambers in permanent teeth[85]
Children with VDRR receiving vitamin D_3 and phosphate later in childhood	• More severe dental manifestations, such as poorly calcified dentin, large pulp chambers in their primary teeth and spontaneous abscessing of primary teeth[85]
Children with pseudohypoparathyroidism receiving vitamin D treatment	• Normal secondary dentin formation[123]

In addition to all of this literature, there are additional reports presenting circumstantial evidence of a possible influence of vitamin D in oral health and dental disease (Table 1.3-2).

Table 1.3-2 – Other circumstantial evidence of a relationship between vitamin D and oral health

Influence of Vitamin D on Oral Health	Nature of Association
Oral cancer and UVB exposure[124,125]	Ecological evidence that oral cancer among Caucasian men is inversely correlated with solar UVB and rural residence[124,125]
Molar-incisor-hypomineraliztion and population-wide vitamin D supplementation[126]	Low prevalence of molar-incisor-hypomineralization may be a result of mass implementation of vitamin D supplementation to children during infancy and childhood in Germany in the past two decades[126]
Ultraviolet light and caries[127-129]	Experimental trial evidence that children exposed to full spectrum ultraviolet lighting had very low or no increase in caries incidence in permanent dentition (DMFT, DMFS)[127] Differences were significantly lower than controls.[127] Elementary school children exposed to full spectrum lighting for 9 months had a lower incidence of caries in permanent first molars than those exposed to cool-white light.[128] Evidence that caries rates in permanent dentition (based on D and M) might be associated with mean annual hours of sunshine[129]
Season and caries[111,130]	Evidence that the incidence of caries is higher in late winter and early spring than during summer periods[111] Children living in cities where the winter temperature was > 30˚F had lower mean caries rates in the permanent dentition than those living in cities with colder winter temperatures[130]
Hours of yearly sunshine and caries and enamel hypoplasia[69,130,131]	Mean caries rates in the permanent dentition of children living in cities with > 2,600 hours of sunshine each year were significantly lower than those receiving < 2,600 hours of sunshine per year[130,131]

Influence of Vitamin D on Oral Health	Nature of Association
Hours of yearly sunshine and caries and enamel hypoplasia (continued)	A statistically significant inverse relationship was reported between pronounced enamel hypoplasia and mean daily hours of sunshine during the third trimester[69]
Geography and caries[36,86,129,130,132-135]	Evidence that geographical distribution of caries may be a result of vitamin D attainment (endogenous & exogenous). Those in southern latitudes and those on high vitamin D diets may have greater immunity to caries[36] Children residing in cities < 40˚N latitude had lower mean caries rates in the permanent dentition than those children living at higher latitudes[130] Greater prevalence of dental caries at higher latitudes[132] Incidence of dental caries among 12 to 14 year old American males increased as latitude increased[133] Retrospective evidence that caries rates in permanent dentition may be related to geography with higher rates at more northern locales[129] Naval personnel from northern United States of American demonstrated more defective teeth affected by caries than those from southern regions[134] Historically low caries rates of natives in tropical regions of the world possibly due to their increased exposure to sunshine[86] Naval midshipmen from the northern United States and New England States were found to have higher DMFT rates than their peers from southern latitudes including the American Southewest[135]

Influence of Vitamin D on Oral Health	Nature of Association
Diet and caries, including odontoclasia[136]	Diets with higher fish intakes may be preventive in the development of odontoclasia, a former term used to describe decalcified bands of enamel caries on primary anterior teeth with stained but non-carious dentin[136]

In summary, this section presents evidence from cross-sectional, observational, and controlled trial studies that vitamin D intakes and status have an influence on oral health in both children and adults. In particular, vitamin D appears to have an effect on enamel hypoplasia and caries. Likewise, vitamin D levels are correlated with periodontal disease, tooth loss, and oral bone loss. Dietary supplementation has been shown to reduce the incidence of caries and halt the progression of lesions, reduce the incidence of enamel hypoplasia, and preserve alveolar ridges. There is also emerging evidence suggesting that some oral health conditions including periodontitis, tooth loss, and perhaps even dental caries rates are associated with VDR gene polymorphisms.

In addition, there is considerable circumstantial evidence pointing to a relationship between oral health and those factors that interfere with the endogenous production of vitamin D, specifically, latitude, season, and hours of sunshine during the year as proposed by East in 1941.[130]

Determining the association between serum 25(OH)D levels and oral health outcomes is relatively new, but offers an opportunity to further our understanding of the role of maintaining vitamin D adequacy for good oral health.

The dental profession and the public health community cannot ignore the volume of research that began during the first half of the twentieth century showing that vitamin

D has an effect on the dentition, particularly in improving resistance to dental caries. While many of these studies would likely not be conducted today because of the ethical considerations of performing such studies with orphaned children residing in institutions we should not ignore these findings. Further, these early studies did not control for many other influencing factors like oral hygiene behaviours, socioeconomics, and access to dental care. Regardless, there is also sufficient evidence to show that the quality of enamel is influenced by vitamin D during periods of enamel formation.[91] The real curiosity is why much of this literature has been forgotten. It may be due to assumptions that the implementation of vitamin D fortified foods would solve the problem of caries or it could be that not including this literature into electronic search engines may have contributed to its loss.

As early as 1936, the Medical Research Council of Great Britain stated that investigations into the role of vitamin D and caries should begin during the prenatal period and extend longitudinally into childhood and adolescence.[100] This review provides important evidence that good oral health outcomes may be achieved with optimizing vitamin D intakes and endogenous synthesis. Therefore, relationships between serum 25(OH)D levels during pregnancy and infant oral health are quite plausible and should be studied.

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Chapter 2 – Methods for a Prospective Study of Prenatal Vitamin D Concentrations on Infant Oral Health

Hypothesis

The hypothesis to be tested in this study was that low maternal 25-hydroxyvitamin D (25(OH)D) concentrations during pregnancy may contribute to the predisposition of enamel hypoplasia in the primary dentition of the infant and may thus increase the risk of developing Early Childhood Caries (ECC).

Methods

A prospective cohort design research study was selected to investigate the association between vitamin D concentrations during pregnancy and enamel hypoplasia and ECC in the primary dentition of infants. The study involved participant recruitment during the second trimester of pregnancy at which time serum testing and a questionnaire were completed. Enrolled women and their infants were followed prospectively until the infant was 12 months of age so that their child's newly erupted primary dentition could be examined. Mothers also completed a follow-up questionnaire at this subsequent study visit.

Objectives

1. To determine the vitamin D and calcium nutritional status of a group of urban dwelling women, primarily of Aboriginal heritage, during pregnancy.

2. To determine the prevalence of enamel hypoplasia and incidence of ECC in the primary dentition, including the maxillary incisors, of the infant as they erupt into the oral cavity.
3. To determine the association between maternal 25(OH)D status during pregnancy and the presence or absence of enamel hypoplasia and ECC in the primary dentition of the infant.

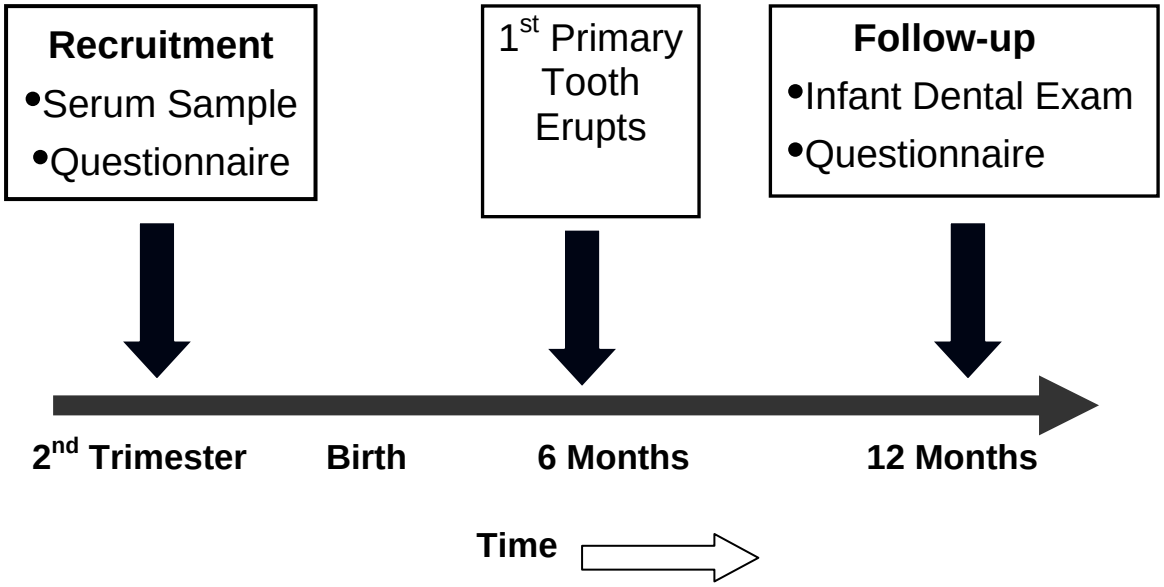
Study Design

A quantitative research strategy was employed and involved women being enrolled in a prospective cohort study during pregnancy (Figure 2.1). A serum sample from each participant was collected during a regular prenatal visit. The collection of these samples for the majority of participants coincided with routine prenatal blood draws. The desired period for serum sampling was during the second trimester of pregnancy, as the maxillary primary incisors (i.e. teeth commonly affected by ECC) begin to develop by six weeks in utero and begin to calcify during weeks 13 and 17 in utero. However, some participants were enrolled during the early third trimester of pregnancy.

Serum analysis was conducted for 25-hydroxyvitamin D (25(OH)D), as this is a reliable means of assessing overall vitamin D status.[1-3] Serum analysis also included assessments of total calcium, inorganic phosphorus, and alkaline phosphatase concentrations. Detectable elevations in circulating serum levels of alkaline phosphatase can be indicative of vitamin D insufficiency[4] while decreased serum phosphorus and calcium in conjunction with parathyroid hormone stimulate the hydroxylation of 25(OH)D to 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), the active form of vitamin D.[5]

Each participant also completed an interviewed questionnaire. This questionnaire was proctored by either the principal investigator or a clinic staff member at one of the participating community or hospital clinic sites. This instrument was based on a previous tool developed to assess nutritional deficiencies in three northern Manitoba First Nations communities.[6] The questionnaire was also constructed with input from senior researchers and community health workers, including a registered dietician. All possessed good knowledge of the study population.

Figure 2.1 – Timeline of this study investigating the relationship between prenatal 25(OH)D status and enamel hypoplasia and ECC.



The questionnaire was composed of specific sections including basic demographics, pregnancy and health, a nutrition and food security assessment, awareness of ECC, and a maternal oral health profile. Other sections posed questions regarding exposure to sunlight, and family composition and finances.

The final component of the study was the infant dental examination, which occurred following the eruption of the primary maxillary incisors. Clinical examination of each infant's erupted primary teeth was conducted by the principal investigator for the presence of developmental defects of enamel (DDE), including enamel hypoplasia at 12 months of age. Criteria used to score enamel hypoplasia in this clinical assessment was based on an established index in the literature discussed in Chapter 1 Section 2 (Table 2.1).[7] Case definitions for ECC and Severe Early Childhood Caries (S-ECC) exist and were adopted for this study (Table 2.2).[8-10]

Table 2.1 – Modified DDE Index for use in epidemiological studies [7]

	Code
Normal	0
Demarcated opacities:	
White/cream	1
Yellow/brown	2
Diffuse opacities:	
Lines	3
Patchy	4
Confluent	5
Confluent/patchy + staining + loss of enamel	6
Hypoplasia:	
Pits	7
Missing Enamel	8
Any other defect	9
Combinations:	
Demarcated and Diffuse	A
Demarcated and hypoplasia	B
Diffuse and hypoplasia	C
All 3 defects	D

Table 2.2 – Recognized case definitions for ECC and S-ECC[8,11]

Age (months)	ECC	S-ECC
< 12	1 or more teeth	1 or more smooth dmf surfaces
12-23	1 or more teeth	1 or more smooth dmf surfaces
24-35	1 or more teeth	1 or more smooth dmf surfaces
36-47	1 or more teeth	dmfs score ≥ 4 OR 1 or more smooth dmf surfaces in the primary maxillary anteriors
48-59	1 or more teeth	dmfs score ≥ 5 OR 1 or more smooth dmf surfaces in the primary maxillary anteriors
60-71	1 or more teeth	dmfs score ≥ 6 OR 1 or more smooth dmf surfaces in the primary maxillary anteriors

dmfs=decayed, missing, filled surfaces in primary teeth

A prospective cohort study design was selected. This allowed the natural progression of outcome to be observed, a useful approach when the exposure is not common. This was beneficial, as serum 25(OH)D status was considered as the exposure of interest. While technically common, 25(OH)D measurements are not and the majority of the population and their health care providers are unaware of their actual vitamin D concentration. This same design also allows multiple outcomes of a single exposure to be examined and ensures that a temporal sequence can be established (Table 2.3).[12-15]

Although there are several benefits to this type of study design, prospective cohort investigations typically require considerable time and expense.[12] There are also challenges in maintaining cohorts over the period of study and the design does not enable the impact of many other possible exposures to be evaluated.[12,13] A description of the

methods used to minimize participant withdrawals and dropouts appears later in this chapter.

Table 2.3 – Advantages and disadvantages of cohort studies (including this investigation of 25(OH)D, enamel hypoplasia, and ECC)[12,14,15]

Advantages	Disadvantages
• Opportunity to observe the natural history of disease (e.g. DDE, enamel hypoplasia, and ECC) • Important when the exposure may be somewhat uncommon (e.g. prenatal vitamin D (25(OH)D) deficiency) • Allow multiple outcomes to be assessed (e.g. enamel hypoplasia, enamel opacities, ECC, birth outcomes) for a single exposure once it is defined (e.g. prenatal 25(OH)D deficiency) • Guarantees the establishment of a chronological timeline (e.g. 2nd trimester to 12 months of age) • Allows for the calculation of incidence/prevalence of disease (e.g. enamel hypoplasia or ECC) among those either exposed or unexposed (e.g. prenatal 25(OH)D deficiency) to determine relative risk of developing the disease/outcome of interest • Reduced bias	• Not useful for studying rare diseases (However, ECC is all too common) • Limited to investigating only a few exposures as the exposure is the start of the starting point (e.g. prenatal 25(OH)D deficiency) • Costly, time consuming, and observational period may be of long duration (e.g. 2nd trimester to 12 months of age) • Logistically complex • May require a large number of participants • Cohort maintenance and prone to loss to follow-up • Difficulty with missing data and subsequent need to account for missing data in statistical analyses

Population

The target population for this investigation was expectant women residing in Winnipeg, Canada located at latitude 49º 53' North. The majority being of self-declared Aboriginal heritage. Many Aboriginal people live at or below the poverty level.[16,17] It is well known that poverty influences food security and that limited food choice can affect the nutritional status of individuals. There are also other factors besides poverty alone that affect women's food purchasing practices.[18] Therefore, it was important to study this population group to determine the outcomes that nutritional deficiencies in utero could have on the infant. Women were excluded if they had any significant medical conditions that would potentially affect their vitamin D status.

Many Aboriginal expectant mothers in remote northern communities have difficulties in sustaining adequate levels of vitamin D during pregnancy with the prevalence of vitamin D deficiency exceeding 80% in some communities.[6,19] One purpose of this study was to determine whether analogous levels of vitamin D deficiency exist in an urban dwelling Aboriginal population of expectant women. This has not been previously evaluated. Another reason for targeting the Aboriginal community was the high prevalence of ECC witnessed in this population[17,20-23], even those residing in urban centres. Enamel hypoplasia, a risk factor for ECC, is also known to be more prevalent in lower socioeconomic (SES) populations.[24-27]

Sample & Calculation of Sample Size

Determining appropriate sample size is important for study results to have statistical significance upon analysis. Sample size for this study was reviewed by a senior

biostatistician to ensure that the method was best suited for the nature of this research and was further validated using PASS 6.0 (Power Analysis & Sample Size) statistical software. The difficulty with this method of estimation is that very little information exists regarding vitamin D concentrations among Canadian urban populations, more specifically expectant urban women, most of whom are Aboriginals. As the known prevalence of vitamin D deficiency in some northern communities in Manitoba was over 80%[6], a reasonable estimate of prevalence in an urban population was 50%. The limitation of this method of sample size calculation is that it is not appropriate for hypothesis testing.

Initial sample size calculation:

Using established estimation methods, the percent of Aboriginal women in an urban center with vitamin D deficiency was calculated to within ± 10 % confidence intervals with 95% confidence.

Using the formula $n = [1.96^2 p (1 - p)] / interval^2$

$n = [1.96^2 0.5 (1 – 0.5)] / 0.01 = 96$

The sample size calculation was verified using PASS 6.0 software:

Numeric Results from PASS 6.0 Software

Precision	C.C. Confidence Coefficient	N Sample Size	P0 Baseline Proportion
0.10000	0.95105	**96**	0.50000

Population size = 10000

Taking into consideration the likelihood of significant dropouts, withdrawals, and losses to follow-up of study participants in this population, the estimated sample size for participant recruitment was doubled to 200 women. Enrolling this number of women

allowed the project to suffer some loss of the cohort while still maintaining an adequate sample size of statistical significance needed for analysis.

Instrumentation

Collected serum samples were sent to Winnipeg's Health Sciences Centre (HSC), Department of Biochemistry and Genetics Laboratory for laboratory analysis. Each single serum sample was analyzed for 25(OH)D, total calcium, inorganic phosphorus, and alkaline phosphatase. 25(OH)D was assessed using radioimmunoassay using the DiaSorin kit (Stillwater, Minnesota). Reference ranges for metabolites when the study began appear in Table 2.4.

Table 2.4 – Reference ranges for 25(OH)D, calcium, phosphorus, and alkaline phosphatase

	Range of Normal
25(OH)D	35 – 105 nnmol/L (winter)[28] 37 – 200 nmol/L (summer)[28]
Calcium	2.10 – 2.60mmol/L
Phosphorus ≤16 years >16 years	 1.29 – 2.26 mmol/L 0.81 – 1.45 mmol/L
Alkaline Phosphatase ≤17 years >17 years	 59 – 422 U/L 30 – 120 U/L

The prenatal questionnaire was proctored by interview by the principal investigator or a clinic staff member. The questionnaire was developed with input from

senior researchers and those health care professionals with knowledge of this population and was pilot tested with the target population. This survey questionnaire was comprised of seven separate sections, each dealing with a specific theme.

The first section was the "participant profile". It was designed to collect basic demographic information from study participants including their date and place of birth, address, and contact information. In addition, participants were asked whether they consider themselves to be of Aboriginal heritage and if so to further identify whether they were Treaty Status, Non-Treaty Status, Inuit, or Métis.

The "pregnancy and health profile" examined perceived prenatal health, and issues relating to prenatal care and well-being. Inquiries regarding multivitamin use were made, including whether their health care provider recommended multivitamins and whether there was compliance with the recommendation. Women were also asked questions, which would possibly give insight as to their vitamin D and calcium status, including clinical symptoms of hypovitaminosis D. As a main objective of this study was to assess serum vitamin D status, participants were also asked whether they had heard of vitamin D and calcium, what they are important for, and what foods contain vitamin D and calcium. Responses to these questions could prove to be useful for those providing supportive nutritional counseling to women during pregnancy, and to assess the knowledge of the cohort.

The "nutrition profile/food security assessment" theme delved into questions relating to food security, whether dietary practices had changed since learning of their pregnancy, and whether women were aware of the "Healthy Baby" Prenatal Benefit from the Province of Manitoba. This is a very modest financial bonus program for expectant

women of limited means to assist with food purchasing ability. If eligible, recipients begin to receive the benefit during their second trimester of pregnancy and it ceases in the last month of pregnancy.[29] Further questioning focused on the consumption of calcium and vitamin D containing food products (e.g. fortified milk and dairy, fish, eggs, etc.), and whether participants were open to various ways to enhance vitamin D and calcium intake. Few foods naturally contain vitamin D, often leaving fortified milk as the main dietary source for a larger proportion of the population.[30] Intake of calcium and vitamin D containing foodstuffs were assessed to determine if women and their developing offspring were at risk of having insufficient vitamin D concentrations.

Two other sections focused on oral health. The "ECC profile" examined whether expectant mothers had heard of ECC or "baby bottle tooth decay" in the vernacular, how they came to learn about it, whether they believed it to be a normal part of childhood, and what they believed were its causes. It also asked mothers whether they believed ECC was preventable. Participant's knowledge of caring for an infant's teeth, including when brushing should first be initiated, when the first dental visit should occur, and issues relating to breast and bottle feeding were assessed. The "maternal oral health profile" assessed dental attendance and whether participants were experiencing dental problems.

The "exposure to sunlight" theme evaluated participant exposure to sunlight during summer months for endogenous production, amount of sun exposure received, and duration of sun exposure. Use of sunscreens, clothing, and insect repellants was also reviewed.

The final theme, the "family & financial profile" recorded their relational status, number of persons in the household, highest level of education, and issues related to

annual income, to establish a socioeconomic profile. This was necessary so that the analysis could control for low SES and related issues as these may influence maternal health and nutrition, enamel hypoplasia, and ECC.

Lactose intolerance is a known issue within this population.[19,31] Therefore, assessing milk consumption alone may not be the most beneficial method of determining risk of inadequate or low vitamin D. Other foods were considered to determine the potential risk for vitamin D and calcium deficiencies. Foods containing vitamin D include fish, eggs, liver, and fortified dairy products including milk and cheese.[32] Calcium rich foods are usually dairy-based, although many other foods contain calcium[32], including some green vegetables. Daily recommendations (adequate intake) of vitamin D and calcium intake during pregnancy when this study began called for 5-10 ug/day (200-400 IU/day) and 1000 mg/day respectively.[32-34] For instance, 1 cup of milk contains approximately 2.3 ug of vitamin D and 300 mg of calcium.[32] However, controversy surrounding the recommended dietary allowances (RDA) for vitamin D exist and the real RDA remains obscure although there is growing consensus that current daily recommendations are set too low.[34-36]

The final component in the research protocol was a **blinded assessment** of the integrity of the primary teeth that were erupted into the infant's oral cavity. The principal investigator served as the dental examiner and was blinded to the prenatal vitamin D level of each infant's mother. The infant dental examination involved an assessment of the primary dentition with particular focus on the maxillary incisors (i.e. those teeth predominantly affected by ECC and S-ECC). Digital photographs were taken when possible. The working definition of enamel hypoplasia used for this study was "a defect

involving the surface of the enamel and associated with a reduced localized thickness of enamel"[7], either as pits or grooves. An index for enamel hypoplasia and other DDE, including enamel opacities, exists and served as references for the assessment of enamel hypoplasia.[7] Specific locations of demarcated opacities, diffuse opacities and hypoplastic defects of the primary teeth were recorded. Both the presence and absence of enamel hypoplasia and opacities, or a combination of the two, was recorded for each erupted tooth. In addition, each infant was categorized as having any evidence of enamel hypoplasia in the entire dentition as well as an overall assessment of whether the child had any developmental defects including both hypoplasia and opacities.

Each tooth was assessed for dental caries following the established research protocol for infants and preschool aged children.[8] While several studies have waited for the cavitation of enamel or dentin to diagnose caries, this study also followed new recommendations to begin to classify early white spot lesions as caries.[8] Children were diagnosed with ECC and S-ECC based upon established case definitions listed in Table 2.2.[8,37] An overall deft score and individual dt score was determined for each infant.

The terms incidence and prevalence are used to describe the occurrence of disease or specific health states in a population. While often used interchangeably, incidence specifically refers to the number of new cases of a disease or health outcome of interest during a particular period of time while prevalence refers to the number of affected individuals in a population at one specific point in time. In reality, the period of time selected to perform assessments for new cases of a health outcome in a cohort study is arbitrary, but is still referred to as cumulative incidence. For the purpose of this prospective birth cohort investigation the time period selected to determine whether

infants had evidence of enamel hypoplasia and ECC in erupted primary teeth was 12 months of age. As ECC only occurs after primary teeth begin to erupt into the oral cavity the incidence of ECC in the study cohort was reported as they were in fact new cases of caries being observed. This is despite the fact that infants underwent only one dental examination. Meanwhile as enamel hypoplasia involving the primary maxillary incisors predominantly develops prior to birth and the eruption of primary teeth it may be argued that since infant dental exams were performed at 12 months of age that it was really prevalence that was determined.

A follow-up questionnaire was also administered at the time of the infant dental examination. This interviewed questionnaire was proctored by the principal investigator. It consisted of several sections including the infant's date of birth, age, sex, along with the required contact information. The "pregnancy and delivery" profile recorded information relating to the infant's birth weight, whether the mother had gestational diabetes, whether the child was premature, the child's health, and use of medications.

The "primary tooth" profile recorded the age, in months, of the eruption of the first primary tooth. This was of interest as altered vitamin D states may delay dental eruption.[21,38] This section also questioned mothers on whether they believed their child had any noticeable dental problems, whether the child had already been to visit the dentist, and oral hygiene practices.

Additional information regarding infant feeding practices, including breastfeeding, bottle-feeding, and training cup use was assessed in another section of the questionnaire, the "infant feeding" profile. Some of the variables collected included the duration of breastfeeding and bottle-feeding, bedtime bottle use, and contents in the bottle

and training cup. In addition, each mother was asked when solid foods were introduced to the child and the frequency of sugary snacks.

The final section of the follow-up questionnaire included variables such as the number of people in the home, whether the mother participated in "Healthy Baby" community programming, received the "Healthy Baby" provincial benefit during pregnancy, and received government assistance.

Implementation

Women seeking prenatal care at various core area community health clinics were invited to participate. Sites serving as centers for the recruitment of study volunteers included the Outpatient Department of Women's Hospital, Health Action Centre, and Mount Carmel Clinic. Women's Hospital is situated at 735 NotreDame Avenue and is part of Winnipeg's Health Sciences Centre (HSC). Health Action Centre, a primary health clinic in the Downtown neighbourhood of Winnipeg, is situated at 425 Elgin Avenue and is also affiliated with HSC. Mount Carmel Clinic, another primary health clinic in the Point Douglas community of Winnipeg, is situated at 886 Main Street. All three clinics are known to serve many members of the Aboriginal population. The sample for this proposed research was obtained through contacts made by primary care obstetricians and family physicians, prenatal nurses, clinic staff, and other health professionals providing prenatal care at these clinics.

The recruitment of research participants commenced during the summer of 2002. The first participant was recruited from Health Action Centre in June 2002. The first participant from the Women's Outpatient Department (OPD) of Health Sciences Centre

was recruited in July of that same year, while recruitment began in September 2002 at Mount Carmel Clinic. Participants were recruited by either existing clinical staff, including nursing staff, physicians, or clerical team members during one of the prenatal care visits, or by the principal investigator (PI) who was present at the site. Informed consent was obtained by clinic staff at the two primary health clinic sites (Mount Carmel Clinic and Health Action Centre) while the PI obtained informed consent from all participants at the Women's OPD. Recruitment into this study closed January 2005.

Serum sampling was utilized to assess prenatal circulating levels of 25(OH)D, calcium, phosphorus, and alkaline phosphatase. Blood samples were drawn by the hospital phlebotomist, the primary care physician, or clinic nurse. The selected period for serum collection was during the second trimester of pregnancy and was planned to coincide with routine prenatal serum screenings to minimize needless punctures. However, in some instances, serum sampling was obtained from participants during the early portion of the third trimester.

Serum analysis was conducted by the Department of Clinical Chemistry, HSC. Analysis included 25(OH)D, total serum calcium, inorganic phosphorus, and alkaline phosphatase (Table 2.4). A preprinted laboratory requisition form was created to facilitate this process. Specimens were forwarded to the laboratory via current transportation and courier arrangements already established between the laboratory and participating health centres. Laboratory reports were forwarded to Dr. Michael E.K. Moffatt to keep the principal investigator blinded to each participant's 25(OH)D status. For ethical reasons, primary physicians were contacted by Dr. Moffatt when a participant's 25(OH)D concentrations was below 25 nmol/L.

The questionnaire was administered during this same prenatal clinic visit by either the principal investigator or an existing staff at the two primary care community clinic sites. The principal investigator performed all of the interviews at Women's Hospital, HSC. The questionnaire was designed to take 30 to 35 minutes to complete.

When the child neared 12 months of age participants were contacted to schedule an assessment of their infant's primary dentition. Dental clinics at Mount Carmel Clinic and Health Action Centre served as sites for the infant dental examination for participants who were originally recruited from those clinic sites. Participants enrolled into this prospective study at Women's Hospital, HSC brought their children to the Manitoba Institute of Child Health adjacent to HSC for the dental evaluation. When possible, digital images of the primary maxillary incisors were taken during the dental examination depending on consent from the mother and the cooperation level of the child. The intent was that this might prove useful in identifying the differential patterns of enamel hypoplasia. The follow-up questionnaire was completed at the start of this scheduled visit. The questionnaire preceded the dental examination in case the child became restless during and following the dental assessment. All exams were performed in a knee-to-knee position.

Cohort retention posed a significant challenge. Follow-up to maintain this cohort involved several contacts by mail, phone, or other means between the researcher, participants, and the clinics from the enrollment stage until the time of the infant's dental examination. The ADT hospital patient database at HSC and Health Action Centre and the clinic database at Mount Carmel Clinic were used to help locate participants who had moved or changed their phone numbers during the study period.

An honorarium was given to each participant to compensate them for out of pocket expenses incurred and to serve as a small incentive for remaining in the study. This honorarium was added on the recommendation of various senior researchers and community health professionals who had experience working with our target population. $15.00 was given at the end of the enrollment visit and another $15.00 following the infant dental exam. This amount was appropriate for the nature of each participant's role.

Ethics

This study protocol was reviewed and approved by the Health Research Ethics Board, University of Manitoba, and the Health Sciences Centre Research Impact Committee. The management of Health Action Centre, and Mount Carmel Clinic also reviewed the proposed research plan and granted permission to recruit study participants on their premises. The Assembly of Manitoba Chiefs was also notified of this research project. The participant information and consent form was approved along with the survey tools. Recruitment posters and follow-up contact letters to enrolled participants were also granted approval for use by the Health Research Ethics Board. All participants gave their consent to take part in this prospective study.

Analysis

Data were entered into a Microsoft Office Access database. This was then transformed into an Excel spreadsheet and was then imported into existing statistical software packages. The packages selected for this study were NCSS Version 2007 (Kaysville, Utah) and SPSS 17.0 (Chicago, Illinois). Statistical analysis included basic descriptive

statistics including frequencies and mean values along with standard deviations (S.D.). Bivariate analysis included Chi Square Analysis, t tests, correlation, Poisson regression, and Analysis of Variance (ANOVA). Multiple logistic regression and multiple regression analyses were also employed. A p value of $\leq .05$ was statistically significant.

Key study variables, both independent (predictor) and dependent (outcome) are listed in Table 2.5. Specific descriptive statistics were performed for mean concentrations of serum metabolites including 25(OH)D, total calcium, alkaline phosphatase, and phosphorus. In addition basic descriptive statistics were employed to determine the prevalence of vitamin D deficiency during pregnancy in this cohort according to values used by the Department of Clinical Chemistry and those purported thresholds in the scientific literature. The prevalence of enamel hypoplasia and incidence of ECC were reported along with the frequency of the various forms of enamel opacities and hypoplasia observed.

Table 2.5 – Sources of independent and dependent variables and key independent and key outcome variables

	Sources of all Variables	**Key Variables**
Independent/Predictor	Prenatal questionnaire Serum analysis Follow-up questionnaire	Maternal 25(OH)D (continuous) Maternal 25(OH)D deficiency (dichotomous)
Dependent/Outcome	Infant dental examination	Enamel hypoplasia (dichotomous) DDE (categorical) ECC (dichotomous) deft and dt (continuous) (rate of decay in primary teeth)

Numerous bivariate associations were investigated. However, major associations that were assessed included the relationship between 25(OH)D concentrations and prenatal factors, 25(OH)D status and the presence of enamel hypoplasia, 25(OH)D and ECC, correlations between the serum concentrations of 25(OH)D, total calcium, inorganic phosphorus, and alkaline phosphatase, and the relationship between enamel hypoplasia and ECC. In order to investigate the relationship between 25(OH)D and enamel hypoplasia several tests were employed. For instance, t tests were used to compare the 25(OH)D levels of mothers and the presence or absence of enamel hypoplasia. Chi Square testing was used to compare the presence or absence of 25(OH)D deficiency with enamel hypoplasia (dichotomous). As both the variables for enamel hypoplasia and ECC were categorical, Chi Square analysis was used to investigate the

relationship between the two. Poisson regression was used to assess the relationship between infant caries rates (dt score) and maternal 25(OH)D levels.

Multiple regression was used to identify those variables that were significantly associated with 25(OH)D levels and logistic regression analysis was performed to identify those variables strongly associated with the presence of enamel hypoplasia and ECC. Additionally, Poisson regression was also used to identify variables associated with the untreated dental caries rate (dt).

Since the number of maternal-infant dyads taking part in this prospective cohort was small numerous logistic and multiple regression analyses were undertaken to prevent over-fitting of the models and avoid potential false associations. These small models for 25(OH)D were based upon themes of serum metabolites, prenatal health and health care, SES, and factors that affect endogenous and exogenous vitamin D attainment. Meanwhile, small regression models for enamel hypoplasia focused on serum metabolites, maternal awareness of calcium and vitamin D, prenatal care and diet, SES and ethnicity, and infant birth characteristics and health. Lastly, models for ECC were based upon themes of serum metabolites, factors influencing vitamin D status, infant feeding practices, SES, and dental status and dental behaviours.

For continuous variables in the regression models, odds ratios and confidence intervals were calculated to reflect a change in one standard deviation unit of the variable. For instance, odds ratios for 25(OH)D and calcium reflected a one unit standard deviation change in their measure, nmol/L.

Funding

Operating grants from the several agencies were awarded to conduct this research. These included the Manitoba Medical Service Foundation (University of Manitoba Grant Number 385 2339 03), the Children's Hospital Foundation of Manitoba Inc. and Manitoba Institute of Child Health, the Dentistry Canada Fund (University of Manitoba Grant Number 387 2339 02), and the Dean's Research Funds, Faculty of Dentistry, University of Manitoba (University of Manitoba Grant Number 611 2335 31). Additional salary support for the principal investigator came in the form of Postdoctoral Fellowship funding from the Children's Hospital Foundation of Manitoba Inc and the Manitoba Institute of Child Health. Further funding was provided through a CIHR Strategic Training Fellowship in the Canadian Child Health Clinician Scientist Program funded by the SickKids Foundation, BC Research Institute for Children and Women's Health, and the Manitoba Institute of Child Health.

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Chapter 3 – Descriptive Results

A total of 207 women were enrolled into this study between 2002 and 2005 (Table 3.1), from the three clinic sites. The majority of research participants were enrolled from the Women's OPD at the Health Sciences Centre (HSC).

Table 3.1 – Distribution of participants recruited during pregnancy

Clinic	**Number**	**%**
Women's Out Patient Department (OPD) Health Sciences Centre	170	82.1
Health Action Centre	24	11.6
Mount Carmel Clinic	13	6.3
Total	207	100.0

Demographic Information

The mean age of women at the time of enrollment in this study was 19.0 ± 4.7 years and ranged from 14 to 37 years. The average age of participants enrolled at Health Action Centre was 22.8 ± 4.8 years, 21.9 ± 4.6 at Mount Carmel Clinic, and 17.7 ± 4.2 years at Women's Hospital, Health Sciences Centre. Participants enrolled at the Women's Hospital clinic site were significantly younger than women enrolled at the other sites ($p<.001$). The distribution of participants according to their ethnic background appears in the following Table 3.2. There were no apparent differences in the ages of women according to ethnic background. A total of 145 women indicated that they were born in Winnipeg, while 190/205 participants (92.7%) reported that they currently resided within city limits.

Table 3.2 – Self-declared heritage of participants

Heritage	**Number**	**%**
Status Indian	128	61.8
Non Status Indian	16	7.7
Métis	41	19.8
Inuit	1	0.5
Other	20	9.7
Missing	1	0.5
Total	207	100.0

A total of 125 participants (60.9%) indicated that this was their first pregnancy (i.e. primigravid). While not all of the women who reported a previous pregnancy went to term with their past pregnancy, 69 women indicated that they had one or more children. These participants had on average 2.2 ± 1.8 children (range 1 – 8) while the median number was one child.

Prenatal Health Status

When asked how they would rate their health during the current pregnancy, 130 women indicated that it was good (63.4%), 70 indicated average health (34.2%) while 5 indicated that their health was poor (2.4%). Nearly one-third (32.2%) of participants indicated that they were worried about their health during this pregnancy. The majority of participants indicated that prenatal care was important (199/205, 97.1%).

Table 3.3 reports data on the use and views of vitamins and supplements by participants. While the majority of women admitted to taking vitamins during their current pregnancy, only 74 (37.2%) were taking them daily even though 92.2% indicated that they felt they should be taking vitamins for good prenatal health. When asked if they had ever heard of vitamin D, 131 respondents (63.9%) indicated that they had, but only

24.2% (40/165) correctly indicated what vitamin D was important for. Fewer women were familiar with dietary sources of vitamin D as only 21.8% correctly identified foods that contained vitamin D. However, participants did have a better understanding of calcium as 72.1% correctly identified foods that contained calcium.

Vitamin D is not only associated with bone health but is also needed for muscle strength and balance. Participants were asked whether they had any signs of bone pain or muscle weakness as a proxy for possible vitamin D deficiency. Only 19.4% of women indicated they suffered from bone pain at the time of the proctored interview while 24.8% reported that their arms or legs felt weak. Only 31 women (15.1%) indicated they had trouble walking, another 9.7% had problems standing, and 11.2% had reported suffering from hip problems.

Table 3.3 – Responses to questions on vitamins and supplements

Question	**Number**	**Valid %**
Did your doctor recommend vitamins during this pregnancy?		
Yes	181	87.9
No	25	12.1
Are you taking vitamins during pregnancy?		
Yes	144	70.6
No	60	29.4
How often are you taking vitamins this pregnancy?		
Often (once a day or more)	74	37.2
Sometimes (once a week or more, but less than once a day)	69	34.7
Rarely (less than once a week)	9	4.5
Never	47	23.6
Do you feel you should take vitamins for good prenatal health?		
Yes	190	92.2
No	3	1.5
Unsure	13	6.3
Would you take vitamins if you were not pregnant?		
Yes	92	45.1
No	105	51.5
Unsure	7	3.4

Question	Number	Valid %
Do you take calcium supplements?		
Yes	11	5.3
No	192	93.2
Unsure	3	1.5

Prenatal Nutrition and Diet

Overall, 158 participants (77.1%) indicated that they thought the foods they were eating were healthy enough for the pregnancy. When asked how they had changed the way they ate since finding out they were pregnant, 66.8% indicated they were eating better and 30.2% the same as before they knew they were pregnant. While the majority of participants indicated they were able to buy all the foods they needed to be healthy during pregnancy, 9.3% reported that they were not able to do so.

Nearly half (45.9%) of the women enrolled indicated they attended "Healthy Baby" Community Support Programs[1] like Healthy Start for Mom & Me. While the majority (88.4%) had heard of the Healthy Baby Prenatal Benefit from the Province of Manitoba only 97 mothers (47.6%) reported receiving this benefit at the time of recruitment into the investigation. Overall, the majority of participants in this study would likely have been eligible for this benefit.

The questionnaire asked participants whether milk or dairy products upset their stomach as a proxy measure for lactose intolerance. A total of 44 women (21.4%) indicated that consuming these food products did upset their stomach. Most of these same women (85.0%) reported consuming less milk and dairy. However, 84.5% of the entire cohort reported that they had been consuming more milk and dairy during the pregnancy.

Overall, 50.0% reported that they drank milk often, meaning that they drank milk daily, whereas, 33.0% drank milk more than once a week, but not daily. The remainder, were either infrequent (9.7%) or non-milk drinkers (7.3%). Responses to other food frequency intakes appear in Table 3.4.

Table 3.4 – Distribution of responses to food intake questions

Food Item	**Frequency of Consumption (Valid %)**			
	Often	**Sometimes**	**Rarely**	**Never**
Fish	1 (0.5)	38 (18.5)	93 (45.2)	74 (35.9)
Liver	-	9 (4.4)	22 (10.7)	175 (84.9)
Animal organ meat	-	2 (1.0)	5 (2.4)	199 (96.6)
Cook with animal bones	3 (1.5)	43 (20.9)	73 (35.4)	87 (42.2)
Eggs	29 (14.2)	134 (65.7)	32 (15.7)	9 (4.4)
Margarine	113 (54.9)	82 (39.8)	7 (3.4)	4 (1.9)
Milk	129 (62.6)	58 (28.2)	9 (4.4)	10 (4.9)
Cheese	54 (26.5)	110 (53.9)	36 (17.7)	4 (1.9)
Yogurt	28 (13.6)	82 (39.8)	46 (22.3)	50 (24.3)
Sour cream	5 (2.4)	59 (28.6)	71 (34.5)	71 (34.5)
Ice cream	17 (8.3)	107 (51.9)	61 (29.6)	21 (10.2)
Green vegetables	53 (25.7)	102 (49.5)	32 (15.5)	19 (9.2)
Broccoli	7 (3.4)	75 (36.6)	61 (29.8)	62 (30.2)
Calcium-rich orange juice	43 (21.0)	73 (35.6)	24 (11.7)	65 (31.7)

Maternal Awareness of Early Childhood Caries

When asked, 77.2% of women indicated that they had heard of Early Childhood Caries (ECC) or its antecedent terms “baby-bottle tooth decay”, “nursing caries”, or “tooth rot”. Forty-eight women (23.5%) thought that ECC was a normal part of childhood while another 36 participants (17.7%) were uncertain. Among those with children, 32.9% (23) indicated that some of their children had experienced ECC, while 65.9% (135) of the entire maternal cohort knew children who had suffered from ECC. Further, 23.9% of women with children (17/71) indicated a child of theirs required dental surgery under general anesthesia. Surprisingly, 88.4% thought ECC could be prevented.

When women were asked when children should see the dentist for the first time only 6.8% responded by the eruption of the first primary tooth, which coincides with current professional recommendations. However, 48.3% indicated that this should occur while the child was one year of age (12-23 months).

Maternal Oral Health

Enrolled participants were asked a select group of questions relating to maternal oral health since dental examinations of mothers were not part of the planned research protocol. Most women (90.3%) reported that they believed their dental health was important during pregnancy. Unfortunately, only 38.5% rated their own current dental health to be good. The majority of participants indicated that they had been to the dentist within the last year (71.4%), while another 14.1% indicated that their last dental appointment was in the past two years. Among those who had never been to the dentist or who had not been seen in the last two years, 21.4% indicated that it was due to a lack of

finances or lack of dental insurance while another 17.9% were either afraid of the dentist or had past bad dental experiences.

In general, 73.3% reported that they usually visited their dentist at least once per year. When asked if they had any existing dental concerns 36.1% (73/202) reported that they currently had dental problems. Forty-two participants indicated that they had cavities, 19 reported bleeding gums, and another 18 were experiencing dental pain.

Maternal Sun Exposure

The prenatal interview included questions relating to sun exposure. Nearly all respondents (91.8%) indicated that they liked to spend time outdoors during the summer, while 78.1% indicated they enjoyed being outside in the sunshine. The majority of participants replied that when outside, their average duration outdoors was one to four hours (62.1%). Early afternoon and late afternoon were the times of day most women spent outdoors. Nearly two-thirds (64.6%) reported that they felt healthier or better after spending time outside, but only 27.0% (55/204) said they would spend more time outdoors than usual if pregnant during the summer period.

Family and Financial Profile

The final section of the questionnaire dealt with family and financial information relating to the participant. The majority of women identified themselves as being single (62.6%), 3.4% were married, and another 33.0% indicated that they were in common-law relationships. The average number of individuals residing in the participant's home at the

time of recruitment was 4.0 ± 2.2 persons. Most (92.2%) had not completed high school and only 2.3% had pursued some post-secondary education.

Serum Results

Serum analysis constituted an essential component of this study. Enrolled participants had serum samples drawn during pregnancy to assess circulating levels of 25(OH)D (nmol/L), total calcium (mmol/L), inorganic phosphorus (mmol/L), and alkaline phosphatase (U/L). Complete laboratory results were available for a total of 200 of the women enrolled into this study. Thus, results from seven women were missing. Some samples were lost due to delays in sending samples to the Department of Biochemistry and Genetics Laboratory or a missed venipuncture (Table 3.5). However, serum 25(OH)D was the main metabolite of interest and results were available for 200 of the women enrolled into the study.

All reports were sent to one of the co-investigators (Dr. Michael Moffatt) rather than to the principal investigator in order to ensure that the primary investigator was blinded to the 25(OH)D status of the mother. This was important so as not to bias or influence the future dental examination of the infant at 12 months of age.

Table 3.5 – Frequency of participants with completed serological assays

Assay	**Number of Participants Completed (%)**	**Number Missing (%)**
25(OH)D (nmol/L)	200 (96.6)	7 (3.4)
Calcium (mmol/L)	198 (95.6)	9 (4.4)
Inorganic Phosphorus (mmol/L)	200 (96.6)	7 (3.4)
Alkaline Phosphatase (U/L)	199 (96.1)	8 (3.9)

Table 3.6 reports mean concentrations along with their respective standard deviations (S.D.), ranges, and median values for the various metabolites of interest. The mean 25(OH)D level for the entire cohort was 48.1 ± 24.4 nmol/L (range 4.7 to 145 nmol/L) while the median value was 43.0 nmol/L.

Various thresholds have been used in the scientific literature to classify 25(OH)D levels. Table 3.7 reports the distribution of participants according to these previous and current thresholds. Nearly 40% of the maternal cohort had prenatal 25(OH)D values that were in the deficient range according to reference values used by the Department of Biochemistry and Genetics Laboratory. When the current threshold proposed in the scientific literature to denote vitamin D deficiency was applied 180 participants were found to have circulating 25(OH)D levels below 80 nmol/L.

Table 3.6– Prenatal serum concentrations of metabolites of interest

Assay	Range of Normal*	Number	Mean ± S.D.	Range	Median
25(OH)D (nmol/L)	35 – 105 (winter) 37 – 200 (summer)	200	48.1 ± 24.4	4.7 – 145	43.0
Calcium (mmol/L)	2.10 – 2.60	198	2.25 ± 0.10	2.01 – 2.57	2.24
Phosphate (mmol/L)	1.29 – 2.26 (<17 years) 0.81 – 1.45 (> 16 years)	200	1.15 ± 0.19	0.69 – 2.28	1.16
Alkaline Phosphatase (U/L)	59 – 422 (≤ 17 years) 30 – 120 (> 17 years)	200	97.9 ± 51.8	34.0 – 372.0	80.0

*Department of Biochemistry and Genetics Laboratory reference values

Table 3.7 – Distribution of participants by vitamin D thresholds

25(OH)D Threshold	Number of Participants	Valid %
< 25 nmol/L	29	14.5
≤ 35 nmol/L	70	35.0
< 37.5 nmol/L	79	39.5
≤ 40 nmol/L	93	46.5
< 80 nmol/L	180	90.0

Infant Questionnaire

135 infants returned with their mother or primary caregiver for the second phase of this prospective study. Overall, 64.3% (n=133) of the cohort was maintained while 74 women, along with their infant(s) were lost to follow-up. Two of the mothers continuing in the study had twins, thus 135 infants participated in this phase of the investigation. The average age was 16.1 ± 7.4 months and ranged from 11 to 46 months. The median age

was 13 months. Males accounted for 55.6% of the infants. Women lost to follow-up did not significantly differ from those who remained in the study with respect to their mean age (p=.24), level of education (p=.74), and whether or not they were of Aboriginal heritage (p=.24).

The mean birth weight was 3489.63 ± 560.85 grams and ranged from 1814.4 to 5159.7 grams. The median birth weight was 3486.99 grams. Seventeen children (12.7%) were reported to have been born prematurely, but when applying the definition for low birth weight (< 2500 grams) only six infants (4.6%) of the cohort were considered to have had low birth weight. A majority of mothers and caregivers reported that their child was in very good health (56.0%), and only eight indicated that their child was in fair health.

A total of 27.8% of parents and caregivers indicated that their child had experienced a medical problem. Seizures were reported for four children, while caregivers indicated that 16 children had asthma. It is likely that these infants really did not have asthma, but may have actually had wheezing which is an early indicator for the onset of asthma in children.

Infant Feeding Practices

Respondents indicated that nearly three quarters (73.5% (97/132)) of infants were breastfed at some point. Of those who were breastfed, only 9.2% were still being breastfed at the time of the follow-up infant examination. Among those children who had stopped being breastfed, the average weaning age was 3.6 ± 4.2 months and ranged from half a week to 24 months of age. The median age of weaning from the breast was 2.0 months.

A total of 77 caregivers responded to the question of whether their infant had ever been breast fed to sleep. Of these, 70.1% (54) indicated that they had done so. Another 53 caregivers provided responses to the frequency of breast feeding their child to sleep with the majority (52.8%) stating that this was a usual practice. Results from the infant questionnaire also determined that many children (39/69) were breast fed on demand, in that the child had the breast whenever they wanted it. It appeared that the majority of children who were breastfed (68.4%) received vitamin D drops.

The majority of infants in this study were bottle-fed (96.3%) with only five respondents indicating that their child did not receive a bottle. One child was tube-fed for medical reasons and was neither breast nor bottle fed. A considerable proportion of infants were given the bottle from birth (40.5%) but the average infant age when the bottle was first introduced was 1.8 ± 2.8 months (median 0.5 months) ranging from birth to 15 months of age. The majority of infants who were bottle fed were still taking the bottle at the time of the study as only 21 caregivers indicated that their child was weaned from the bottle. Among those who were already weaned from the bottle, the average weaning age was 14.2 ± 4.8 months.

The majority of participants indicated that their infant had gone to bed with a bottle (82.3%) and that the frequency of this practice was usually (62.3%). Fortunately, only 39.8% of caregivers indicated that their infant received the bottle whenever they demanded it.

Common contents in the bottle included formula (93.9%), water (90.0%), cow milk (88.5%), and apple juice (74.6%). Other contents included human breast milk (39.2%), tea (13.1%), and pop (9.2%). Interestingly, 40.6% of respondents indicated that

their infant had sugar added to the bottle. Overall, the beverage that was most frequent in the bottle was milk (74.8%).

Nearly all (94.0%) infants participating in this phase of the study had used a sippy cup with 89.3% still using a sippy cup at the time of this phase of the investigation. A total of 84 infants had used a soother, but fortunately, only 8.6% of caregivers reported that they dipped the soother into sweets before giving it to their child.

Essentially, all of the infants (98.5%) had been introduced to solid foods with the average age when they first received solid food being 6.7 ± 2.8 months. The majority of infants were receiving sugary snacks on a daily basis (123/133), but most were receiving only one sugary snack per day (61.7%).

Infant Family Life

The average number of individuals in the home, including the parent or caregiver was 4.4 ± 2.0 persons (median 4.0). The majority lived independently (64.2%), while 34.3% resided with family. A total of 94 women indicated that they had moved during pregnancy or since their child was born and the median number of times a mother moved during pregnancy was once. However, 19.4% moved more than 2 times during pregnancy.

One third (33.6%) of participants in this study did not attend community-based Healthy Baby Community Support programs. Further, only 74.4% of participants received the Healthy Baby Prenatal Benefit. Nearly one third of caregivers (34.3%) reported that they had utilized a food-bank since their child was born while 64.2%

indicated that they were receiving government assistance. The majority were earning less than $1,000 each month (58.2%) with only 11.9% earning above $1,500 per month.

Dental History of Mother

The majority of caregivers (63.3%) reported that they had not visited their dentist during pregnancy, but 53.1% had experienced dental problems during pregnancy or since their infant was born. Dental problems in descending order included cavities (55.4%), extractions (20.0%), dental pain (12.3%), wisdom teeth (9.2%), gum problems (1.5%), and infection (1.5%).

Infant Oral Health Profile

The average age given for the eruption of the first primary tooth for infants in this cohort was 5.5 ± 2.3 months of age, and ranged from birth to 12 months of age with the median age being 5.3 months. When asked, only two caregivers (1.5%) indicated that their child's teeth did not look healthy when they erupted into the oral cavity and another 30 caregivers (22.2%) believed their child had dental problems. The main types of problems identified included decay (46.9%), chipped or discoloured teeth (15.6%), and crooked baby teeth (18.8%). Even though 22.2% of caregivers indicated that their child had a dental problem only 15 children (11.1%) had been to the dentist for a first visit. Among those who had already been to the dentist, the main reason was due to cavities and not for a check-up examination (58.3% vs. 41.7%).

While 88% of caregivers indicated that they had started to clean their child's teeth and mouth, the age when this needed behaviour was initiated ranged from birth to 30

months of age. The average age when cleaning began was 9.0 ± 5.5 months of age while the median age was 8.0 months. Half of the caregivers (49.6%) indicated that they used toothpaste to clean their child's teeth. Mothers were the primary caregiver looking after the child's oral hygiene (83.6%). None of the children had received fluoride drops during infancy.

The infant oral examination provided an opportunity to identify whether children exhibited developmental defects of enamel (DDEs), including enamel hypoplasia. In accordance with the modified DDE index[2], normal teeth were given the code 0 while opacities were categorized from 1 to 6. Enamel hypoplasia was rated with codes from 7 to 9 while combinations of opacities and hypoplasia were scored as A, B, C or D. Frequencies appear in Tables 3.8 and 3.9.

A figure of the primary dentition and corresponding tooth numbers appears in Figure 3.1. Those primary maxillary teeth with the highest prevalence of DDEs were the primary maxillary central incisors. Similarly, more cases of DDEs were observed in the primary maxillary incisors. Primary mandibular teeth that had the greatest number of reported cases of opacities and hypoplasia were the incisors. The vast majority of DDEs were identified on the buccal surfaces of primary teeth, particularly the maxillary incisors. Among the children who were examined, 20 had DDEs on the buccal surface of tooth #52 (13 opacities, 4 hypoplasia, and 3 combinations of opacities and hypoplasia). 45 primary maxillary right central incisors (#51) were found to have DDEs on the buccal surface (35 opacities and 9 hypoplasia). Meanwhile, 25 children had DDEs on the buccal surface of tooth #62 (17 opacities, 7 hypoplasia, and 1 combination of opacity and hypoplasia), while 47 children had DDEs on the buccal surface of tooth #61 (36 opacities

and 11 hypoplasia). DDEs were also present on the buccal surfaces of the primary mandibular incisors, but were less common; #72 (1 opacity and 1 hypoplasia), #71 (5 opacities and 5 hypoplasia), #81 (3 opacities and 5 hypoplasia), and #82 (1 hypoplasia).

Figure 3.1 – FDI primary teeth codes (WHO Oral Health Surveys)

Enamel hypoplasia was identified in 21.6% of the cohort (29/134). The main forms of hypoplasia in the primary maxillary teeth included pits, followed by missing enamel. In the primary mandibular teeth, pits, missing enamel, and other enamel defects were observed. The distribution of enamel hypoplasia appears in Tables 3.8 and 3.9. The majority of the enamel hypoplastic defects in the maxillary arch (32/34) were found on the primary maxillary incisors. Six children had hypoplastic pits on tooth #51 while eight had pits on #61. Meanwhile, enamel hypoplasia in the mandibular arch was limited to the primary central incisors.

Table 3.8 – Frequency of DDEs by primary maxillary teeth

Modified DDE Index Code	**Number of Children Affected by Primary Tooth Number**									
	55	**54**	**53**	**52**	**51**	**61**	**62**	**63**	**64**	**65**
0 (Normal)	13	71	35	104	88	82	102	33	74	14
Opacity										
1 (White-cream)		1		5	5	11	5	1		
2 (Yellow-brown)				2	4	1	2			
3 (Lines)			1	2	6	5	2			
4 (Patchy)			4	3	18	17	6	5	1	
5 (Confluent)										
6 (Confluent/patchy)										
Hypoplasia										
7 (Pits)		1		3	6	8	7	1		
8 (Missing enamel)				1	3	2				
9 (Other defect)					1	1				
Combinations										
A										
B				2						
C								1		
D										

Table 3.9 – Frequency of DDEs by primary mandibular teeth

Modified DDE Index Code	**Number of Children Affected by Primary Tooth Number**									
	75	**74**	**73**	**72**	**71**	**81**	**82**	**83**	**84**	**85**
0 (Normal)	15	69	33	109	121	120	112	33	64	15
Opacity										
1 (White-cream)	1				2	2		1		1
2 (Yellow-brown)					4	4				
3 (Lines)				1						
4 (Patchy)			2					3		
5 (Confluent)										
6 (Confluent/patchy)										
Hypoplasia										
7 (Pits)					2	1				
8 (Missing enamel)				1	2	3	1			
9 (Other defect)					3	2				
Combinations										
A										
B										
C										
D										

Caries rates for children in this prospective cohort appear in Table 3.10. Overall, 31 infants (23.0%) had ECC when applying the current case definition and when limited to cavitated enamel lesions.[3] However, when white spots were considered, 49 infants (36.3%) had ECC. Because of the young ages of these children essentially all were classified as having the more severe form of ECC termed Severe Early Childhood Caries (S-ECC). The average d for children with ECC, including white spot lesions was 3.44 ± 2.04.

Table 3.10 – Mean d, e, f, and deft for infants

Caries Rate	Number	Mean ± S.D.	Range	Median
d	134	1.23 ± 2.05 (3.44 ± 2.04*)	0 – 10	0
e	134	0.07 ± 0.49	0 – 4	0
f	134	0.17 ± 1.11	0 – 9	0
deft	134	1.47 ± 2.80	0-17	0

*includes white spot lesions

The primary maxillary teeth with the highest prevalence of caries were the primary maxillary incisors (Table 3.11). Among the children who were examined, 92 were found to have sound tooth surfaces on tooth #52, while 17 had initial white spot lesions that were not cavitated. Six children had enamel lesions, six had lesions involving dentin, one had already received a restoration on this tooth while three children had this tooth extracted. Ninety-nine children did not have any caries on the primary maxillary right central incisor (#51), but 18 had white spot lesions, six had enamel lesions, eight had caries involving dentin, and two had this tooth extracted. A similar pattern was witnessed for tooth #61 as 17 children had white spot lesions, seven had caries involving

enamel, ten had caries into dentin, and two had this tooth extracted. Ninety-seven infants had sound surfaces on tooth #62, while 15 had white spot lesions, seven had caries involving the enamel, five had caries that had progressed to the dentin layer, two had undergone extraction, and one child had this tooth restored. The majority of the infants had sound primary mandibular teeth (Table 3.12).

Table 3.11 – Frequency of caries by primary maxillary teeth

Caries Index Code	**Number of Children with Affected by Primary Tooth Number**									
	55	**54**	**53**	**52**	**51**	**61**	**62**	**63**	**64**	**65**
A (sound)	11	71	34	92	99	95	97	34	70	12
B (white spot lesion)			3	17	18	17	15	3		
C (enamel lesion)	3	1	3	6	6	7	7	2	1	1
D (dentin lesion)		1		6	8	10	5		1	
E (filled, but decay present)										
F (filled, no decay)	2	3		1			1		3	1
G (missing due to caries)			1	3	2	2	2			
U (trauma)										

Table 3.12 – Frequency of caries by primary mandibular teeth

Caries Index Code	**Number of Children with Affected by Primary Tooth Number**									
	75	**74**	**73**	**72**	**71**	**81**	**82**	**83**	**84**	**85**
A (sound)	12	66	33	109	128	128	109	34	62	12
B (white spot lesion)	1	1	1	1	3	4	2	1		1
C (enamel lesion)	1				1				1	
D (dentin lesion)		1								
E (filled, but decay present)										
F (filled, no decay)	3	3	1			1	1	1	3	3
G (missing due to caries)										
U (trauma)					1					

The next chapter presents results arising from bivariate analysis of data with particular emphasis on the key objectives of this prospective study.

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Chapter 4 – Results: Associations of Interest Results

This chapter focuses on reporting the relationships between variables collected in this prospective study, including serological markers and those collected from interviews with participants at the time of recruitment and during the follow-up phase. Particular emphasis has been placed on reporting results from the specific objectives set forth in Chapter 2.

I. Associations Between Metabolites

Correlation analysis was performed to determine whether there were any significant associations between maternal levels of 25(OH)D and the other metabolites assayed in this investigation, including calcium, phosphorus, and alkaline phosphatase. No significant relationships between 25(OH)D and calcium ($p=.42$), 25(OH)D and phosphorus ($p=.10$), and 25(OH)D and alkaline phosphatase ($p=.42$) were identified. However, a significant inverse relationship was found between calcium and alkaline phosphatase levels in these participants (Figure 4.1, $p=.01$) and calcium and phosphorus (Figure 4.2, $p<.001$). There was no significant correlation between levels of phosphorus and alkaline phosphatase ($p=.33$).

Figure 4.1 – Correlation of calcium and alkaline phosphatase levels

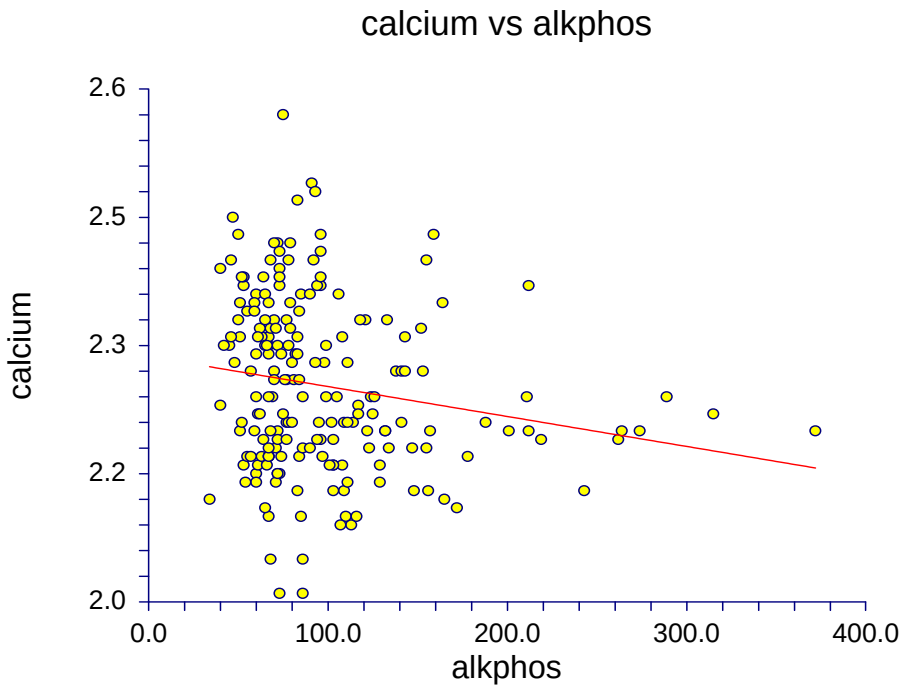
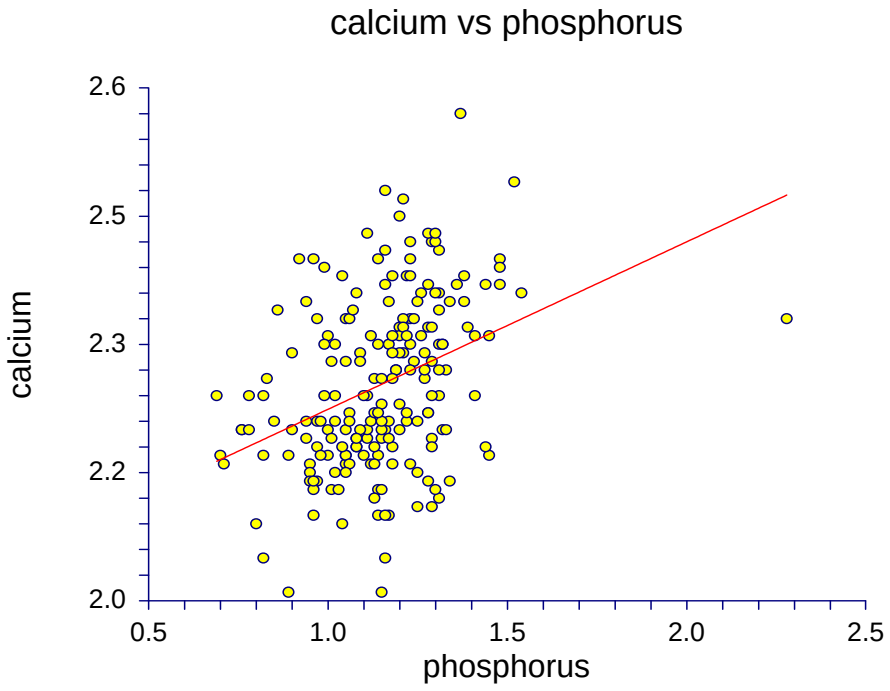


Figure 4.2 – Correlation of calcium and phosphorus levels



II. Relationship Between 25(OH)D Levels and Study Variables

Participant Profile

Statistical tests were employed to determine whether 25(OH)D concentrations were associated with variables relating to the participant at the time of recruitment into the study. Correlation analysis revealed that 25(OH)D levels were not associated with maternal age ($p=.68$) at enrollment. No significant difference was also found for mean 25(OH)D concentrations between women residing in or outside the city of Winnipeg (48.4 ± 24.9 nmol/L vs. 49.8 ± 22.5, $p=.42$).

There was a significant difference in the 25(OH)D status of women according to their ethnic background. Aboriginal women enrolled into this study had statistically lower mean levels of 25(OH)D than did women who were not identified as being Aboriginal (45.9 ± 22.2 nmol/L vs. 68.8 ± 33.4, $p<.001$). Analysis of variance (ANOVA) also revealed a statistically significant relationship between ethnicity and 25(OH)D status ($p<.001$), where Tukey's multiple comparison test revealed significant differences between Status Indians and non-Aboriginals (42.7 ± 21.7 nmol/L vs. 68.8 ± 33.4). Overall, Chi-square analysis indicated that 98.5% of those with 25(OH)D levels ≤ 35 nmol/L were Aboriginal compared to 85.5% with levels > 35 nmol/L. Similarly, 97.8% of those with concentrations ≤ 40 nmol/L were Aboriginal compared with 83.3% of those with levels > 40 nmol/L ($p<.001$). A similar pattern appeared with the new threshold for vitamin D adequacy of 80 nmol/L, where 92.1% with levels below this threshold were Aboriginal compared with 71.4% of those with levels ≥ 80 nmol/L ($p<.005$).

Maternal 25(OH)D levels were also associated with the month and season of sample collection. Table 4.1 reports mean values of 25(OH)D by the month of collection.

Since endogenous synthesis of vitamin D in northern regions of the United States and Canada is only possible between the months of May and October, an additional variable was created categorizing months of the year into a dichotomous variable called season. The winter season included months where there is no endogenous 25(OH)D synthesis (i.e. November to April). Results from t test analysis appear in Table 4.2.

Table 4.1 – Relationship between maternal 25(OH)D levels and month of measurement

Month	**Number**	**Mean ± S.D. (nmol/L)**	**P value**
January	16	43.2 ± 25.6	< .0001
February	13	40.9 ± 33.1	
March	12	34.3 ± 10.1	
April	8	25.7 ± 19.1*	
May	10	47.9 ± 12.9	
June	15	47.9 ± 24.0	
July	20	57.5 ± 20.7	
August	24	69.7 ± 29.5†	
September	27	52.3 ± 20.5	
October	24	46.6 ± 22.1	
November	23	38.4 ± 16.0	
December	8	44.1 ± 21.1	

*Significantly differs from July
†Significantly differs from January, February, March, April, October, November

Table 4.2 – Relationship between maternal 25(OH)D levels and season of measurement

Season	**Number**	**Mean ± S.D. (nmol/L)**	**P value**
Winter (November – April)	80	38.4 ± 21.8	<.0001
Summer (May – October)	120	54.6 ± 24.0	

Pregnancy & Health Profile

No significant differences were found between mean 25(OH)D levels for women who indicated that this was their first pregnancy and those who did not (p=.99). Correlation analysis revealed that there was no relationship between participants' levels of 25(OH)D and the number of children they reported they had (p=.64).

ANOVA involving 25(OH)D and maternal health rating identified a significant association (p=.043) with Tukey's multiple comparison test indicating differences between those rating their health as good and those rating their health as average (51.5 ± 24.3 nmol/L vs. 42.3 ± 24.3). This variable was further dichotomized to contrast those claiming to be in good health with all others. Results clearly indicated that women rating their health as good during the pregnancy had statistically higher 25(OH)D concentrations than those rating their health as average or poor during the pregnancy (51.5 ± 24.3 nmol/L vs. 42.6 ± 23.9, p=.006). Similarly, women who indicated that they were worried about their health during pregnancy had significantly lower levels than those who had no concerns (43.7 ± 24.1 nmol/L vs. 50.4 ± 24.5, p=.036). A statistically significant association was found between vitamin D status and whether women were worried about their health during the pregnancy. Women with 25(OH)D levels ≤ 40 nmol/L were significantly more likely to be worried about their health than those with concentrations > 40 nmol/L (p=.045).

Chi-square analysis was performed to investigate the relationship between these same variables and vitamin D states. Those with concentrations > 35 nmol/L and > 40 nmol/L were significantly more likely to rate their health as good compared with those with levels ≤ 35 nmol/L and ≤ 40 nmol/L (p=.014 and p<.01 respectively).

While those who were uncertain about the benefits of prenatal care had lower levels of 25(OH)D compared with those who thought prenatal care was important, this relationship was not statistically significant, even though it did approximate the threshold of significance (p=.055). A significant relationship was also found between maternal vitamin D status and maternal views of the importance of prenatal care, with significantly more women unsure of the importance of prenatal care having levels ≤ 35 nmol/L (p=.01).

Participants who indicated that their physician recommended vitamin use during pregnancy had significantly higher levels of vitamin D than those who were not informed (49.7 ± 24.9 nmol/L vs. 37.9 ± 18.3, p=.012 one-tailed). Chi-square analysis revealed that significantly more women with levels ≤ 40 nmol/L reported that their doctor did not recommend vitamins (p=.05). Further, results from t test analysis also showed that those taking prenatal vitamins during pregnancy had significantly higher 25(OH)D levels than those who did not (52.7 ±25.7 nmol/L vs. 38.7 ±17.6, p<.001). Likewise, chi-square analysis revealed significant associations between vitamin D states and whether women were taking recommended vitamins (Table 4.3).

Table 4.3 – Relationship between vitamin D states and use of vitamins

Vitamin D State	**Taking Recommended Vitamins**		**P value**
	No (%)	**Yes (%)**	
> 35 nmol/L	28 (21.7)	101 (78.3)	<.001
≤ 35 nmol/L	31 (45.6)	37 (54.4)	
> 40 nmol/L	19 (17.8)	88 (82.2)	<.001
≤ 40 nmol/L	40 (44.4)	50 (55.6)	
≥ 80 nmol/L	2 (10.0)	18 (90.0)	.04
< 80 nmol/L	57 (32.2)	120 (67.8)	

The frequency of vitamin intake during pregnancy also appeared to be a determinant of overall maternal 25(OH)D status ($p<.001$). Tukey's multiple comparison test indicated that women who reported never taking vitamins during pregnancy had significantly lower levels than those who took them often (once a day or more), and who took them sometimes (once a week or more, but not daily) (37.1 ± 15.7 nmol/L compared with 57.1 ± 25.9 and 48.8 ± 25.0). However, whether participants felt they should be taking vitamins or not for good prenatal health had no influence on serum 25(OH)D levels ($p=.3$).

Knowledge and familiarity with vitamin D and calcium had little influence on maternal vitamin D status. Having heard of vitamin D before participating in the study was not associated with better 25(OH)D status ($p=.89$), while ANOVA revealed that knowledge of foods containing vitamin D also did not predict higher 25(OH)D levels ($p=.50$). Likewise, mothers who reported to have heard about calcium did not have significantly different 25(OH)D concentrations than those who had not heard about calcium ($p=.86$). No significant associations were found on ANOVA between circulating 25(OH)D and what participants identified calcium to be important for ($p=.39$) and having correctly identified foods that contained calcium ($p=.89$). There was also no significant difference in 25(OH)D and participant use of calcium supplements ($p=.33$).

The prenatal questionnaire also included some questions pertaining to subclinical symptoms associated with vitamin D deficiency and insufficiency. There was an apparent pattern witnessed among participants between 25(OH)D and some common symptoms of clinical 25(OH)D deficiency, particularly difficulty walking and weakness in arms or legs, but not complaints of bone pain. For instance, participants who indicated that their

arms and legs felt weak had significantly lower 25(OH)D concentrations (37.9 ± 19.1 nmol/L vs. 51.5 ± 25.1, p<.001) and those who reported trouble walking also had significantly lower vitamin D levels (38.6 ± 18.6 nmol/L vs. 50.0 ± 25.0, p=.016). Similarly, women who did not report weakness in their arms or legs were significantly more likely to have concentrations greater than 35 nmol/L and 40 nmol/L compared to those reporting these symptoms (p<.001 and p<.001, respectively).

Smoking appeared to have a negative influence on maternal vitamin D status since women who smoked had significantly lower values than non-smokers (45.2 ± 23.0 nmol/L vs. 52.5 ± 26.2, p=.04). Non smokers were significantly more likely to have concentrations in excess of 40 nmol/L (p=.028). Results from ANOVA revealed that maternal diabetes did not result in significantly different 25(OH)D levels (p=.94).

Nutrition Profile/ Food Security Assessment

No significant difference was found between 25(OH)D levels and participant responses to whether they thought the foods they ate were healthy enough for their pregnancy (p=.35). Similarly, no significant differences were observed on ANOVA between 25(OH)D and how participants responded to the question of how they had changed their eating habits since finding out they were pregnant (p=.16). There was also no difference in vitamin D levels and whether participants bought their groceries in the community where they resided (p=.77).

Grocery shopping patterns and habits appeared to have no direct influence on maternal 25(OH)D. Although women who indicated they had visited a food-bank during pregnancy had lower levels of 25(OH)D, they did not statistically differ from those who

did not (42.9 ± 21.2 nmol/L vs. 49.2 ± 25.1, p=.18). However, those who indicated that they were unable to purchase all the foods they needed to be healthy during pregnancy had significantly lower mean vitamin D levels (38.7 ± 19.4 nmol/L vs. 49.2 ± 24.8, p=.038). Their participation in Healthy Baby Community Support programming did not have an influence either on circulating levels (p=.49), nor did their awareness of the Manitoba Healthy Baby Prenatal Benefit program (p=.70). No significant differences in mean levels of 25(OH)D were noted upon ANOVA between those receiving the benefit (48.7 nmol/L), those not (51.6 nmol/L), and those who had applied but had yet not received the stipend (39.6 nmol/L) (p=.23).

Dietary determinants of maternal 25(OH)D during pregnancy essentially centred upon milk consumption (Table 4.4), as no other food intakes appeared to be predictive. Maternal concentrations were not significantly related to fish, liver, and egg intakes or margarine use. Similarly, although not fortified with vitamin D, intake of other dairy products also did not relate with measured 25(OH)D levels.

Chi-square analysis was performed to determine the relationship between vitamin D states and milk intake. Women who drank milk on a daily basis were significantly more likely to have 25(OH)D levels above 35 nmol/L and 40 nmol/L (p=.032 and p<.001, respectively). Overall milk consumption was also associated with maternal vitamin D status, as those who consumed milk daily were significantly more likely to have levels > 40 nmol/L (p=.037).

Unfortunately, individuals who indicated that milk and other dairy products upset their stomachs had significantly lower mean 25(OH)D levels than those who did not indicate they had an intolerance to dairy (38.6 ± 16.9 nmol/L vs. 50.9 ± 25.5, p<.005).

Similarly, chi-square analysis revealed that those who indicated that dairy upset their stomach were significantly more likely to have 25(OH)D levels below 80 nmol/L, 40 nmol/L, and 35 nmol/L ($p=.047$, $p=.011$, and $p<.01$, respectively).

Table 4.4 – Relationship between 25(OH)D and milk intake

Food Item	**N**	**Mean 25(OH)D ± S.D. (nmol/L)**	**P Value**
Overall milk use			<.001 [a]
Often	124	53.6 ± 26.0*	
Sometimes	57	39.1 ± 18.9	
Rarely	8	38.3 ± 21.6	
Never	10	41.8 ± 15.2	
Overall milk use			<.001[b]
Often	124	53.6 ± 26.0	
Sometimes/Rarely/Never	75	39.4 ± 18.6	
Drink milk			<.001[a]
Often	99	55.7 ± 25.8†	
Sometimes	66	42.3 ± 21.1	
Rarely	19	34.0 ± 16.3	
Never	15	43.1 ± 22.8	
Cook with milk			.031[b]
Often/Sometimes	150	50.3 ± 25.1	
Rarely/Never	49	41.7 ± 21.2	
Milk with cereal			.23[a]
Often	89	51.3 ± 25.8	
Sometimes	85	46.1 ± 24.6	
Rarely	19	47.9 ± 17.5	
Never	6	33.0 ± 10.6	
Milk in coffee or drinks			.13 [a]
Often	30	49.6 ± 21.9	
Sometimes	40	46.6 ± 20.0	
Rarely	24	38.0 ± 16.1	
Never	105	50.8 ± 27.6	

[a]ANOVA
[b]T test
* Significantly differs from Never
† Significantly differs from Sometimes & Rarely

Early Childhood Caries (ECC) Profile

While maternal knowledge of ECC, familial experience with ECC, and views about prevention did not appear to be significantly associated with maternal vitamin D levels, the only variable in this section of the prenatal survey instrument that was found to be significantly predictive of a participant's mean 25(OH)D level was whether any of the respondents' children had undergone pediatric dental surgery under general anesthesia (37.8 ± 15.8 nmol/L vs. 49.3 ± 26.8, p=.041). Although participants who had heard of ECC had marginally lower concentrations, the difference was not statistically significant (46.8 ± 24.1 nmol/L vs. 53.2 ± 25.1, p=.066).

Maternal Oral Health Profile

There was no significant association between 25(OH)D concentrations and whether subjects thought their dental health was important during pregnancy (p=.77). Similarly, there were also no association between maternal 25(OH)D status and when they had last seen a dentist (p=.61), and the frequency between dental visits (p=.40).

Meanwhile, ANOVA revealed a significant relationship between 25(OH)D and how participants rated their oral health (p=.014). The fair oral health and poor oral health groups were then combined and further t test analysis revealed that those who rated their oral health as good had significantly higher vitamin D levels (54.6 ± 28.8 nmol/L vs. 44.4 ± 20.6, p<.01). Interestingly, although there was no apparent significant difference between those who indicated they had dental problems and those who did not, the difference did approach, but did not meet the threshold of significance (44.9 ± 22.1 nmol/L vs. 50.4 ± 25.7, p=.063).

Exposure to Sunlight

Only one of the questions asked in this section of the prenatal questionnaire, which were to serve as proxy measures for overall sun exposure, appeared to have an influence on circulating levels of 25(OH)D in this cohort. T test analysis determined that those who reported spending their outside activities in the sunshine had significantly greater 25(OH)D levels than those who did not (62.3 ± 33.2 nmol/L vs. 47.3 ± 23.6, p=.040). However, when participants were stratified by season there was no statistically significant difference in mean vitamin D concentrations between those spending time outside in the sunshine and those who did not (63.7 ± 33.7 nmol/L vs. 53.8 ± 23.1, p=.23). Meanwhile, use of a hat, sunscreen, and clothing had no influence on vitamin D levels.

Family and Financial Profile

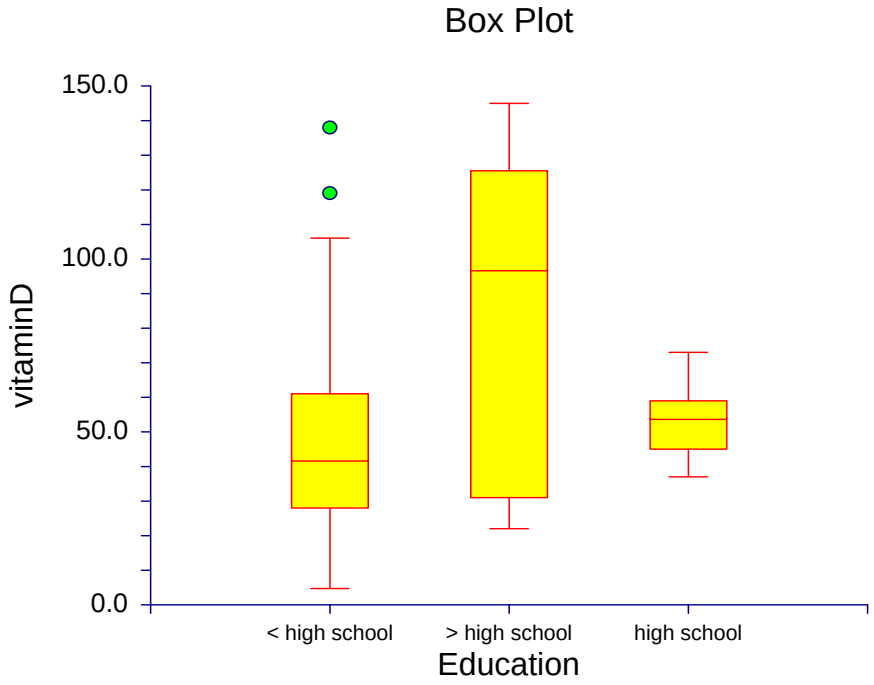
Relational status was not a predictor of participant 25(OH)D levels as no significant differences were exhibited on ANOVA between those indicating that they were single, married, divorced, or living in common law relationships (p=.52). Family size was also found not to be correlated with maternal 25(OH)D (p=.19). ANOVA indicated that there was a significant association between the education level of subjects and their vitamin D levels (Figure 4.3, p<.005) with Tukey's multiple comparison test revealing differences between those who had not completed high school and those who had pursued postsecondary education.

Similarly, results from chi-square analysis revealed that those who had completed high school and those who pursued post-secondary education were significantly more

likely to have vitamin D levels at or above the 80 nmol/L threshold (p<.001) than those who did not complete high school.

Income and financial assistance also appeared to have an influence on maternal 25(OH)D levels. For instance, ANOVA revealed a significant relationship between vitamin D status and household income (p<.005); with those individuals who indicated that their family's main source of household income was full-time employment had significantly higher mean 25(OH)D values than those who indicated government assistance (59.3 ± 25.9 nmol/L vs. 44.5 ± 23.4). Those who received financial help from relatives or friends also had significantly higher levels (51.6 ± 24.9 nmol/L vs. 45.4 ± 23.8, p=.036). Finally, yearly income was significantly associated with a mother's vitamin D status (ANOVA, p<.001), with those earning ≥ $26,000/year having higher mean 25(OH)D levels than the other groups.

Figure 4.3 – Relationship between 25(OH)D and education level of participants



Chi-square analysis revealed that women who reported the main source of overall household income as full-time employment were significantly more likely to have levels > 35 nmol/L ($p<.001$) and > 40 nmol/L ($p<.001$). Likewise, those who received financial help from others were significantly more likely to have 25(OH)D levels > 35 nmol/L ($p=.033$) and > 40 nmol/L ($p<.01$).

III. Associations Between Maternal 25(OH)D and Infant Oral Health

Considering that there were several losses to follow-up, there was no significant difference in the 25(OH)D levels of women who remained in the study and those who were lost to follow-up, although levels were marginally lower among those who did not return with their infant (49.8 ± 26.3 nmol/L vs. 45.1 ± 20.1 nmol/L, $p=.081$). As mentioned in Chapter 3 there were no differences in age, level of education, or heritage between women lost to follow-up and those who remained in the study.

Associations between mean 25(OH)D levels and key infant oral health outcome measures, including developmental defects of enamel (DDEs), enamel hypoplasia, ECC, decayed tooth (d) score, and the eruption of the first primary tooth were investigated. Likewise, other associations between these dental outcomes and vitamin D states, including deficiency, insufficiency, and adequacy were explored.

Table 4.5 reports the relationship between mean 25(OH)D levels and infant dental outcomes of DDE, enamel hypoplasia, and ECC. Results from t test analysis indicated that there was no significant relationship between circulating levels of maternal 25(OH)D and the presence of DDEs among infants ($p=.91$). Although mothers of infants with enamel hypoplasia had what appeared to be lower mean concentrations of 25(OH)D, they

did not statistically differ from values associated with children who did not have enamel hypoplasia (p=.072). However, mothers of infants who were classified as having ECC, based upon the presence of cavitated caries lesions, had significantly lower serum levels of 25(OH)D than children who were caries free (p=.022).

Chi-square analyses were also performed to determine whether there were any significant relationships between infant dental outcomes and vitamin D thresholds of deficiency, insufficiency, and adequacy. No significant associations were found between mothers with concentrations > 35 nmol/L or ≤ 35 nmol/L and DDEs (p=.65), enamel hypoplasia (p=.18), and ECC (p=.29). Likewise, no significant associations were found between whether mothers had concentrations > 40 nmol/L or ≤ 40 nmol/L and DDEs (p=.98), enamel hypoplasia (p=.29), and ECC (p=.12). In addition, there were no apparent associations between whether mothers had concentrations < 80 nmol/L or ≥ 80 nmol/L and DDEs (p=.92), enamel hypoplasia (p=.31), and ECC (p=.51).

Table 4.5 – Relationship between oral health outcomes and maternal 25(OH)D

	Maternal 25(OH)D			
Oral Health Outcomes	**N**	**Mean ± S. D.**	**Median**	**P value**
Developmental Defects of Enamel				.91
Yes	122	50.3 ± 26.5	46	
No	7	49.1 ± 25.5	45	
Enamel Hypoplasia				.072[a]
Yes	28	43.2 ± 21.1	39.5	
No	104	51.4 ± 27.4	46.5	
ECC (cavitated lesions)*				.022[a]
Yes	30	41.4 ± 20.4	39	
No	103	52.4 ± 27.4	47	
ECC (including white spot lesions)				
Yes	48	45.8 ± 23.9	40.5	.088 [a]
No	85	52.2 ± 27.5	46	

T test analysis
[a]One-tailed
*2 tailed p=.045

Poisson regression was used to examine the relationship between the average number of infant primary teeth that were affected by decay (dt) and maternal levels of vitamin D during pregnancy. This relationship was found to be statistically significant (p=.0002). Higher 25(OH)D levels during pregnancy were inversely related to the number of primary teeth affected by caries.

The following examples and Figure 4.4 demonstrate this relationship for three different thresholds of vitamin D:

1) 25 nmol/L, associated with severe deficiency (Deficient)

$dt = e^{(B\ intercept)} \times e^{(B\ vitamin\ D)(vitamin\ D\ level)}$

$= e^{(0.83)} \times e^{(-0.014)(25)}$

$= 2.3 \times 0.71 = 1.65$ teeth affected with caries

2) 50 nmol/L, the mean value for vitamin D for mothers in this Poisson regression (Mean Level)

$dt = e^{(B\ intercept)} \times e^{(B\ vitamin\ D)(vitamin\ D\ level)}$

$= e^{(0.83)} \times e^{(-0.014)(50)}$

$= 2.3 \times 0.51 = 1.17$ teeth affected with caries

3) 80 nmol/L, associated with vitamin D adequacy (Adequate)

$dt = e^{(B\ intercept)} \times e^{(B\ vitamin\ D)(vitamin\ D\ level)}$

$= e^{(0.83)} \times e^{(-0.014)(80)}$

$= 2.3 \times 0.34 = 0.78$ teeth affected with caries

Figure 4.4 – Predicted number of decayed primary teeth by 25(OH)D level

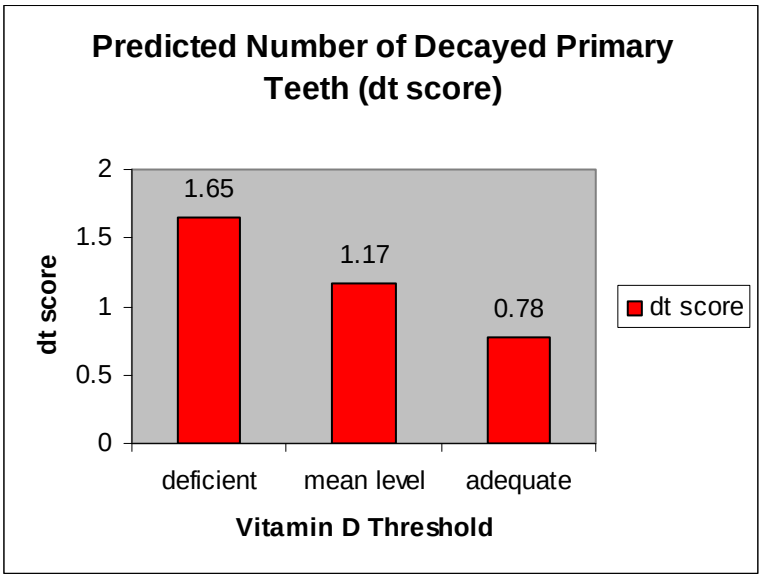
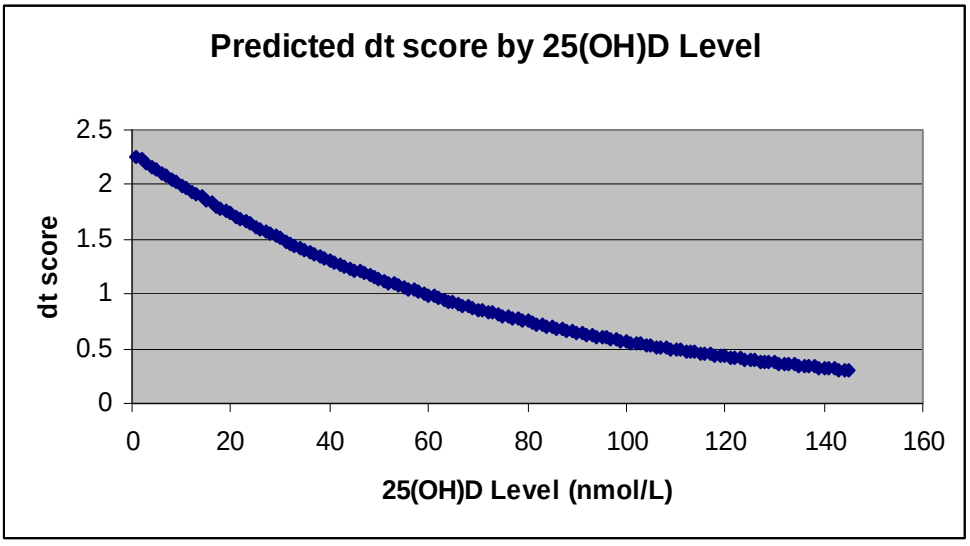


Figure 4.5 is a graphic display of the overall relationship between infant dt score and maternal vitamin D concentrations indicating the decline in caries rates with increasing 25(OH)D levels.

Figure 4.5 – Predicted dt score by maternal 25(OH)D level



T test analyses were undertaken to assess the relationship between the number of decayed teeth (d) score and various 25(OH)D threshold levels. There was no significant difference in the mean number of primary teeth with decay among infants of mothers who had concentrations ≤ 35 nmol/L and > 35 nmol/L (Table 4.6). Similarly, there was no significant difference when the threshold of 40 nmol/L was selected. Interestingly, infants of mothers who had 25(OH)D levels ≥ 80 nmol/L had a statistically lower mean d score than those who had levels below this threshold (p=.032).

Table 4.6 – Relationship between dt score and maternal 25(OH)D

25(OH)D	**dt score**			
	N	**Mean ± S.D.**	**Range**	**P value**
25(OH)D Threshold – Deficiency				.099
> 35 nmol/L	85	1.07 ± 1.95	0 – 10	
≤ 35nmol/L	47	1.55 ± 2.24	0 – 9	
25(OH)D Threshold – Insufficiency				.092
> 40 nmol/L	73	1.03 ± 1.99	0 – 10	
≤ 40 nmol/L	59	1.51 ± 2.14	0 – 9	
25(OH)D Threshold - Adequacy				.032*
≥ 80 nmol/L	17	0.65 ± 1.22	0 – 4	
< 80 nmol/L	115	1.33 ± 2.15	0 – 10	

*Aspin-Welch Unequal-Variance Test

A similar series of analyses was undertaken for the mean number of decayed tooth surfaces (ds) with these selected thresholds for 25(OH)D (Table 4.7). While mean ds values did not statistically differ between infants of mothers with levels > 35 nmol/L vs. ≤ 35 nmol/L and > 40 nmol/L vs. ≤ 40 nmol/L, they did significantly differ at the higher threshold of adequacy. Infants of mothers who had concentrations of 25(OH)D below 80 nmol/L had significantly higher ds scores than infants whose mothers had serum levels at or above this threshold (p=.028). These results seem to indicate that infant dental caries is associated with maternal 25(OH)D status.

Table 4.7 – Relationship between ds score and maternal 25(OH)D

25(OH)D	**ds score**			
	N	**Mean ± S.D.**	**Range**	**P value**
25(OH)D Threshold – Deficiency				.78*
> 35 nmol/L	81	1.67 ± 4.54	0 – 34	
≤ 35nmol/L	47	1.85 ± 2.93	0 – 13	
25(OH)D Threshold – Insufficiency				.91*
> 40 nmol/L	70	1.70 ± 4.83	0 – 34	
≤ 40 nmol/L	58	1.77 ± 2.75	0 – 13	
25(OH)D Threshold - Adequacy				.028*
≥ 80 nmol/L	15	.67 ± 1.40	0 – 4	
< 80 nmol/L	113	1.88 ± 4.22	0 – 34	

*Aspin-Welch Unequal-Variance Test

Associations between enamel hypoplasia and ECC with other study variables were explored. Table 4.8 report variables from the prenatal questionnaire which were found to be associated with enamel hypoplasia among infants while Table 4.9 reports prenatal variables associated with ECC.

Table 4.8 – Prenatal variables associated with enamel hypoplasia

Variable	**No Enamel Hypoplasia (%)**	**Enamel Hypoplasia (%)**	**P value**
Doctor recommended vitamins			.012
No	6 (50.0)	6 (50.0)	
Yes	99 (81.1)	23 (18.9)	
Heard of vitamin D			.01
No	34 (66.7)	17 (33.3)	
Yes	71 (85.5)	12 (14.5)	
Identified what calcium important for			.021
Correct	78 (83.9)	15 (16.1)	
Didn't Know	25 (69.4)	11 (30.6)	
Incorrect	2 (40.0)	3 (60.0)	
Weak arm/leg			.030
No	88 (82.2)	19 (17.8)	
Yes	17 (63.0)	10 (37.0)	

Variable	No Enamel Hypoplasia (%)	Enamel Hypoplasia (%)	P value
Drink milk			<.001
Often	58 (86.6)	9 (13.4)	
Sometimes	32 (74.4)	11 (25.6)	
Rarely	4 (33.3)	8 (66.7)	
Never	11 (91.7)	1 (8.3)	
Milk upset stomach			.016
No	87 (82.9)	18 (17.1)	
Yes	18 (62.1)	11 (37.9)	
Eat margarine			.011
Often	61 (85.9)	10 (14.1)	
Sometime	41 (71.9)	16 (28.1)	
Rarely	1 (25.0)	3 (75.0)	
Never	2 (100.0)	0	
Overall Milk Consumption			<.01
Often	71 (84.5)	13 (15.5)	
Sometime	26 (72.2)	10 (27.8)	
Rarely	1 (20.0)	4 (80.0)	
Never	7 (77.8)	2 (22.2)	
Heard of ECC			.044
No	25 (92.6)	2 (7.4)	
Yes	80 (74.8)	27 (25.2)	
Older children had general anaesthesia for dental surgery			<.005
No	31 (83.8)	6 (16.2)	
Yes	4 (40.0)	6 (60.0)	
Household income			.034
Full-Time	34 (91.9)	3 (8.1)	
Government Assist	51 (72.9)	19 (27.1)	
Part-Time	7 (58.3)	5 (41.7)	
Other	13 (86.7)	2 (13.3)	

Overall, mothers of children without enamel hypoplasia were significantly more likely to have heard of vitamin D, more likely to have been recommended to take vitamins by their physician, likely to have consumed milk and margarine more

frequently, and less likely to have had older children undergo general anesthesia for pediatric dental surgery.

Table 4.9 – Association between prenatal variables and ECC

Variable	**No ECC* (%)**	**ECC* (%)**	**P value**
Heritage			.020
Aboriginal	88 (73.9)	31 (26.1)	
Other	16 (100.0)	0	
Health rating			.014
Good	72 (83.7)	14 (16.3)	
Average/Poor	32 (65.3)	17 (34.7)	
Worried about health			<.005
No	76 (84.4)	14 (15.6)	
Yes	28 (62.2)	17 (37.8)	
Doctor recommended vitamins			.020
No	6 (50.0)	6 (50.0)	
Yes	98 (79.7)	25 (20.3)	
Identified foods containing calcium			.016
Correct	80 (83.3)	16 (16.7)	
Incorrect	11 (64.7)	6 (35.3)	
Didn't Know	12 (57.1)	9 (42.9)	
Calcium important during pregnancy			.032
No	0	1 (100.0)	
Yes	94 (79.7)	24 (20.3)	
Unsure	8 (57.1)	6 (42.9)	
Weak arm/leg			<.005
No	88 (82.2)	19 (17.8)	
Yes	16 (57.1)	12 (42.9)	
Shopping with kids limits what can buy			.042
No	17 (63.0)	10 (37.0)	
Yes	8 (100.0)	0	
Food-bank now			.021
No	90 (80.4)	22 (19.6)	
Yes	12 (57.1)	9 (42.9)	

Variable	No ECC* (%)	ECC* (%)	P value
Able to buy all foods			.021
No	6 (50.0)	6 (50.0)	
Yes	97 (79.5)	25 (20.5)	
Overall milk consumption			.012
Often	70 (82.4)	15 (17.6)	
Sometime	26 (72.2)	10 (27.8)	
Rarely	1 (20.0)	4 (80.0)	
Never	7 (77.8)	2 (22.2)	
Other children with ECC			<.005
No	28 (84.8)	5 (15.2)	
Yes	5 (41.7)	7 (58.3)	
Prevent ECC (can be prevented)			.0099
	2 (100.0)	0	
No	95 (80.5)	23 (19.5)	
Yes	7 (46.7)	8 (53.3)	
Unsure			
Enjoy sunshine			.0054
No	16 (57.1)	12 (42.9)	
Yes	87 (82.1)	19 (17.9)	
Household income			.043
Full-Time	34 (89.5)	4 (10.5)	
Government Assist	54 (77.1)	16 (22.9)	
Part-Time	7 (58.3)	5 (41.7)	
Other	9 (60.0)	6 (40.0)	
Annual income			<.005
< $18,000	93 (76.9)	28 (23.1)	
$18,000-26,000	0	3 (100.0)	
> $26,000	2 (100.0)	0	
Didn't Know	9 (100.0)	0	

*ECC excluding white spots

Results of the chi-square analyses reveal that mothers of infants who were found to have ECC were significantly more likely to be Aboriginal, more likely to rate their

health as average or poor, and more likely to worry about their health. Similarly, they were more likely to have used a food-bank, but less likely to consume milk frequently.

Correlation analysis was performed for the age of eruption of the first primary tooth and maternal 25(OH)D concentrations yielding a statistically insignificant result (p=.39). Further, the mean age of the eruption of the first tooth was not significantly associated with the thresholds of deficiency (p=.54), insufficiency (p=.75), and adequacy (p=.91).

Finally, results from chi-square analysis reveal that enamel hypoplasia and ECC were significantly associated as infants with enamel hypoplasia were significantly more likely to have ECC (73.3% vs. 26.7%, p<.001).

IV. Relationship between ECC, Enamel Hypoplasia, and Infant Variables

Both Chi-square and t test analyses were undertaken to determine whether any of those variables collected during the follow-up infant examination study visit were associated with enamel hypoplasia or ECC. No significant age differences existed between infants with and without enamel hypoplasia (14.8 ± 5.2 months vs. 16.4 ± 7.9, p=.018). Likewise, there was no significant association between gestational diabetes and enamel hypoplasia (p= .77) or prematurity and enamel hypoplasia (p= .28). Overall, no significant associations were found between the presence of enamel hypoplasia and the remaining variables collected in the infant questionnaire.

Children with ECC were significantly older than those who were caries free (19.4 ± 10.0 months vs. 14.3 ± 4.4, p=.0013). While no relationship was found between enamel

hypoplasia and gestational diabetes, infants of mothers who indicated that they had diabetes during pregnancy were significantly more likely to have ECC (p=.0088).

None of the infant feeding practices appeared to be significantly associated with the presence of ECC. ECC was not significantly associated with breastfeeding (p=.35) or bottle-feeding (p=.86). At the time of the infant examination study visit, of the 87 infants who had already stopped breastfeeding there was no significant difference with respect to the age when the child was weaned from the breast and the presence or absence of ECC (4.3 ± 5.7 months vs. 3.3 ± 3.1, p=.39). Among the 21 infants who were reported to have stopped bottle-feeding, there was no significant difference between the presence and absence of ECC and weaning age although this did approach the threshold of significance (16.0 ± 5.1 months vs. 12.5 ± 4.0, p=.051). However, a relationship was found between sippy-cup use and ECC. Those who were still using their sippy-cup were significantly less likely to have ECC (p=.0012).

Significantly more infants whose parent/caregiver indicated that they believed their child had dental problems had ECC than those who did not think their child had dental problems (p<.001). Of those who had already had a dental visit unrelated to this study (n=15), significantly more were in the ECC group (p=.043). Similarly, of these 15 infants, significantly more had been to the dentist because of cavities (p=.0038).

117 caregivers indicated that their infant's teeth were being cleaned. Significantly fewer children whose teeth were being cleaned had ECC compared to those who were not being cleaned (p=.019). However, there was no significant difference in the mean infant age when caregivers began to clean their teeth between the ECC and caries free groups (10.6 ± 7.5 months vs. 8.2 ± 4.0, p=.067). This became significant when restricted to one-

tail (p=.034) suggesting that early commencement of oral hygiene reduces the risk for ECC.

Chapter 5 – Multivariate Regression Results

This chapter reports results from multiple and logistic regression analyses for key outcome variables of interest in the study, specifically 25(OH)D, enamel hypoplasia, and early childhood caries (ECC). Since the number of maternal-infant dyads taking part in this prospective cohort was small numerous logistic and multiple regression analyses were undertaken to prevent over-fitting of the models and avoid potential false associations. Description of these models appears in the methods chapter of this thesis.

I. Multiple Regression Analysis for 25(OH)D

Multiple regression analyses were conducted for maternal 25(OH)D status. Table 5.1 reports the results from the first model that included serum metabolites of calcium, alkaline phosphatase, and phosphorus that were measured from the maternal blood draw taken during pregnancy. It was apparent that none of these metabolites were significantly correlated with 25(OH)D concentrations in this model. Further, this model only explained 0.4% of the variance.

Table 5.1 – Multiple regression analysis for 25(OH)D concentration – Serum metabolites of alkaline phosphatase, calcium, and phosphorus

Variable	**Regression Coefficient**	**± 95% Confidence Interval**	**P value**
Intercept	10.61		
Alkaline Phosphatase	0.034	0.067	.32
Calcium	7.94	37.47	.68
Phosphorus	14.44	19.73	.15

Adjusted R^2 = 0.4%

The next series of multiple regression analyses for 25(OH)D levels explored potential relationships between 25(OH)D levels of participants and independent variables gleaned from the prenatal questionnaire completed with subjects at the time of enrolment. Table 5.2 reports findings of a regression model that included factors relating to prenatal health and prenatal care. This model included those prenatal independent variables that were significantly associated with maternal vitamin D levels like maternal health rating during pregnancy, whether their physician recommended prenatal vitamins, their use of prenatal vitamins, and whether participants believed prenatal care to be important. This model revealed that both maternal health rating and use of prenatal vitamins were significantly predictive of 25(OH)D concentrations.

Another two similar multiple regression models were created with 25(OH)D concentrations as the dependent outcome and included socioeconomic factors like food security, employment and income, education, and heritage of the mother at the time of enrollment. The difference between the two models was that the first model included overall household employment status (Table 5.3) while the second included the mother's annual income (Table 5.4). Results from the model presented in Table 5.3 indicated that Aboriginal heritage, household employment, and maternal education level were significant predictors of 25(OH)D concentrations during pregnancy. Those who were Aboriginal, had not completed high school, and lived in a home where no one was employed on a full-time basis had lower levels of vitamin D.

Table 5.2 – Multiple regression analysis for 25(OH)D concentration – Prenatal health and healthcare

Variable	Regression Coefficient	± 95% Confidence Interval	P value
Intercept	17.27		
Doctor recommended vitamins (reference: no)	3.60	11.30	.53
Maternal health rating (reference: average/poor)	8.31	6.87	**.018**
Prenatal care important (reference: no)	14.71	19.23	.13
Took vitamins during pregnancy (reference: no)	12.16	7.82	**.0025**

Adjusted R^2 = 9.2%

Table 5.3 – Multiple regression analysis for 25(OH)D concentration – Socioeconomic factors including full-time household employment

Variable	Regression Coefficient	± 95% Confidence Interval	P value
Intercept	58.71		
Able to purchase food during pregnancy (reference: no)	7.99	11.04	.16
Not of Aboriginal heritage (reference: no)	15.08	11.76	**.012**
Financial help from family & friends (reference: no)	4.89	6.50	.14
No one with full-time employment in household (reference: no)	-9.80	8.05	**.017**
Low education level (reference: ≥ high school)	-15.51	11.80	**.010**

Adjusted R^2 = 13.2%

Results from the second model appear in Table 5.4 and reveal that Aboriginal heritage and maternal education were significant predictors of 25(OH)D concentrations during pregnancy while annual income was not. As in Table 5.3, those who were Aboriginal and had not completed high school had significantly lower prenatal vitamin D levels.

Table 5.4 – Multiple regression analysis for 25(OH)D concentration – Socioeconomic factors including annual income

Variable	**Regression Coefficient**	**± 95% Confidence Interval**	**P value**
Intercept	57.43		
Able to purchase food during pregnancy (reference: no)	9.04	11.21	.11
Not of Aboriginal heritage (reference: no)	20.55	10.87	**<.001**
Low annual income (reference: > $18,000)	-8.30	15.24	.28
Financial help from family & friends (reference: no)	4.22	6.63	.21
Low education level (reference: ≥ high school)	-14.76	12.14	**.017**

Adjusted R^2 = 11.1%

Another small regression model was constructed including some independent variables that are known to influence vitamin D attainment, from both endogenous and exogenous sources (Table 5.5). Participants who reported drinking milk often, defined as once a day or more, had significantly higher 25(OH)D levels. Likewise, those who took recommended prenatal vitamins had higher serum levels of vitamin D. However, the frequency of vitamin intake did not appear to have an effect on 25(OH)D levels when

controlling for the influence of other independent variables. Those who were sampled during winter months (November – April) had significantly lower 25(OH)D concentrations.

Table 5.5 – Multiple regression for 25(OH)D concentration – Factors influencing attainment from diet and exposure to sunlight

Variable	**Regression Coefficient**	**± 95% Confidence Interval**	**P value**
Intercept	37.55		
Drink milk (reference: < often)	13.71	6.09	**.000**
Frequency of prenatal vitamin use (reference: < often)	6.36	6.83	.068
Season (reference: summer)	-13.90	6.22	**.000**
Took vitamins during pregnancy (reference: no)	10.29	7.33	**.0062**

Adjusted R^2 = 24.9%

The final model examined the relationship between 25(OH)D concentrations during pregnancy and those signs and symptoms possibly indicative of vitamin D deficiency (Table 5.6). The only question that appeared to be significantly associated with 25(OH)D concentrations during pregnancy was whether participants experienced any weakness in their arms or legs at the time of recruitment into the study ($p<.01$). Those who reported weakness in their arms or legs during pregnancy had significantly lower vitamin D levels. This may be a useful question to screen for individuals who may be at risk for vitamin D deficiency when serum analysis is not possible.

Table 5.6 – Multiple regression status for 25(OH)D concentration – Possible signs and symptoms of deficiency

Variable	Regression Coefficient	± 95% Confidence Interval	P value
Intercept	48.45		
Maternal health rating (reference: average/poor)	5.87	7.07	.10
Trouble walking (reference: no)	-8.70	9.36	.068
Weak arms or legs (reference: no)	-11.05	8.07	**.0076**

Adjusted R^2 = 7.5%

II. Logistic Regression Analysis for Enamel Hypoplasia

This next section reports results of regression analyses for enamel hypoplasia exhibited in the primary teeth of infants from mother-infant dyads. Several small regression models were undertaken to prevent over-fitting of the models. The first logistic regression model examined the association between enamel hypoplasia and serum metabolite levels from blood samples obtained from mothers during pregnancy. Of interest was whether any had an influence on enamel hypoplasia in the primary teeth of offspring. Odds ratios less than one are protective against enamel hypoplasia while those greater than one indicate an increase in the risk for enamel hypoplasia.

Table 5.7 shows the results from this model, which indicated that only serum calcium was significantly associated with enamel hypoplasia in infants (p=.05). Mothers with lower calcium levels during pregnancy were significantly more likely to have infants with enamel hypoplasia. Further, results of backwards logistic regression analysis also confirmed this relationship as only calcium remained in the model (p=.04) (data not shown).

Table 5.7 – Logistic regression for enamel hypoplasia* – Serum metabolites of alkaline phosphatase, calcium, phosphorus, and 25(OH)D

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Alkaline Phosphatase	-0.0057	0.0057	51.79	0.74	0.42, 1.33	.32
Calcium	-4.84	2.47	0.10	0.62	0.38, 1.00	**.050**
Phosphorus	-0.14	1.24	0.19	0.97	0.61, 1.54	.91
25(OH)D	-0.013	0.0093	24.44	0.72	0.47, 1.14	.16

*Enamel Hypoplasia reference = yes R^2= 6.0%

The next series of models looked at the relationship between variables collected during the prenatal phase of data collection as well as those originating from the infant dental visit phase of data collection. Because of the small sample size of maternal-infant pairs, four separate logistic regression models were initially performed based upon themes of 1) maternal awareness of calcium and vitamin D, 2) prenatal care and diet, 3) socioeconomic status (SES) and ethnicity, and 4) the infant's birth and health status. Significant variables from these five regression models were then entered into a final model for enamel hypoplasia.

The first model for enamel hypoplasia included variables of whether participants had previously heard of vitamin D and whether they knew what calcium was important for (Table 5.8). These were independent variables uncovered from the bivariate analyses undertaken in the previous chapter. Results of the logistic regression for enamel hypoplasia revealed no significant relationship with maternal awareness of calcium or

vitamin D. However, it did suggest that offspring of participants who had heard of vitamin D were less likely to have enamel hypoplasia (p=.053).

Table 5.8 – Logistic regression for enamel hypoplasia* – Maternal awareness and knowledge of calcium and vitamin D

Variable	Regression Coefficient (b)	Standard Error b	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Heard of vitamin D (reference: no)	-0.88	0.45	0.42	0.17, 1.01	.053
Knew what calcium is important for (reference: no)	-0.72	0.46	0.48	0.20, 1.19	.11

*Enamel Hypoplasia reference = yes R^2= 6.4%

Another regression model was constructed to examine the relationship between prenatal diet and prenatal care factors and risk for enamel hypoplasia in offspring (Table 5.9). Variables considered for this model included both calcium and 25(OH)D levels of mothers during pregnancy, whether mothers experienced gestational diabetes, intakes of milk and margarine, both known to be fortified with vitamin D, and whether mothers were receiving the Healthy Baby prenatal government subsidy during pregnancy. Those variables that were found to be significantly associated with the presence or absence of enamel hypoplasia in infants were prenatal serum calcium levels of mothers (p=.019) and margarine use (p=.022). Those who did not eat margarine often, defined as once a day or more, were more than three times as likely to have an infant with enamel hypoplasia (OR = 1/0.31= 3.2). Results from a backwards logistic regression model involving the same variables included in the model in Table 5.9 identified three variables as being significantly associated with enamel hypoplasia, namely maternal calcium levels (p=.01),

not drinking milk often (p=.009), and not consuming margarine often (p=.017) (data not shown).

Table 5.9 – Logistic regression for enamel hypoplasia* – Prenatal care and diet

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Calcium	-6.35	2.70	0.10	0.53	0.31, 0.90	**.019**
Gestational diabetes (reference: no)	0.004	0.73		1.00	0.24, 4.21	1.00
Drink milk (reference: < often)	-1.04	0.55		0.35	0.12, 1.05	.061
Doctor recommended vitamins (reference: no)	-0.48	0.72		0.62	0.15, 2.52	.50
Eat margarine (reference: < often)	-1.18	0.52		0.31	0.11, 0.84	**.022**
Received Healthy Baby benefit (reference: no)	0.41	0.64		1.50	0.43, 5.22	.52
Milk upsets stomach (reference: no)	0.74	0.56		2.10	0.70, 6.29	.19
25(OH)D	-0.0051	0.010	24.44	0.88	0.53, 1.45	0.62

*Enamel Hypoplasia reference = yes R^2= 17.6%

Table 5.10 reports findings from the regression model constructed to determine the association between enamel hypoplasia and household employment status and ethnicity of the participants (Table 5.10). Household employment status was included in the model as this relationship was significant at the bivariate level. No other independent economic variables like annual income were included in this model as they were not

significant at the bivariate level and the sample was too small to allow the regression model to include more variables. No significant associations were identified. However, when a separate model was run for enamel hypoplasia with the economic variables (not shown) like participant's annual income during pregnancy (<$18,000 vs. ≥ $18,000), full-time employment (not full-time vs. full-time), and whether the parent at the time of the infant's examination was receiving government assistance (yes vs. no), only those not benefiting from someone in the home working full-time during pregnancy was significantly associated with enamel hypoplasia (p= .019).

Table 5.10 – Logistic regression for enamel hypoplasia* – SES & ethnicity

Variable	**Regression Coefficient (b)**	**Standard Error b**	**Adjusted Odds Ratio**	**± 95% Confidence Interval**	**P value**
Not of Aboriginal heritage (reference: no)	-0.86	1.12	0.42	0.047, 3.83	.45
No one with full-time employment in household (reference: no)	1.21	0.68	3.35	0.88, 12.71	.076

*Enamel Hypoplasia reference = yes R^2= 5.1%

Table 5.11 reports findings from a logistic regression analysis involving independent variables relating to birth characteristics and child health status. This included birth weight, whether the infant was born prematurely, and whether the infant had experienced any serious medical problems since birth. No significant associations were identified between infant birth characteristics and health status and enamel hypoplasia.

Table 5.11 – Logistic regression for enamel hypoplasia* – Infant birth characteristics and health status

Variable	Regression Coefficient	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Birth weight (grams)	0.0005	0.00039	560.8	1.00	0.99, 1.00	.21
Premature (reference: no)	0.99	0.62		2.70	0.80, 9.10	.11
Serious medical problem(s) (reference: no)	0.52	0.45		1.68	0.69, 4.09	.25

*Enamel Hypoplasia reference = yes R^2= 4.9%

A more rigorous model for enamel hypoplasia included dietary intakes of milk and margarine, the metabolites of calcium and 25(OH)D, along with child health status and history of serious medical conditions, and knowledge of vitamin D (Table 5.12(a)). Drinking milk and having heard of vitamin D were included as they did approach the threshold of significance in Table 5.9 and Table 5.8, respectively. 25(OH)D was included in this model as it was a variable of major interest, while the variable of serious medical problems in infancy was included as the literature has often implicated infant illness as a risk factor for enamel hypoplasia. Results indicated that those independent variables that were significantly associated with enamel hypoplasia included maternal serum calcium levels during pregnancy, margarine intake, and maternal awareness of vitamin D, while not drinking milk often just failed to reach the threshold for significance (p=.057).

Table 5.12(a) – Logistic regression for enamel hypoplasia* – Final model

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Calcium	-5.53	2.61	0.10	0.57	0.35, 1.52	**0.034**
Drink milk (reference: < often)	-1.07	0.56		0.34	0.11, 1.03	.057
Eat margarine (reference: < often)	-1.14	0.51		0.32	0.12, 0.86	**.024**
No one with full-time employment in household (reference: no)	1.43	0.83		4.16	0.82, 21.03	.085
Heard of vitamin D (reference: no)	-1.03	0.49		0.36	0.14, 0.94	**.036**
Serious medical problem(s) (reference: no)	0.038	0.55		1.04	0.36, 3.03	.94
25(OH)D	-0.0017	0.011	24.44	0.96	0.89, 1.04	.87

*Enamel Hypoplasia reference = yes R^2= 19.9%

When the variable of serious medical problem(s) during infancy was removed from the model (since it was not significant in Table 5.11), these same variables identified in Table 5.12(a) remained significant. However, not drinking milk often did become statistically significant (p=.05). Overall, lower calcium concentrations were associated with enamel hypoplasia. Infants of mothers who lived in a household where no one was employed full-time during pregnancy were at greater risk for having enamel hypoplasia while those whose mothers ate margarine, drank milk frequently, and had

heard of vitamin D were significantly less likely to display enamel hypoplasia in their primary teeth.

Table 5.12(b) – Backwards logistic regression for enamel hypoplasia* – Final model

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Calcium	-5.57	2.57	0.10	0.57	0.35, 0.95	**.030**
No one with full-time employment in household (reference: no)	1.47	0.80		4.33	0.91, 20.69	.066
Drink milk (reference: < often)	-1.11	0.52		0.33	0.12, 0.92	**.034**
Eat margarine (reference: < often)	-1.14	0.51		0.32	0.12, 0.86	**.023**
Heard of vitamin D (reference: no)	-1.04	0.49		0.36	0.14, 0.92	**.033**

*Enamel Hypoplasia reference = yes

Backwards logistic regression analysis for enamel hypoplasia using the same variables included in Table 5.12(a) revealed that the final iteration contained five of these variables, but only four were significantly associated with hypoplastic defects (Table 5.12(b)). The five remaining variables included low maternal calcium levels (p=.030), not having heard of vitamin D (p=.033), not drinking milk frequently (p=.034), and not using margarine daily (p=.023), and no household full-time employment during pregnancy (p=.066).

III. Multivariate Regression Analysis for ECC and Caries Rate

The final section of this chapter examined regression relationships between independent variables collected from both prenatal and infant stages of this longitudinal cohort study and the dependent outcomes of ECC and caries tooth rates (dt). Because of the small sample size of maternal-infant dyads returning to complete this longitudinal study, several separate logistic regression models were constructed. One model explored the relationship between ECC in infants and serum metabolites of mothers during pregnancy, when primary teeth begin to develop and calcify. Other models included several well known risk factors for ECC such as the presence of enamel hypoplasia, age of the child, household employment, and infant feeding practices.

Table 5.13 reports results from a logistic regression analysis performed for ECC that only examined the serum metabolites of 25(OH)D, calcium, alkaline phosphatase, and phosphorus that were obtained from women during pregnancy. Results from this small model suggested no particular association between these four metabolites with the presence of ECC in infants. However, backwards logistic regression for ECC with these same variables was performed. Results of this model (not shown) revealed that 25(OH)D was the only metabolite remaining in the last iteration of the model as it approached the threshold of being statistically significant with ECC (p=.063).

Table 5.13 – Logistic regression for ECC* (excluding white spot lesions) – Serum metabolites of alkaline phosphatase, calcium, phosphorus, and 25(OH)D

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Alkaline Phosphatase	-0.001	0.005	51.79	0.95	0.36, 0.99	.81
Calcium	-2.06	2.36	0.10	0.81	0.49, 1.29	.38
Phosphorus	-0.76	1.22	0.19	0.86	0.24, 0.61	.53
25(OH)D	-0.016	0.009	24.44	0.67	0.55, 1.36	.088

*ECC reference = yes R^2= 4.2%

Another regression model was constructed to explore the associations between factors influencing endogenous synthesis and exogenous attainment of vitamin D and ECC. This model included milk and margarine intakes during pregnancy, frequency of prenatal vitamins use, season of blood sample collection, and the administration of vitamin D drops to the child during infancy (Table 5.14). Results revealed that only milk consumption was significantly associated with ECC with frequent milk intake reducing the risk for ECC (p=.024). This was also confirmed through backwards logistic regression as drinking milk was the only variable remaining in the final iteration of the model and was significantly associated with ECC (p=.026) (data not shown).

Table 5.14 – Logistic regression for ECC* (excluding white spot lesions) – Factors influencing vitamin D status

Variable	Regression Coefficient (b)	Standard Error b	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Drink milk (reference: < often)	-1.31	0.58	0.27	0.086, 0.84	**.024**
Eat Margarine (reference: < often)	0.50	0.57	1.64	0.54, 5.04	.38
Frequency of prenatal vitamin use (reference: < often)	0.30	0.57	1.35	0.44, 4.14	.61
Season (reference: summer)	-0.18	0.57	0.84	0.27, 2.58	.76
Infant received vitamin D drops (reference: no)	0.60	0.65	1.81	0.51, 6.49	.36

*ECC reference = yes R^2= 8.6%

An additional model was constructed to examine the relationship between infant feeding practices and ECC in infants (Table 5.15). Traditionally, these infant feeding methods have often been implicated in contributing to the onset of decay in the primary dentition of infants. Two variables for infant feeding were created to handle exclusive bottle feeding, exclusive breastfeeding, and mixed feeding methods, with mixed feeding serving as the reference category. The model also included sippy cup use. None of these variables were significantly associated with ECC.

Table 5.15 – Logistic regression for ECC* (excluding white spot lesions) – Infant feeding practices

Variable	Regression Coefficient (b)	Standard Error b	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Infant Feeding (Bottle) (reference: mixed)	-0.31	0.46	0.73	0.30, 1.82	.51
Infant Feeding (Breast) (reference: mixed)	-0.74	1.25	0.48	0.041, 5.57	.56
Sippy cup use (reference: no)	-1.28	0.75	0.28	0.064, 1.21	.088

*ECC reference = yes R^2= 9.9%

Additional items such as the age when solid foods were first introduced to the child and soother use were added to the logistic regression model appearing in Table 5.15 to examine their influence on the presence of ECC (Table 5.16). None of the independent variables in this model were found to be significantly associated with the presence of ECC.

Table 5.16 – Logistic regression for ECC* (excluding white spot lesions) – Infant feeding practices and soother use

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Age solids introduced (months)	0.027	0.080	2.82	0.93	0.60, 1.44	.74
Infant Feeding (Bottle) (reference: mixed)	-0.20	0.49		0.82	0.31, 2.16	.68
Infant Feeding (Breast) (reference: mixed)	-0.80	1.27		0.45	0.038, 5.39	.53
Soother use (reference: no)	-0.40	0.47		0.67	0.27, 1.67	.39
Sippy cup use (reference: no)	-1.02	0.79		0.36	0.076, 1.71	.20

*ECC reference = yes R^2= 6.9%

Table 5.17 presents results from the logistic regression model that examined the relationship between personal and family finances and ECC. Specifically, it included both the annual income of the mother and overall household employment status of the mother during pregnancy. Results indicate that mothers with an annual income < $18,000 per year were not significantly more likely to have an infant with caries (p=.058). However, those who were from a household where someone was not employed full-time were over three times more likely to have a child with ECC (p=.031). Similar results also emerged from the backwards logistic regression model that included these same variables plus receiving government assistance. The final iteration of this backwards regression

only included annual income and overall household employment with both being significantly associated with ECC (p=.049 and p=.017, respectively) (data not shown).

Table 5.17 – Logistic regression for ECC* (excluding white spot lesions) – Finances and full-time employment

Variable	Regression Coefficient (b)	Standard Error b	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Low annual income (reference: > $18,000)	-1.90	1.00	0.15	0.021, 1.07	.058
No one with full-time employment in household (reference: no)	1.31	0.60	3.70	1.13, 12.09	**.031**

*ECC reference = yes R^2= 6.7%

Another model examined the relationship between ECC and the dental status of the infants and their family's dental history and awareness (not shown). Initially, variables in this model were whether the infant had enamel hypoplasia, their age at the time of the dental examination (dichotomized), whether the infant had experienced a dental problem, whether the infant's teeth were being brushed and cleaned, mother's attitude of whether ECC is preventable and if the infant's siblings had been diagnosed with ECC. However, since enamel hypoplasia is the strongest single predictor of ECC, enamel hypoplasia was removed from the model (Table 5.18). As reported earlier, infants with enamel hypoplasia were eight times more likely to suffer from ECC. Results from Table 5.18 indicate that infants who were ≥ 14 months old were 5.8 (1/0.17) times more likely to have ECC (p=.047).

Table 5.18 – Logistic regression for ECC* (excluding white spot lesions) and dental status and behaviour of child and his/her family

Variable	Regression Coefficient (b)	Standard Error b	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Other siblings had ECC (reference: no)	1.58	0.96	4.87	0.74, 31.66	.10
Infant's teeth being cleaned or brushed (reference: no)	-0.65	1.17	0.52	0.053, 5.10	.57
Child had dental problem (reference: no)	1.50	1.19	4.48	0.43, 46.16	.21
Infant's age at time of dental examination (reference: ≥ 14 months)	-1.76	0.89	0.17	0.030, 0.98	**.047**
Mother believed ECC preventable (reference: no)	-0.19	1.16	0.82	0.084, 8.08	.87

*ECC reference = yes R^2= 60.7%

An overall regression model was constructed that only included those variables that were significantly associated with ECC in the earlier models in this Chapter. 25(OH)D was also added to this model as it was of particular interest in this prospective study. Results appearing Table 5.19(a) reveal that the presence of enamel hypoplasia and the age of the infant at the time of the dental assessment for this study were significantly associated with ECC. While not statistically significant, 25(OH)D did approach the threshold of significance. However, interesting results were found when a backwards logistic regression using these same variables was performed. The final iteration of the model retained three of these variables, all showing a significant association with ECC;

enamel hypoplasia (p<.001), infant age (p=.002), and lower 25(OH)D levels during pregnancy (p=.019) (Table 5.19(b)).

Table 5.19(a) – Logistic regression for ECC* (excluding white spot lesions) – Significant variables in this chapter

Variable	**Regression Coefficient (b)**	**Standard Error b**	**Standard Deviation of Variable in Sample**	**Adjusted Odds Ratio**	**± 95% Confidence Interval**	**P value**
Low annual income (reference: > $18,000)	-2.19	1.59		0.11	0.005, 2.51	.17
Drink milk (reference: < often)	-0.35	0.57		0.71	0.23, 2.18	.55
Enamel hypoplasia (reference: no)	2.05	0.60		7.73	2.41, 24.84	**.0006**
No one with full-time employment in household (reference: no)	0.91	0.86		2.49	0.46, 13.39	.29
Infant's age at time of dental examination (reference: ≥ 14 months)	-1.67	0.57		0.19	0.061, 0.57	**.0034**
25(OH)D	-0.022	0.012	24.44	0.59	0.32, 1.06	.077

*ECC reference = yes R^2= 29.7%

Table 5.19(b) – Backwards logistic regression for ECC* (excluding white spot lesions) – Significant variables in this chapter

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Enamel hypoplasia (reference: no)	2.33	0.57		10.30	3.37, 31.49	**<.0001**
Infant's age at time of dental examination (reference: ≥ 14 months)	-1.74	0.56		0.18	0.059, 0.52	**.0020**
25(OH)D	-0.027	0.012	24.44	0.52	0.29, 0.92	**.019**

*ECC reference = yes

A final and more exploratory model for ECC was constructed including 11 different variables collected either from serum analysis or interview at the time of enrollment or obtained during the infant clinical examination visit (Table 5.20(a)). Some variables were reported to be significantly associated with ECC in this chapter. Other variables were either significant at the bivariate level or are commonly identifiable contributors to increased risk for ECC. Some other variables that influence vitamin D status were also included. Results indicated that the presence of enamel hypoplasia was strongly associated with ECC (p=.0011) and that the age of infants at the time of their dental examination was also significantly associated with ECC, with those being 14 months of age or older at the time of the dental assessment being more likely to have ECC. Finally, 25(OH)D levels of mothers during pregnancy were also found to be significantly associated with ECC (p=.049). As with other models presented in this

chapter, backwards logistic regression analysis was also performed with these same variables included in the expanded model reported in Table 5.20(a). The final backwards regression model only included three variables, namely, enamel hypoplasia, infant age, and maternal 25(OH)D levels during pregnancy (Table 5.20(b)). All were found to be significantly associated with ECC in infants; enamel hypoplasia ($p<.001$), infant age ($p=.001$), and vitamin D levels ($p=.015$).

Table 5.20(a) – Logistic regression for ECC* (excluding white spot lesions) – Final model

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Low annual income (reference: > $18,000)	-2.47	1.49		0.085	0.0045, 1.57	.098
Child health (reference: < very-good)	-0.61	0.60		0.55	0.17, 1.76	.31
Infant's teeth being cleaned or brushed (reference: no)	1.29	1.04		3.63	0.47, 28.07	.22
Drink milk (reference: < often)	-0.36	0.60		0.70	0.21, 2.29	.55
Enamel hypoplasia (reference: no)	2.18	0.67		8.89	2.40, 32.87	**.0011**
No one with full-time employment in household (reference: no)	0.99	0.91		2.70	0.45, 16.24	.28
Government assistance (reference: no)	-0.48	0.60		0.62	0.19, 1.99	.42
Infant age at time of dental examination (reference: ≥ 14 months)	-1.60	0.62		0.20	0.060, 0.68	**.0098**
Infant feeding (Bottle) (reference: mixed)	0.25	0.64		1.28	0.36, 4.51	.70
Infant feeding (Breast) (reference: mixed)	-0.14	1.62		0.87	0.037, 20.63	.93
Season (reference: summer)	-0.40	0.62		0.67	0.20, 2.27	.52
25(OH)D	-0.029	0.015	24.44	2.02	1.00, 4.08	**.049**

*ECC reference = yes R^2= 32.9%

Table 5.20(b) – Backwards logistic regression for ECC* (excluding white spot lesions) – Final model

Variable	Regression Coefficient (b)	Standard Error b	Standard Deviation of Variable in Sample	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Enamel hypoplasia (reference: no)	2.30	0.57		9.97	3.25, 30.61	**<.001**
Infant age at time of dental examination (reference: ≥ 14 months)	-1.78	0.56		0.17	0.056, 0.51	**.001**
25(OH)D	-0.029	0.012	24.44	0.49	0.52, 0.87	**.015**

*ECC reference = yes

Finally, Poisson regression models were constructed to examine potential risk factors for caries tooth rates (dt) of infants participating in this longitudinal study. In the first model, serum metabolites of calcium, alkaline phosphatase, phosphorus, and 25(OH)D were included (Table 5.21). Results indicated that vitamin D levels during pregnancy were significantly associated with infant caries rates (p=.0005). Similarly, maternal calcium levels were also significantly associated with the dt score (p=.0018). Lower maternal 25(OH)D and calcium levels were associated with higher dt scores. Alkaline phosphatase was also significantly associated with the dt score; higher alkaline phosphatase concentrations were associated with a greater number of primary teeth with untreated decay.

Table 5.21 – Poisson regression for dt (caries tooth rate) and metabolites

Variable	Regression Coefficient	± 95% Confidence Interval	P value
Intercept	6.20		
Alkaline Phosphatase	0.0030	0.0026	**.023**
Calcium	-2.12	1.71	**.0018**
Phosphorus	0.32	0.81	.44
25(OH)D	-0.012	0.0067	**<.0010**

Poisson regression was also used to examine the relationship between the average number of infant primary teeth that were affected by decay (dt) and both maternal levels of vitamin D during pregnancy and the presence of enamel hypoplasia in the primary dentition. Both enamel hypoplasia and vitamin D were found to be significantly associated with the rate of untreated decay (dt) (<.001 and p=.0037, respectively). Higher vitamin D levels during pregnancy were inversely related to the number of primary teeth affected by caries.

The following examples demonstrate this relationship for three different thresholds of vitamin D and the presence or absence of enamel hypoplasia by predicting the number of primary teeth with decay (Figure 5.1):

Enamel hypoplasia (Yes = 1, No = 0)

Vitamin D Levels:

1) 25 nmol/L, associated with severe deficiency
2) 50 nmol/L, the mean value for vitamin D for mothers in this Poisson regression
3) 80 nmol/L, associated with vitamin D adequacy

If enamel hypoplasia is present and vitamin D = 25nmol/L:

$dt = e^{(\text{B intercept})} \times e^{(\text{B enamel hypoplasia})(\text{enamel hypoplasia})} \times e^{(\text{B vitamin D})(\text{vitamin D level})}$

$= e^{(0.32)} \times e^{(1.14)(1)} \times e^{(-0.011)(25)}$

$= 1.38 \times 3.13 \times 0.77 = 3.30$ teeth affected with caries

If enamel hypoplasia is absent and vitamin D = 25nmol/L:

$dt = e^{(\text{B intercept})} \times e^{(\text{B enamel hypoplasia})(\text{enamel hypoplasia})} \times e^{(\text{B vitamin D})(\text{vitamin D level})}$

$= e^{(0.32)} \times e^{(1.14)(0)} \times e^{(-0.011)(25)}$

$= 1.38 \times 1 \times 0.77 = 1.06$ teeth affected with caries

If enamel hypoplasia is present and vitamin D = 50nmol/L:

$dt = e^{(\text{B intercept})} \times e^{(\text{B enamel hypoplasia})(\text{enamel hypoplasia})} \times e^{(\text{B vitamin D})(\text{vitamin D level})}$

$= e^{(0.32)} \times e^{(1.14)(1)} \times e^{(-0.011)(50)}$

$= 1.38 \times 3.13 \times 0.59 = 2.53$ teeth affected with caries

If enamel hypoplasia is absent and vitamin D = 50nmol/L:

$dt = e^{(\text{B intercept})} \times e^{(\text{B enamel hypoplasia})(\text{enamel hypoplasia})} \times e^{(\text{B vitamin D})(\text{vitamin D level})}$

$= e^{(0.32)} \times e^{(1.14)(0)} \times e^{(-0.011)(50)}$

$= 1.38 \times 1 \times 0.59 = 0.81$ teeth affected with caries

If enamel hypoplasia is present and vitamin D = 80nmol/L:

$dt = e^{(B\ intercept)} \times e^{(B\ enamel\ hypoplasia)(enamel\ hypoplasia)} \times e^{(B\ vitamin\ D)(vitamin\ D\ level)}$

$= e^{(0.32)} \times e^{(1.14)(1)} \times e^{(-0.011)(80)}$

$= 1.38 \times 3.13 \times 0.42 = 1.83$ teeth affected with caries

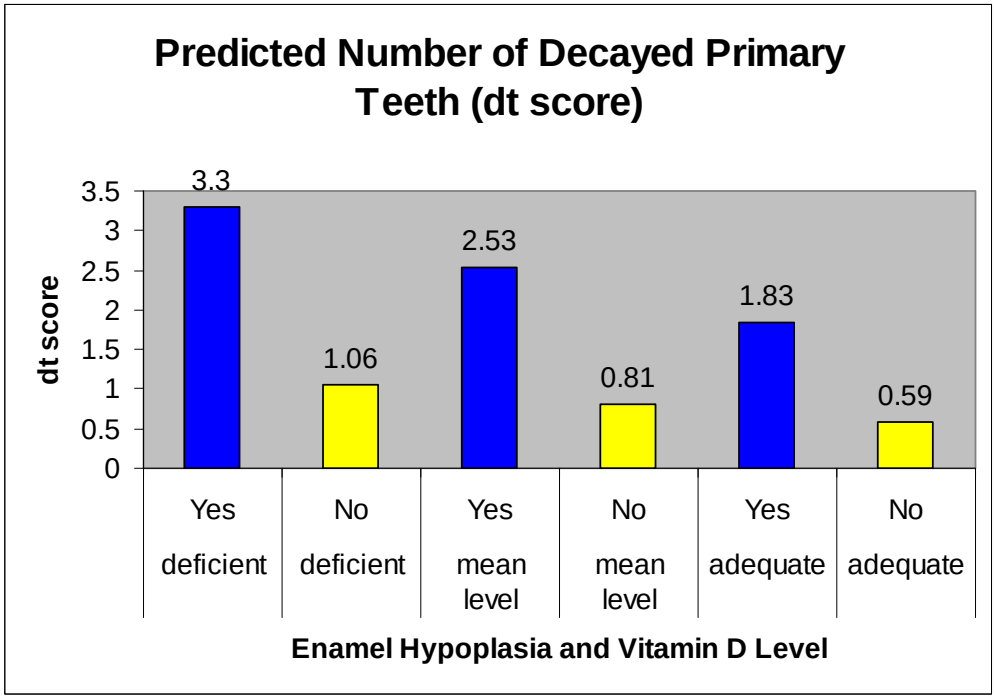
If enamel hypoplasia is absent and vitamin D = 80nmol/L:

$dt = e^{(B\ intercept)} \times e^{(B\ enamel\ hypoplasia)(enamel\ hypoplasia)} \times e^{(B\ vitamin\ D)(vitamin\ D\ level)}$

$= e^{(0.32)} \times e^{(1.14)(0)} \times e^{(-0.011)(80)}$

$= 1.38 \times 1 \times 0.42 = 0.59$ teeth affected with caries

Figure 5.1 – Predicted number of decayed primary teeth by 25(OH)D level and enamel hypoplasia status



The final Poisson regression model (Table 5.22) included those same independent variables that appeared in the expanded logistic regression model for ECC. Results reveal

that infant age, the presence of enamel hypoplasia, and maternal 25(OH)D levels during pregnancy were significantly associated with the presence of ECC in infants. The association between ECC and the health of the child was also significant (p=.037).

Table 5.22 – Poisson regression for dt (caries tooth rate)

Variable	**Regression Coefficient**	**± 95% Confidence Interval**	**P value**
Intercept	1.68		
Low annual income (reference: <$18K)	-0.28	0.73	.45
Child health (reference: < very-good)	-0.35	0.33	**.037**
Infant's teeth being cleaned or brushed (reference: no)	-0.13	0.51	.60
Drink milk (reference: < often)	0.054	0.36	.77
Enamel hypoplasia (reference: no)	1.02	0.37	**<.001**
No one with full-time employment in household (reference: no)	0.39	0.59	.20
Government assistance (reference: no)	0.13	0.38	.50
Infant age at time of dental examination (reference: ≥ 14 months)	-1.03	0.38	**<.001**
Infant feeding (Bottle) (reference: mixed)	0.031	0.37	.87
Infant feeding (Breast) (reference: mixed)	-0.53	0.81	.20
Season (reference: summer)	-0.32	0.42	.13
25(OH)D	-0.013	0.0085	**.0021**

Since maternal calcium levels were found to be significantly inversely associated with infant dt scores (Table 5.21) this variable was also added to the Poisson regression model appearing in 5.22. Results of this model (data not shown) indicated that enamel hypoplasia, infant age of ≥ 14 months, and maternal vitamin D levels were significantly and independently associated with untreated primary tooth decay rates (dt) ($p<.001$, $p<.001$, and $p=.0074$, respectively).

Chapter 6 – Discussion

There is no doubt that optimal vitamin levels are associated with improved health outcomes. It also appears that oral health is influenced by vitamin D concentrations (as reviewed in Chapter 1, Section 3). For instance, we know that vitamin D regulates the body's use of calcium and plays a key role in craniofacial development and the maintenance of good oral health. Vitamin D has a role in enamel and dentin formation and also is associated with periodontal and oral bone health.[1,2] Higher serum levels of 25(OH)D are generally associated with improved periodontal health outcomes for adults.

Several early studies identified a connection between vitamin D fortified diets and caries and enamel hypoplasia in school aged children. As reviewed in Chapter 1, Section 3, research from the first half of the twentieth century suggested that school-aged children receiving cod liver oil on a daily basis had a lower incidence of caries in both their primary and permanent dentition after two years.[3] In addition, children receiving radiostol (vitamin D) had significantly lower incidence and extent of caries in the permanent dentition than controls.[3] Further, cod liver oil and sunbathing during infancy and early childhood was shown to be effective in decreasing the incidence of enamel hypoplasia in the permanent dentition.[4]

Unfortunately, most of these studies were not controlled trials and did not involve large samples of children needed for statistical power. They also did not correlate actual 25(OH)D levels with caries or developmental defects of enamel (DDE) like enamel hypoplasia. Few studies have reported associations between vitamin D status (e.g. neonatal tetany as a result of maternal vitamin D deficiency, chronic disorders of calcium and phosphate homeostasis like Vitamin D Dependent Rickets (VDDR), privational

vitamin D deficiency, and rickets)[5-9] or supplementation during periods of tooth development and enamel hypoplasia.[10,11] However, no study has ever examined the relationship between actual circulating levels of 25(OH)D during periods of tooth development and outcomes like enamel hypoplasia and caries. Although one study reported that maternal supplementation of vitamin D during pregnancy lowered the incidence of enamel hypoplasia, a risk factor in the development of caries, their analysis was restricted to a very small subset of the entire study cohort.[10] Further, their assessments for hypoplasia were not all performed during infancy and the statistics used were limited.[10] This was only reported at the bivariate level and not confirmed through multivariate analysis. Therefore, low vitamin D levels during this period are a plausible risk factor for enamel hypoplasia and may theoretically increase the risk for caries.

The purpose of this longitudinal blinded study was to determine the vitamin D status of a cohort of pregnant urban women and to determine whether there was an association with the presence of enamel hypoplasia and dental caries in the primary teeth of their offspring. A total of 207 women were enrolled into this prospective study. The majority were of self-declared Aboriginal heritage residing in urban Winnipeg, Canada. Many were young women with this being their first pregnancy. The first phase of this study involved a serum draw and completion of an interviewed questionnaire. Overall, nearly two-thirds of the cohort (64.3%) was maintained for the second phase of this study. A total of 135 infants returned for the dental examination by an examiner blind to the mother's vitamin D level during pregnancy. Attrition of the cohort was expected at the outset of the study and considering the different life challenges faced by many of

these participants the loss of one-third of the cohort was not unexpected. However, as discussed later in this chapter it still is a limitation of this study.

Determining the vitamin D status of these participants was a key objective of this prospective investigation. Very few participants indicated that they took prenatal vitamins during pregnancy even though many did report that they believed this to be important for good prenatal health. One possible explanation for this disconnect may be the fact that several participants were recruited at their first prenatal visit and might not have previously received any advice on the need to add supplements to their diets. However, it does signify a need to ensure that consistent and early messaging on vitamin use during the prenatal period is provided to all expectant women from their primary caregiver. Many in this study indicated that they did not receive any such advice. A recent article on the vitamin D status of Albertans suggests that the medical community has been slow to incorporate new revelations on vitamin D and health into practice.[12] Physicians may not be fully aware of the latest Canadian Paediatric Society (CPS) recommendations on supplementation.[13] Unfortunately, these recommended intakes are still too low to have any real effect.[14]

Overall, there appeared to be a relatively low level of participant knowledge about vitamin D and its role in health. While nearly two-thirds of respondents had heard of vitamin D less than 25% correctly stated what vitamin D was important for. Even fewer could identify dietary sources of vitamin D. There is uncertainty about whether vitamin D awareness is lacking only in this segment of the population or whether overall public awareness is low. One possible way to improve and sustain maternal vitamin D levels during pregnancy may be to raise awareness of vitamin D; its role in health and also ways

to obtain it. Health literacy may be an issue and may need to be part of any preventive strategy. Fortunately, Manitoba Health is now funding a demonstration project to provide expectant women in urban Winnipeg with high doses of vitamin D_3 (100,000 IU) during pregnancy in an attempt to improve perinatal and infant health.

The mean 25(OH)D level for participants in this study was 48.1 ± 24.4 nmol/L; ranging from 4.7 to 145.0 nmol/L. The relatively low mean vitamin D levels in this study is concerning although it mirrors levels recently reported for expectant women in southern Manitoba.[15,16] Vitamin D deficiencies, defined as levels < 37.5 nmol/L, were present in 46% of mothers and 36% of infants at the time of delivery at the Health Sciences Centre in Winnipeg.[15] Deficiencies were more prevalent among non-white women.[15] The average vitamin D level for participants in this prospective investigation are similar to wintertime vitamin D concentrations for a small group of nonwhite women (e.g. Asian, Aboriginal, and Indo-Asian) participating in a study in Toronto, Canada.[17]

Data from a recent case-control pilot study contrasting Winnipeg preschool children with Severe Early Childhood Caries (S-ECC) and those caries-free reported an average 25(OH)D of 59.8 ± 21.5 nmol/L (range 29 – 105 nmol/L) during the months of August and September.[18] However, the mean 25(OH)D level of women enrolled into this study were still higher than concentrations of expectant mothers residing in three northern First Nations communities during the late 1990s.[19] Median 25(OH)D concentrations for St. Theresa Point, Garden Hill, and Norway House were 21 nmol/L, 18 nmo/L, and 24 nmol/L, respectively.

Meanwhile, results from the current Canadian Health Measures Survey by Statistics Canada reveals higher average vitamin D levels among participants age six to

70 years (66.9 nmol/L) than what is reported in this longitudinal cohort investigation.[20] In general, vitamin D deficiency appears to be a growing concern among pregnant women in Canada as the ability to endogenously produce vitamin D is restricted to only a few months during the summer. The need for vitamin D supplementation is quite apparent. In addition, these participants may have future increased risk for other chronic health conditions.[21]

Many participants exhibited vitamin D levels consistent with vitamin D deficiency, depending on the different thresholds proposed. A recent recommendation to adopt values < 80 nmol/L as evidence of vitamin D deficieny is based on the inverse relationship between 25(OH)D and parathyroid hormone (PTH) levels; the threshold where PTH levels begin to plateau. When this threshold was applied to our participants, 90% were found to have deficient concentrations. This is somewhat alarming given the fact that the developing fetus is entirely dependent on maternal vitamin D concentrations. However, even when the different thresholds for deficiency that appeared in Chapter 3 were applied there were still a considerable proportion of participants that had levels in the deficient range; 46.5% (≤ 40 nmol/L) and 35.0% (≤ 35 nmol/L).

Questionnaire responses relating to milk consumption were encouraging since it is vitamin D fortified. Half of those women recruited into this study were consuming milk on a daily basis while another one-third was drinking milk a few times per week. This is a positive sign considering concerns over possible lactose intolerance in some Aboriginal populations. Milk is a good source of vitamin D for women residing in urban centres, where milk is generally affordable compared with extreme prices in the northern regions of Canada.[22] Apart from dietary supplements, milk use may be a good means of obtaining

vitamin D from the diet as dietary intake of other vitamin D containing foods like fish and yogurt are not consumed as frequently. A recent Canadian study reported that the number of glasses of milk consumed daily was a strong determinant of overall vitamin D levels.[12]

The majority of participants were single at the time of recruitment into the study and most had not completed high school (92.2%). This further reinforces the different life challenges faced by many young urban women and their vulnerability. Educational levels likely influence oral health literacy, and may pose another challenge in reaching young expectant mothers with health promotional materials that are clearly understood and easy to follow in the home setting with infants.

Most participants in this study admitted to previously hearing of Early Childhood Caries (ECC) (77%). However, considering the socioeconomic and ethnic composition of this cohort, this comes as little surprise. The prevalence of ECC among urban disadvantaged groups in Manitoba is similar to that for some northern populations.[23,24] While 90.3% in this study felt that their own dental health was important during pregnancy, only 38.5% rated their oral health as being good. Further, 36.1% reported having dental problems at the time of enrollment. This suggests that oral health may be a concern for many expectant mothers. Access to care may be limited and other life challenges may make it difficult to seek dental care. This may even become more limited during pregnancy. With emerging evidence that maternal oral health during pregnancy may be associated with negative birth outcomes and the fact that cariogenic bacteria are most commonly transmitted vertically from mother to infant, improving the oral health status of high-risk women is important.[25-27]

The vast majority of infants returning for follow-up were reported to be in good health. Surprisingly 12.7% of infants returning for the second phase of this study were reported to be premature and 4.6% were of low birth weight at birth. The incidence of preterm birth in this cohort appears to be higher than provincial statistics.[28] Between the 2001/02 and 2005/06 fiscal years the reported preterm birth rate was 7.7%.[28] The high rate in our study may be a reflection of the lower socioeconomic background of many of our participants. In fact, preterm birth rates for the Winnipeg neighbourhoods of Point Douglas and Downtown range from 9.4% to 10%.[28] Very few infants in this study had any medical issues although 16 children were reported to have signs of early asthma. This was likely wheezing rather than specialist diagnosed asthma.

Infant feeding behaviours revealed that many were breastfed, although for those who had already been weaned at the time of dental examination, breastfeeding ceased before four months of age. Very few were still being breastfed at the time of their dental examination. The data reveal that bottle feeding was routine as a feeding method in this birth cohort. The majority were still bottle fed at the time of the dental follow-up examination as only 21 were weaned from the bottle. Common bottle contents included formula, water, milk and, apple juice. Such infant feeding practices have been reported to increase a child's risk of developing ECC. However, we found no relationship between infant feeding practice and caries status in offspring of women enrolled into this investigation.

A considerable number of the mothers in this study were living independently and many moved during the period from enrollment into the cohort until the infant dental examination visit. The pressures of single parenting combined with the lack of a stable

home environment can be determinants of childhood health and well-being during early childhood.

A total of 135 infants underwent a dental screening examination at an average age of 16.1 months. Since this longitudinal cohort study involved a considerable proportion of Aboriginal participants it provides a unique snapshot of the prevalence of enamel hypoplasia and incidence of ECC in this population in the Winnipeg region. Not surprising, teeth showing the greatest prevalence of DDEs were the primary maxillary incisors as incisors are the only erupted teeth at this age. Approximately one-fifth of infants examined had evidence of enamel hypopolasia in their primary teeth. An epidemiological study partnering with a northern Manitoba First Nation reported that 50% of the preschool cohort had obvious signs of hypoplastic enamel.[22] A study involving Australian Indigenous preschool children reported that virtually all children (98%) had at least one tooth affected by an enamel defect, either an opacity or hypoplasia.[29] On average, children in that study had at least four primary teeth with hypoplasia.[29] Another study by the same investigator reported a much lower prevalence of DDE; only 20% of this small sample of Aborigine were affected.[30] This is closer to the prevalence reported in this prospective Canadian birth cohort study.

A total of 31 infants (23.0%) were determined to have ECC when using cavitated lesions as the definition for caries. When incipient (i.e. white spot) lesions were included, 49 infants were found to have ECC. New efforts have attempted to standardize caries diagnosis, classification, and reporting for epidemiological purposes.[31] The newly proposed International Caries Detection and Assessment System (ICDAS) allows for early incipiencies be recorded as decay.[32] Although these incipient lesions may be

remineralized with fluoride they still serve as markers of considerable cariogenic activity and risk for the young child. The incidence of ECC in this infant cohort is high compared to that of urban dwelling Canadian children of similar ages.[33] However, the social and physical environment in which these children reside may be contributing to their heightened risk. In fact, the incidence of ECC is comparable to previously reported prevalence rates of ECC in Manitoba. A 2005 study reported a prevalence of ECC of 30.4% among infants < 24 months of age.[23] The average deft score was 1.47 ± 2.80 (range 0-17) with over 80% being attributable to the decayed teeth score (dt). The data reveal that a minority of children bore the bulk of the caries burden in this cohort. Very few had undergone treatment for ECC.

A more rampant form of ECC is S-ECC. This sub-classification is reserved for children with more severe forms of caries, and is site, pattern, and age specific.[34,35] However, any caries experience, even one isolated decayed smooth tooth surface is enough to garner the diagnosis of S-ECC among children under 36 months of age. This in fact means that 23% of the infants in this study actually met the criteria for S-ECC at a mean age of 16.1 months. This is concerning as children with S-ECC frequently need to undergo rehabilitative treatment in hospital under general anesthesia (GA), primarily because of their young ages and the volume of dental treatment they require.

Correlation analysis revealed that there was no statistically significant relationship between 25(OH)D levels and calcium, phosphorus, or alkaline phosphatase. The other metabolites were anticipated to be associated with vitamin D levels as others have used some of these metabolites as pseudo markers for 25(OH)D.[19,36,37] However, no such relationship was substantiated. Recent research reports that biomarkers like parathyroid

hormone, calcium absorption and bone density are good predictors of overall 25(OH)D concentrations.[38,39]

Several variables collected in this study were found to be associated with participant 25(OH)D levels. Women of Aboriginal heritage were found to have significantly lower vitamin D levels and a larger proportion was below the threshold for vitamin D deficiency than others enrolled in the study. Results of multivariate analyses further confirmed the relationship between low vitamin D levels and Aboriginal heritage when controlling for other variables including ability to purchase food, annual income, and education. The First Nations Bone Health Study has published results on the vitamin D status of Aboriginal and non-Aboriginal women (25-76 years of age) residing in urban or rural and northern regions of Manitoba.[40] The prevalence of vitamin D insufficiency (defined as 25(OH)D <37.5 nmol/L) was only 19% in urban white women, but rose to 30% in urban Aboriginal and 32% in rural Aboriginal women.[40]

Not surprising was the fact that 25(OH)D levels were dependent on the month and season of sample collection. Those who were sampled during winter periods (November to April) had significantly lower concentrations than those sampled in summer months. The influence of season on circulating vitamin D is well documented and further supported by results of multiple regression modeling in this study when controlling for other factors like milk intake and use and frequency of vitamin supplements.

Analysis of Variance (ANOVA) revealed that maternal ratings of good health, including oral health, were significantly associated with higher circulating levels of 25(OH)D. A randomized controlled trial concluded that there is a definite connection between circulating concentrations of vitamin D and a sense of well-being.[41] However,

after controlling for other health outcomes believed to be linked to low vitamin D in our study, such as difficulty walking and weakness in the limbs, multiple regression analysis was not able to support a relationship between participant ratings of health and vitamin D levels. Multiple regression analysis for 25(OH)D and possible subclinical signs of deficiency did reveal a significant relationship with weakness in the arms and legs. This is consistent with other reports and reviews that vitamin D deficiencies may be associated with loss of muscle control, strength, pain, fatigue, and balance.[21,42,43]

The frequency and use of prenatal vitamins was also found to be associated with higher serum levels of 25(OH)D. Those participants who took prenatal vitamins were significantly more likely to have vitamin D levels above all of the various proposed thresholds for vitamin D deficiency and insufficiency. While the relationship with frequency of vitamin intake was not confirmed through multiple regression analysis, statistical modeling did reveal that prenatal vitamin use, season, and milk consumption were significantly and independently associated with participant 25(OH)D levels. Those who used vitamins and drank milk had higher levels. The relationship between vitamin intake and vitamin D is not surprising since prenatal vitamins do contain vitamin D. In fact, for many North Americans dietary supplementation accounts for the greatest intake of vitamin D, even though endogenous production is recognized as the prime physiologic method of attainment for humans.

It was apparent that the only dietary practice found to be significantly associated with vitamin D status in this study population was milk consumption. No other foods were found to have a relationship with 25(OH)D. Milk is fortified with vitamin D in Canada and the United States and provides the public with additional exposure to this

important nutrient. For many, this may be the only regular and affordable source of vitamin D in the diet. Results from our regression analysis revealed that daily consumption of milk was strongly associated with higher 25(OH)D concentrations during pregnancy. This is consistent with another recent publication from western Canada.[12]

Another strong predictor of participants vitamin D levels during pregnancy was their socioeconomic status (SES). ANOVA revealed that maternal education level was associated with 25(OH)D levels; those who had not completed high school had significantly lower concentrations. This observation was also confirmed through multiple regression analysis suggesting that education and vitamin D levels are associated. Little published data exist on the influence of socioeconomics and educational achievements on 25(OH)D levels.[16] A study involving women at term in Riyadh, Saudi Arabia reported significantly higher median levels of vitamin D for mothers in the upper class than those in middle and lower classes.[44] Further, there were no cases of extreme vitamin D deficiency (defined as $\leq$ 3 ng/ml ($\approx$ 7.5 nmol/L)) among those in the upper SES group.[44] Hillman & Haddad (1976) investigated the relationship between 25(OH)D and SES by creating their own definition of social class based upon education and occupation.[45] While their study attempted to measure the association between social class and serum 25(OH)D they were unable to show that the relationship was statistically significant. Another investigation of ethnic differences of vitamin D in the Netherlands concluded that vitamin D levels were not confounded by socioeconomics of the mother.[46]

Regardless, education is a strong predictor of SES and may result in greater earning potential for women. Perhaps women with more education may make better decisions concerning their diet and nutrition and have greater purchasing ability to

acquire nutritious foods. While the income of participants and their households was found to be associated with vitamin D levels and vitamin D deficiency on bivariate analyses, this was not confirmed through multiple regression analysis controlling for other socioeconomic factors like education, Aboriginal heritage, and receiving financial assistance from friends and family.

A final prominent factor influencing prenatal vitamin D concentrations included the season during which the serum sample was obtained. Multiple regression analysis for 25(OH)D concentration revealed that season had a significant influence when also controlling for milk consumption and use of prenatal vitamins. Those who had samples taken during winter periods had significantly lower levels. This is not a new finding, but does reinforce the overwhelming contribution endogenous synthesis can have on vitamin D attainment.

Infant oral health was another key outcome of interest in this longitudinal investigation. Very few studies have examined the influence of the prenatal period and environment on early childhood dental status. Bivariate analysis revealed that there was no apparent relationship between prenatal vitamin D levels of participants and the presence of the very broad classification of DDE in their infants. Similarly, maternal vitamin D levels did not appear to be associated with the presence of enamel hypopolasia in infants. However, a one-tailed t test revealed that mothers of infants with enamel hypoplasia had slightly lower concentrations of 25(OH)D than those whose infants did not have hypoplastic enamel (43.2 ± 21.1 nmol/L vs. 51.4 ± 27.4, p=.072). This did approach the threshold of significance set for this prospective study and would suggest that our original hypothesis may be plausible. This is the first investigation to report on

this potential relationship with actual circulating 25(OH)D levels. The study by Cockburn and colleagues did report significantly less enamel hypoplasia among infants whose mothers regularly took vitamin D supplements during pregnancy.[10] As discussed in Chapter 1, Section 3, only a small subset of children in that study underwent the dental examination and the statistics used may have been limited.

Several other variables were reported to be associated with the presence of enamel hypoplasia in this study. As enamel hypoplasia directly correlates with periods of tooth formation and maturation, we restricted our statistical analyses to those factors corresponding to the prenatal and birth periods. Some of the prenatal variables associated with enamel hypoplasia included not having heard of vitamin D, infrequent milk intake, infrequent margarine use, and no recommendation from their physician to take vitamins during pregnancy. Results of logistic regression were able to further substantiate these relationships. We undertook several smaller regression models for enamel hypoplasia based upon the themes of prenatal care and diet, SES and ethnicity, and awareness of calcium and vitamin D.

The final model for enamel hypoplasia revealed that infrequent milk consumption and infrequent margarine use were significantly associated with increased risk. It is possible that mothers who consumed little of these two fortified dietary items had lower maternal vitamin D concentrations since the regression model for vitamin D did confirm milk intake as a significant predictor for 25(OH)D. This potentially may have resulted in disturbances of primary enamel formation during fetal development. Additionally, awareness of vitamin D also appeared to be related to enamel hypoplasia. Those with little awareness of vitamin D were more likely to have infants with enamel hypoplastic

defects. Low awareness of vitamin D may be a proxy measure for dietary and supplemental intake of vitamin D. These individuals may not be consciously looking for foods rich in vitamin D when they shop for groceries and may not be taking supplements. It is plausible that women who are generally unaware of vitamin D, its sources, and its role in health have lower vitamin D levels, which may have an impact on their infant's oral health.

As discussed in Chapter 1, Section 2, enamel hypoplasia and other developmental defects of enamel in the primary dentition can arise from a variety of genetic conditions and disturbances resulting from infections, nutritional deficiencies and metabolic disorders, perinatal disturbances (e.g. premature birth and low birth weight), and chemical agents including antibiotics.[47-49] One particular study examined 455 exfoliated primary incisors to evaluate the influence of both antenatal and postnatal factors on enamel hypoplasia.[47] The results of that study suggest that women who first present for prenatal care following the first trimester of pregnancy are significantly more likely to have a child who displays enamel hypoplasia in the primaray dentition.[47] It also reported that children who were born premature were more likely to have enamel defects in their primary incisors.[47] This certainly reinforces the importance of early access to prenatal care as it may have an impact for infant and childhood oral health.

While others have reported that infant illness and medical conditions may be associated with an increased prevalence of hypoplastic enamel, we were unable to confirm such a finding in this prospective study. Our study collected information on possible risk factors for DDEs either directly or indirectly. However, this prospective investigation was limited as it did not directly capture information regarding specific

genetic conditions, childhood medical disorders (e.g. inherited disorders of calcium metabolism), or infections during pregnancy and infancy (e.g. infant infection during the first 35 days of life, cytomegalovirus infection, maternal rubella, and postnatal measles). When controlling for other variables in our final logistic regression model, serious childhood medical problems was not significantly associated with hypoplasia. This differs from others who did find a clear relationship (as reviewed in Chapter 1, Section 2). In hindsight, it would have been useful to ask about specific medications infants received (e.g. antibiotics including amoxicillin) and whether the child was intubated following delivery as these have been identified as contributors to DDEs.

Results of the final logistic regression model revealed that maternal levels of calcium were significantly associated with enamel hypoplasia. It appeared that participants with lower calcium levels were more likely to have infants with detectable enamel hypoplasia. Despite the fact that none of the mothers in this prospective study were found to have extremely low or elevated calcium levels (range 2.01 – 2.57, median 2.24), results at the bivariate level revealed that mothers who had infants with enamel hypoplasia had significantly lower calcium levels during pregnancy than those whose infants had no enamel defects (2.23 ± 0.10 mmol/L vs. 2.27 ± 0.10, p=.036). Further, it does concur with findings of other groups that hypocalcemia is a risk factor for enamel hypoplasia.[50,51]

Some of these relationships were confirmed through logistic regression analysis. Those who had heard of vitamin D, consumed margarine often, and drank milk often were significantly less likely to have an infant with enamel hypoplasia while controlling

for additional factors such as the child's medical history and prenatal 25(OH)D concentration.

An interesting finding from the bivariate analysis was the fact that participants who had a previous child undergo pediatric dental surgery in hospital to treat ECC had significantly lower vitamin D levels. These same participants were also significantly more likely to have infants with noticeable enamel hypoplasia. While we are unaware of these participants' vitamin D status during their previous pregnancies it is possible that their vitamin D levels would not differ greatly from one pregnancy to the next. Their dietary and lifestyle choices may be static over time. Low levels of vitamin D during a past pregnancy may have resulted in their older child having enamel hypoplasia that placed them at increased risk for dental surgery under GA because of ECC. However, this hypothesis cannot be substantiated in this current investigation.

In addition to enamel hypoplasia, statistical analyses also explored associations with the presence of both ECC and caries rates, namely the decayed tooth rate (dt score). Several small regression models were constructed for ECC. The first involved the measures of serum metabolites obtained from the blood sample provided by participants during pregnancy. No one metabolite emerged to be significantly associated with ECC; although vitamin D levels were significantly associated with ECC at the bivariate level. However, results of the backwards logistic regression including all four serum metabolites did suggest that low 25(OH)D levels during pregnancy were associated with an increased risk for ECC ($p=.063$). No other study has attempted to correlate maternal prenatal levels of these metabolites with ECC development in offspring. Another regression model for ECC controlled for factors known to have a direct influence on

vitamin D status including milk intake, season when the serum sample for vitamin D was collected, and the frequency of prenatal vitamin use. Only milk consumption emerged as being significantly associated with ECC. Participants who drank milk often during pregnancy were significantly less likely to have an infant who developed ECC (p=.024). No other variables in this model were identified as being significantly associated with ECC. Previous models in this study revealed that milk intake was a strong predictor of both maternal 25(OH)D and enamel hypoplasia.

Another two models were constructed to assess the impact of infant feeding methods on caries risk. In addition to infant feeding methods, the other regression model also included the age of the child when solids were first introduced along with soother and sippy cup use. However, none of the independent variables were found to be significantly associated with ECC in either of these two models. Recent reviews of the literature have concluded that breastfeeding is not a strong determinant of ECC and may instead offer a protective effect.[52,53] Further, results from the analysis of the 1999-2002 National Health and Nutrition Examination Survey (NHANES) for a large sample of 2-5 year olds concluded that there was no evidence to support the association of breastfeeding and its duration as independent risk factors for caries. [54]

Since ECC is known to be influenced by SES, a logistic regression model incorporated variables like the participant's annual income, whether anyone in the household had full-time employment during the mother's pregnancy, and whether participants were receiving government assistance. Chi square analysis did reveal that there were more infants with ECC from households where someone did not have full-time employment. The findings from the logistic regression model also support this

relationship. However, infants belonging to mothers who had an annual income of less than $18,000 per annum appeared to be less likely to have ECC, although this was not significant. It is unsure whether annual income is a more reliable indicator of each participant's actual SES as it directly pertained to the mother, while full-time employment status was based on what was reported for the entire household unit. Neither was significantly and independently associated with ECC upon further regression analyses. However, it does indicate that future research must also further examine maternal variables that influence ECC risk, including family finances. Households for many of these participants may have often been in a state of flux and what was reported by participants may not have been entirely accurate. Additionally, the results from this investigation revealed that only 34 respondents were from such homes.

The dental status and dental behaviours of the infant and family were also examined as possible factors associated with ECC. From this regression model, two variables emerged as being significantly associated with ECC. The age of children at the time of their dental visit was found to be associated with ECC. Specifically, those who were examined at or beyond 14 months of age were at increased odds of being afflicted by ECC. Age is a recognized predictor of ECC; older infants and preschool children are at increased risk for developing caries.[23,55,56] This is due to more teeth being exposed to the oral environment and cariogenic bacteria for longer periods of time. The other risk factor identified in this logistic regression model was enamel hypoplasia, which has been shown to be a strong predictor of ECC. [30,57,58] Children with enamel hypoplasia were at tremendous odds of also having ECC. These enamel defects are preferentially colonized

by cariogenic oral flora.[49] Since enamel hypoplasia was so strongly associated with ECC in this model, it was excluded from the model presented in Table 5.18 in Chapter 5.

Backwards logistic regression including variables that were significantly associated with ECC in the earlier logistic regression models revealed that three factors were significantly associated with ECC, namely enamel hypoplasia, the age of the infant at the time of the dental assessment, and maternal 25(OH)D levels during pregnancy. Further, the final logistic regression model for ECC factored many of the previously identified variables associated with ECC from this investigation along with some other factors that have frequently been believed to contribute to caries risk. However, after controlling for income and government assistance, oral hygiene, and infant feeding practices, only three variables were found to be significantly associated with ECC. Enamel hypoplasia, infant age (≥ 14 months), and 25(OH)D levels were significantly associated with ECC. Enamel hypoplasia and age are already recognized risk factors for ECC. However, this is the first time that any association between actual circulating levels of 25(OH)D and caries has been reported. This blinded prospective study suggests that infants of mothers with lower 25(OH)D levels are significantly more likely to develop ECC. The exact mechanism is not clear, but it is likely that lower levels of vitamin D during periods of tooth development and maturation result in enamel that is less resistant to caries and more hypoplaisa.

The last series of Poisson regression models that were constructed for dental caries in infants used the dt score as the dependent outcome. The first model only considered the relationship between circulating levels of metabolites measured from the serum sample obtained during pregnancy. This regression model revealed that there was

a significant association between 25(OH)D levels and the untreated decay rate (dt) (p=.046) while controlling for the influence of other serum metabolites. In addition, calcium levels were also significantly associated with dt scores. Lower prenatal calcium levels were indicative of higher dt scores.

The second model included those same independent variables that were in the expanded logistic regression model for ECC. Again, the same variables of enamel hypoplasia, infant age, and 25(OH)D were found to be significantly associated with the untreated caries rate when adjusted for the other potential influencing variables. Lower vitamin D levels were associated with increased untreated primary tooth caries rates.

Naturally, there were some limitations to this study that are worth mentioning. One of the limitations of this study was the relatively small sample size and attrition of the birth cohort. However, considering the nature of the study population, a retention rate of nearly two-thirds is remarkable. We had in fact anticipated a loss of half of the cohort. In order to deal with the foreseeable loss of participants, the decision was made to double our desired sample size at the outset of this prospective investigation. The small cohort size did restrict our ability to develop complex multivariate models for our outcomes of interest.

Overall, several women and their offspring were lost between the prenatal phase and infant study phase. This likely translated into limited statistical power and did result in several smaller regression models due to the small sample of infants. Fortunately, even though there were several losses to follow-up, it did not appear to negatively impact on vitamin D assessments as there was no significant difference in vitamin D levels between those who remained in the study and returned with their infant and those lost to follow-up

(49.8 ± 26.3 nmol/L vs. 45.1 ± 20.1 nmol/L, p=.081). Had we enrolled a much larger sample of participants and had we maintained a greater proportion of the offspring a statistically significant association between 25(OH)D and enamel hypoplasia may have been observed. Recall that the results of a one-tailed t test for maternal 25(OH)D and enamel hypoplasia in infants just failed to reach the threshold of significance, but suggested that mothers of infants with enamel hypoplasia had slightly lower vitamin D levels during pregnancy (43.2 ± 21.1 vs. 51.4 ± 27.4, p=.072).

The average age of infants at the time of the dental assessment was 16.1 ± 7.4 months of age, which is slightly higher than our targeted age of 12 months. Older children are known to be at greater risk for ECC as teeth have been in the oral cavity for a longer period of time. It is possible that some children who attended this phase of study at an older age might have already had ECC involving their primary maxillary incisors, which may have made it difficult to determine whether these teeth erupted into the oral cavity with signs of enamel hypoplasia.

In addition, rather than measuring 25(OH)D levels of mothers during the second trimester we could have opted to recruit participants and measure cord blood samples at the time of delivery. This would likely not have been a practical approach as women delivering infants are normally not in a position to provide informed consent for research, complete an interviewed questionnaire; while clinic staff can easily forget to collect a cord sample during the drama of the delivery process. This might have resulted in better preservation of the cohort as the gap in time from recruitment to the point of the infant dental exam would have been shortened by approximately four months. However, that would have led to increased recall bias as the questionnaire about prenatal health and diet

would have had to be administered at birth. It is possible that 25(OH)D assays from cord blood samples would be a better indicator of perinatal and newborn vitamin D status than sampling only during the second trimester of pregnancy. Had there been no budgetary constraints we could have proposed measuring 25(OH)D at two time points; during the second trimester and at birth (i.e. cord blood). This would have provided some data as to whether mothers improved their vitamin D status in the latter half of pregnancy. Unfortunately, most women in this study with an average concentration of 48.1 nmol/L would have had to have taken over 2,000 IU of vitamin D daily on a regular basis to raise their vitamin D level to 80 nmol/L.[39] It is doubtful that many women actually achieved a significant increase in their vitamin D levels during the last trimester of pregnancy.

Radioimmunoassay was the method utilized to analyze the serum samples for 25(OH)D in this investigation. This is the most commonly used assay for 25(OH)D. In recent years there has been an international effort to standardize vitamin D assessments to assist with comparing results between different populations.[59] The international Vitamin D Quality Assessment Scheme (DEQAS) has been monitoring the performance of 25OHD assays since 1989.[60] While Diagnostic Services of Manitoba (DSM) did not participate in this initiative when this study first began it has moved forward on this issue and now subscribes to this protocol. Fortunately, for the purpose of this investigation all vitamin D testing was conducted by one laboratory to minimize variability of the results.

During the course of this investigation, physicians of participants who were identified to have extremely low vitamin D concentrations were notified by a member of the study team without compromising the blinding of the dental assessments. This provided participants with an opportunity to improve their vitamin D status during

pregnancy, which may have influenced our final results. It is unlikely that any such women participating in this study would have changed their vitamin D status that drastically through improved diet and dietary supplements. Daily doses of 400 IU/day of vitamin D_3 for a period of eight weeks generally only causes and increase of 11 nmol/L in healthy adults.[42] Therefore, intakes of cholecalciferol in the range of 400 IU and fortified foods are unable to produce marked increases in 25(OH)D levels to the range of ≥ 80 nmol/L if baseline levels are low.[39] An intake of 1000 IU/day for a period of four months potentially can raise 25(OH)D levels by 25 nmol/L[43], but if someone was initially very deficient prior to supplementation, this regimen still would be inadequate to raise concentrations to the desirable range.

The questionnaires used in this cohort study were also limited and may not have collected adequate data on the numerous risk factors for enamel hypoplasia as reviewed in Chapter 2. This prevented a thorough analysis of potential contributors to DDEs and enamel hypoplasia in this population of infants. In hindsight, the questionnaires also did not fully explore many potential confounders that may have had an influence on infant oral health status, particularly caries risk. Therefore, we were unable to control for several confounders in our analyses. Future studies will need to collect more variables that are believed to influence enamel hypoplasia and ECC. In addition, rather that relying on maternal self-reported oral health, future studies could actually include maternal examinations to determine caries experience and determine oral levels of cariogenic microorganisms. The infant questionnaire was not based upon a previously validated instrument and thereby may have limited any attempts to accurately determine whether there were any associations between infant feeding practices and ECC. Another issue is

the possibility that better maternal vitamin D levels may be associated with better health behaviours that could have translated into the establishment of early preventive infant oral hygiene regimens at home thereby lowering their infant's risk of developing caries.

Generalizability of the findings from this study may be limited. This was not a random sample of women during pregnancy, but rather one of convenience. Despite this, it does provide glimpses into both the nutritional status of expectant women during pregnancy and the oral health of infants in Winnipeg. In fact, this may not be a weakness of the study, but rather a strength. We purposely targeted a population at high risk for both low vitamin D and ECC. In fact, this study is generalizable to this very urban Aboriginal population that all too commonly displays these conditions. Prospective studies can yield important information, but they can be expensive and cohort maintenance and preservation are a constant challenge.

Finally, assessments for enamel hypoplasia can be challenging. For instance, saliva and dental plaque can mask enamel defects, poor lighting conditions, and lesions associated with caries can make identification difficult.[61] Fortunately, standardized indices do exist and one such index was used for the purpose of this study.[62] However, one notable limitation is that the index selected does not clearly differentiate enamel opacities and defects from dental fluorosis.

Despite these limitations this study did have some strengths. Overall, it was a moderate size study involving an identified high risk population of mostly urban Aboriginal participants. The selection of this particularly vulnerable group was deliberate. The prospective study design was a considerable strength as it allowed the natural history of infant oral health (i.e. enamel hypoplasia and caries onset) to be

observed and it allowed multiple outcomes to be assessed for a single exposure (i.e. vitamin D status). Additionally, a temporal sequence was established spanning pregnancy through infancy. Cohort studies also have the advantage of reduced bias.

Another notable strength of this study was that the infant dental assessments were made while being blinded to maternal 25(OH)D levels. This was a unique aspect of this study design ensuring that the dental examiner was not biased by prenatal vitamin D concentrations of mothers when performing the infant dental examination for enamel hypoplasia and caries.

Findings from this study may have implications for early childhood oral health policy. Health policy is loosely defined as the actions of key players such as decision-makers, the public, and various levels of government that are concentrated on improving the overall health of the population.[63] Policy development is not a static process. It is continually changing and subjected to several factors including social and political influences, technological advancements, changes in the general population, the overall importance of a health issue and the population at-risk, and the ongoing debate between a preventive view of health (i.e. promoting wellness) versus a fixation with treatment (i.e. disease based approach).[64] Undoubtedly, these forces are at play with oral health policy for infants and preschool aged children.

Assuming that low maternal calcium and vitamin D concentrations during pregnancy predispose enamel hypoplasia and caries in offspring, translating this knowledge in an effective manner may assist in shaping needed oral health policy for young children. Research evidence already links these DDEs with ECC.[30,65-69]

Over the last decade ECC has garnered much attention in the province of Manitoba and at times has become a politically charged issue.[70-73] Unfortunately, there is little policy in the area of preschool oral health in the province. For children's oral health to gain importance some have suggested that ECC should increasingly be viewed as a public health dilemma[74] or a broader pediatric health issue[75] that can influence overall childhood well-being rather than a dental problem so those in policy circles take note. It makes sense to view early childhood oral health in the context of an overall healthy living strategy. ECC is known to negatively impact child health and well-being.[76]

Good health policy for young children and expectant women must be based on credible evidence. The evidence-based movement has resulted in the formulation of clinical practice guidelines, forever changing the way health care professionals care for their patients.[77-80] Evidence-based health care has led to the establishment of hierarchical methods of reviewing and rating the scientific literature, with randomized controlled trials (RCT) being touted as having the most superior evidence. Prospective cohorts also provide valuable evidence. However, there are clear distinctions between evidence-based health care practice and evidence-based policy as neither evidence-based medicine, nor strong scientific findings lead to direct policy change.[81] While basing policy decisions on sound information is the ultimate goal, both researchers and decision-makers must be cognizant of additional factors that influence how evidence is utilized to shape public health policy. They must also be aware of other barriers that can hinder the formulation and adoption of policy for early childhood oral health.

These factors should be properly considered when designing a comprehensive policy strategy to prevent ECC. While this longitudinal investigation has provided some

scientific evidence linking prenatal calcium and vitamin D concentrations with infant oral health, this does not translate into immediate policy change. A host of other issues influence the process throughout the various policy development stages.

Preterm birth and nutritional deprivation of vitamin A and D are known to be associated with an increased prevalence of enamel hypoplastic defects in the primary dentition.[5,6,9,48,49,82,83] There is also evidence that vitamin D supplementation during pregnancy (400 IU/day) may offer some protection.[84] This information along with the findings from this longitudinal cohort looking at actual 25(OH)D levels and infant oral health help to build further evidence of the importance of vitamin D for childhood health. Considering this evidence, although of differing degrees of quality, attempts to improve nutrition during periods of fetal tooth formation in utero and early childhood should be examined as a potential strategy to improve host resistance (i.e. enamel integrity) against decay.

Not only is it important that oral health policy and planning be based on scientific evidence, it also must be culturally appropriate. Ideally, it should be incorporated into existing health and wellness activities (e.g. prenatal and post natal programs, Head Start, community nutrition) to improve the chances it becomes adopted and sustained. Finally, it must undergo regular assessments and modifications as required.[85]

It is probably fair to state that true preventive and needs-based early childhood oral health care has received little attention from the dental profession, governments, and the general public. Only recently have national and provincial dental organizations begun to highlight the need for better prevention and treatment for at-risk preschool children. The profession has been too preoccupied with treating dental caries and has forgotten that

ECC is theoretically preventable. Similarly, governments continue to address the continuing waitlists for pediatric dental surgery under GA. Effective oral health promotion has not been a priority and as such there is little oral health policy in place to ensure that all children remain caries-free during infancy and preschool life. Coordination is required at all levels including government, the dental profession, and other health service providers if early childhood oral health is to emerge as a priority. Partnerships are required to implement far reaching and effective oral health policy that benefits children. The Canadian Dental Association has recently struck a Task Force on ECC to facilitate the development of a national advocacy strategy.[86,87] Recommendations from this Task Force include the need for informing the public of ECC prevention, promoting the concept for a first dental visit by 12 months of age, and partnering with the broader health services community to promote early childhood oral health.

Ultimately, timely knowledge translation of evidence into practice is needed. Practitioners, policy makers, and the public must be regularly updated on epidemiological evidence and outcomes from health promotion initiatives and clinical trials in the area of children's oral health.[85] As well, a favorable policy environment can go a long way to seeing the adoption and implementation of necessary health policy. The creation of our Oral Health and the Aboriginal Child Knowledge Transfer site is one small step forward in this direction (http://oralhealth.circumpolarhealth.org/) to share information.[88]

Early childhood oral health is important as it sets the foundation for oral health along the continuum of childhood. ECC can have negative consequences for childhood health and well-being.[76] It is imperative to educate the public and health providers that ECC is preventable, and is costly to treat. Further, the public and providers need to be

cognizant that children who undergo dental surgery under GA often experience new or recurrent decay following surgery and those experiencing decay during the preschool period are significantly more likely to experience decay throughout childhood and adolescence.[89,90] Future improvements in infant and preschool oral health may eventually reduce the high demand for GA and free up surgical time for other pediatric disciplines.

As this study has shown that low calcium and vitamin D levels during pregnancy are associated with enamel hypoplasia and ECC, it suggests that prevention should really begin during the prenatal period. Bolstering maternal nutrition during prenatal periods, either through improved dietary intakes or supplementation with vitamin D is one place to start in the hope that this will translate into improved host resistance to caries development. The province of Manitoba implemented a Healthy Baby prenatal benefit program a few years ago aimed at improving prenatal nutrition.[91] Our results suggest that many women with limited incomes who are recipients of Employment and Income Assistance (EIA) are not presently benefiting from this subsidy.[91] Only 75% of these high risk mothers returning with their infants (99/133) in our study indicated that they had received this supplement. This form of financial assistance should arguably be expanded given the growing evidence that many expectant women in Manitoba and Winnipeg have inadequate vitamin D levels. However, those receiving this benefit at the time of enrollment into this study did not have statistically higher 25(OH)D levels. Vitamin D supplements could be recommended to government as an affordable means to improve maternal and infant health.

Findings from this investigation also suggest that a first dental visit during the first year of life may be an important preventive practice to adopt in order to minimize

caries-risk. This is not a universal practice in Manitoba as seen by a recent survey of practicing dentists.[92] However, this is now the recommended practice according to national professional organizations.[93,94] This has prompted the Manitoba Dental Association to develop a first dental visit promotional program for children under three years of age with particular emphasis on the first visit by 12 months of age.[95] This program will compliment the recommendations coming from the Canadian Dental Association's Task Force on ECC.[86]

Results from the vitamin D assessment of participants in this thesis have already been shared with members involved in the Maternal and Child Healthcare Services (MACHS) Task Force. The growing concern over the vitamin D status of young children in Manitoba prompted MACHS to raise this issue with the Minister of Health for the Province of Manitoba. MACHS recommended that attention to improving the vitamin D status of newborns and examining the relationship between vitamin D status and childhood oral health is needed.[96] As such, Manitoba Health is now funding an upcoming high dose (100,000 IU vitamin D_3) supplementation pilot project for expectant mothers in Winnipeg. The eventual goal, should this project prove to be beneficial and feasible is to include high dose vitamin D supplementation as part of routine prenatal health care in this province. While this might improve host resistance to caries this does not necessarily eliminate caries-risk as ECC is still a complex multifactorial disease that is heavily influenced by dietary practice, oral hygiene, and the social determinants of health.

Unfortunately, crowded political healthcare agendas and limited government funds may ultimately dictate whether a provincial government will ever undertake a comprehensive and needs-based preventive approach to deal with ECC. Such a

preventive program would need to begin during the prenatal period and continue throughout early childhood anticipating key development milestones. Other pressing health issues may overshadow the importance of infant and preschool oral health. For instance, the fear of a potential H1N1 pandemic may leave ECC low on the list of priorities. In addition, lobby groups have some influence, which can both have positive and negative consequences on the research evidence. Past demands by pediatric dentists for more operating room time has given the problem of ECC some public exposure in the media. However, these treatment solutions to dealing with the problem often overshadow the real need for effective preventive solutions. A balanced approach makes sense and appears to be the current government strategy to dealing with ECC.[97,98]

It was the burden of ECC and the growing wait lists for dental surgery under GA in Manitoba that prompted the development of the Manitoba Collaborative Project for the Prevention of Early Childhood Tooth Decay.[99] This project, now more commonly known as Healthy Smile Happy Child (HSHC) formed in 1999, adopting a population health and community-development approach to foster community solutions to ECC prevention.[23,99] It is a true example of a community-based knowledge transfer strategy. Emphasis has been placed on building capacity within existing communities and local services to ensure oral health promotion is sustained. HSHC methods, similar to the PRECEDE-PROCEED model, have been to engage the community as a key participant in prevention.[100] HSHC wanted communities to understand the importance of early childhood oral health, proceed to obtain skills and capacity needed to act, and ultimately develop ECC prevention strategies.[101-104] The project has developed contacts with community members and identified natural community leaders and service providers who

were able to assist with strategies to reduce the incidence and severity of ECC. Three guiding pillars have been 1) community identification and relationship building, 2) oral health promotion and education, and 3) research and evaluation.

HSHC capacity-building and educational activities are undertaken with existing programs and services reaching infant, preschool and prenatal populations, including pre- and postnatal programs, Aboriginal HeadStart, parenting programs, and daycares. Capacity-building methods include informational meetings, presentations on the causes and consequences of ECC, and hands-on demonstrations on how to use resources developed by communities. The goal is to encourage community-developed promotional activities that can be sustained and integrated into existing services. In 2005, the Minister of Health for the Province of Manitoba announced funding in the amount of $1.2 million to expand this program across Manitoba for a period of two fiscal years.[97,98] This project also received an additional third year of funding ending in March 2009 and the partnership is now developing a sustainability and legacy plan.

Successful translation of the findings of this research study will depend upon how well information is shared with various stakeholder groups, including the HSHC partnership, and how well they are engaged. Informing community members and interest groups (e.g. Aboriginal groups and high-risk populations) of these findings may result in improving the public's awareness of preventing ECC, may help to improve prenatal nutrition, and may help to raise the profile of early childhood oral health. Media coverage may also raise the profile of ECC.

An ongoing dialogue with those in decision-making circles is also required. Follow-up investigations are warranted and it may be beneficial to partner with decision-

makers in the formative stages. As mentioned earlier, the pilot project of MACHS that is looking at high dose vitamin D supplementation during pregnancy is one example as it has brought together decision-makers, clinicians, and researchers. Decision-makers and politicians may also find the cost-effectiveness of improving vitamin D status during pregnancy to improve host resistance to caries a compelling argument as treatment for ECC under GA is costly.

This growing knowledge also needs to be transferred to a broad range of healthcare professionals including physicians (e.g. obstetricians, pediatricians, and family practitioners), nurses and nurse practitioners, midwives, dieticians, dentists, and other dental professionals. Research evidence can be reported through various channels apart from scientific publications such as professional newsletters, presentations, forums and Grand Rounds to raise awareness that improved prenatal nutrition can have consequences on infant health, including oral health. Meetings with local public health professionals will be necessary to inform them of the link between prenatal nutrition and infant oral health.

Finally, the evidence needs to be shared with both dental and medical organizations (e.g. physician groups, nursing organizations, and others that have an interest in maternal and preschool health) as a coordinated team-approach to ECC prevention is needed. These organizations have the necessary funds, influence, and credibility to truly act on these findings. As mentioned earlier, the Canadian Dental Association is presently working on a national strategy to deal with this problem.

Knowledge transfer is a complex process, but is fundamentally necessary in today's society if research evidence is to help develop and modify health policy. Several

factors determine whether this data will help shape early childhood oral health policy in Manitoba. Considering that ECC has garnered tremendous negative attention for the provincial government and that treatment approaches are costly the current environment in the province may be receptive to affordable preventive strategies to curb the scourge of ECC. In fact, the former Minister of Health for the province of Manitoba was aware of the potential of inadequate vitamin D concentrations during pregnancy and dental decay among infants (Unscheduled Speech by Manitoba Minister of Health Tim Sale at the Conference on Wait Times - January 16, 2006). This coupled with the recent MACHS Task Force Report suggests that decision-makers in this province are open to early preventive approaches beginning during pregnancy to improve childhood health. In addition, such a partnership as mentioned above does exist with the HSHC project, which has caught the provincial government's attention and has prompted a commitment to improving the oral health of children in this province.[33,34]

Results of this study reveal that many expectant women in Manitoba have low vitamin D levels during pregnancy. This is of great concern as the developing fetus is dependent on the mother to meet its requirements for proper growth and development. Maternal 25(OH)D concentrations were influenced by ethnicity, education, milk intake, season, and intake of prenatal vitamins. While no direct association between enamel hypoplasia was found following logistic regression analyses, the relationship is still plausible. Although mothers of infants with enamel hypoplasia had lower 25(OH)D levels than those who did not exhibit these defects, this just failed to reach the threshold established for significance in this study ($p=.072$). However, we did determine that prenatal calcium levels are associated with enamel hypoplasia in their offspring.

Surprisingly, the final regression model for enamel hypoplasia identified maternal awareness of vitamin D, milk intake, and margarine use as predictors. Finally, regression analyses for ECC and dt score revealed that enamel hypoplasia, infant age at the time of follow-up, and 25(OH)D concentrations were independent predictors of caries-risk.

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Chapter 7 – Conclusions

This thesis focused on the relationship between vitamin D and oral health. Of particular interest was the influence that prenatal vitamin D concentrations could have on infant oral health, specifically enamel hypoplasia and Early Childhood Caries (ECC). Vitamin D deficiency is common in some Manitoba populations and ECC is also prevalent. A review of the ECC literature in Chapter 1, Section 1 revealed that Aboriginal children in Canada bear a higher burden of caries. It is not just a phenomenon in remote First Nations communities, but is also prevalent among Aboriginal children residing in urban centres.

While ECC is a complex and multifactorial disease affecting infants and preschool children that is heavily influenced by the determinants of health, a key determinant is the presence of enamel defects, termed enamel hypoplasia. These defects are easily colonized by cariogenic microorganisms that can increase susceptibility to caries development. Numerous risk factors for enamel hypoplasia have been identified and were reviewed in Chapter 1, Section 2. These defects are the result of disturbances to ameloblasts that form enamel. It has been reported that low calcium and vitamin D concentrations can increase the risk for such developmental defects of enamel.

Some of the earliest work in this area dates back to the first half of the twentieth century. Chapter 1, Section 3 reviewed this evidence relating to vitamin D and oral health outcomes including enamel hypoplasia and caries. In fact, there is historical literature reporting that vitamin D has an effect on the dentition, particularly in improving resistance to dental caries. Vitamin D fortified diets appeared to reduce and arrest caries in some children. Although there is evidence that vitamin D supplementation during

pregnancy may reduce the incidence of enamel defects, the missing link is that no research group has ever attempted to investigate whether circulating serum vitamin D and calcium levels during periods of tooth development with outcomes of hypoplasia and caries. This thesis is the first study to ever attempt to prospectively investigate the relationship between maternal 25-hydroxyvitamin D (25(OH)D) levels during pregnancy and the presence of enamel hypoplasia and ECC in offspring.

Findings from the prospective investigation undertaken in this thesis reveal that vitamin D deficiency and insufficiency were common in this cohort of primarily urban dwelling Aboriginal pregnant women. Many women were identified to have inadequate 25(OH)D levels; 35.0% were identified to be vitamin D deficient when the former definition was applied and as many as 90% had inadequate levels when the new guidelines were applied. Overall median and mean concentrations suggest a need to improve maternal vitamin D levels during pregnancy to promote perinatal health.

Prenatal vitamin D concentrations of participants in this study were influenced by several factors including the ethnic background of the participant, personal ratings of their health during pregnancy, their intake of vitamins during pregnancy, and their socioeconomic status. In particular, Aboriginal women, those who were not part of a household where someone was employed full-time, and those who had not completed high school were significantly more likely to have lower concentrations. Further, those who drank milk daily and took prenatal vitamins had significantly higher 25(OH)D levels, while those who were recruited and sampled during the winter months had significantly lower concentrations.

This study provided an interesting glimpse of the oral health of infants in Winnipeg, Canada. Nearly one fifth of the infants returning for the infant dental assessment were identified as having enamel hypoplasia in their primary anterior teeth. A total of 31 infants (23.0%) also had cavitated caries lesions involving primary teeth. When early incipiencies were included in the definition for ECC, 49 children (36.3%) were reported to have ECC.

Several factors were identified to be associated with the presence of enamel hypoplasia in infants. Overall, lower calcium levels during pregnancy were significantly associated with the presence of enamel hypoplastic defects while drinking milk daily, daily margarine use, and maternal awareness of vitamin D were significantly protective against the presence of enamel hypoplasia. Unfortunately, despite the fact that there was a trend at the bivariate level towards lower maternal vitamin D levels and the presence of enamel hypoplasia, this was not substantiated upon logistic regression analyses. Prenatal 25(OH)D levels were not found to be associated with the presence of enamel hypoplasia in infants in this study.

Daily milk consumption during pregnancy was identified to be protective against ECC while the absence of full-time employment in the household during pregnancy was significantly associated with ECC. Overall, key factors identified to be associated with ECC in these infants included their age at the time of the dental visit, the presence of enamel hypoplasia, along with lower maternal 25(OH)D levels during pregnancy. This relationship was identified at both the bivariate and multivariate levels. Further, the overall caries rate was also associated with lower prenatal vitamin D levels.

This is the first prospectively designed birth cohort study that has attempted to investigate the relationship between maternal levels of vitamin D and calcium and the presence of enamel hypoplasia and caries in the offspring of study participants. Evidence from this study suggests that maternal calcium and vitamin D may be significant influencing factors in the development of enamel defects and caries. Further longitudinal assessment of this cohort is important. Operating grant funding from the Dairy Farmers of Canada provided an additional opportunity to assess this same birth cohort during preschool life to collect more evidence of whether maternal vitamin D status has a significant influence on enamel hypoplasia and ECC.

Naturally, this research has the potential to shape health policy for pregnant women in Manitoba. Milk and fortified dairy products may be a simple, feasible, and affordable way to supplement women with much needed vitamin D and calcium during pregnancy. As maternal compliance with daily multivitamin supplementation can often be problematic and our endogenous production is severely restricted, fortified dairy products may remain a viable route to ensure women improve and maintain higher vitamin D concentrations so that their developing fetus has the best possible environment for healthy development. Providing expectant women from low income areas of Winnipeg with increased access to milk during pregnancy may not only improve their vitamin D and calcium levels, but may even impact the dental health of their offspring. In addition to the Healthy Baby Benefit that some qualifying individuals receive during pregnancy, decision-makers should consider piloting or expanding interventions such like providing regular and affordable access to milk to all women of reproductive ages

residing in disadvantaged communities. However, for those who are intolerant or adverse to milk, other nutrients for improving vitamin D intake would warrant consideration.

Finally, this investigation also has implications for future research. This is the first study that has examined the relationship of prenatal 25(OH)D and calcium levels and infant oral health outcomes of enamel hypoplasia and ECC. This initial evidence provides rationale to justify complementary epidemiological investigations including small interventional studies aimed at bolstering prenatal and perinatal nutrition and assessing its impact on infant dental health. The Maternal and Child Health Services (MACHS) Task Force sponsored prenatal vitamin D supplementation pilot project that was discussed in Chapter 6 will also contribute to our understanding of the relationship between vitamin D and infant oral health. Further investigations are also warranted and are planned. Translating the arising knowledge to decision-makers, clinicians, and researchers will be essential.

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. R. Schroth

Protocol Reference Number: H2001:126
Date: August 21, 2001

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following are approved for use:

- **Protocol**
- **Informed Consent Form, dated July 18, 2001**
- **Questionnaire, Version 2, dated August 2001**

The above was approved by Dr. A. Katz, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated August 10, 2001. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba.

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for reapproval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Nicholas Anthonisen, MD, Ph.D.
Chair, Biomedical Research Ethics Board

For:
Alan Katz, MB., Ch.B., MSc., CCFP, FCFP.
Chair, Health Research Ethics Board

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to REB Secretary
Telephone: (204)789-3883 / **Fax**: (204)789-3414

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Robert Schroth

Protocol Reference Number: H2001:126
Date of Approval: August 24, 2004
Date of Expiry: August 24, 2005

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following is/are approved for use:

- **Annual Approval**
- **Amendment per letter dated August 9, 2004**
- **Research Participant Information and Consent Form (Version 8, dated May 8, 2002)**

The above was approved by Dr. Ken Brown, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated August 9, 2004. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

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Sincerely yours,

Ken Brown, MD, MBA
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary
Telephone: (204) 789-3255/ **Fax:** (204) 789-3414

UNIVERSITY OF MANITOBA | Faculty of Medicine

Department of
Community Health Sciences
750 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Fax (204) 789-3905

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: "Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population".

Principal Investigator: Robert J Schroth, DMD
D341 – 780 Bannatyne Avenue
University of Manitoba
Winnipeg, MB R3E 0W2
Phone: (204) 975-7764 Cell: (204) 981-5041
Fax: (204) 789-3913

Co-Investigator: Michael EK Moffatt, MD, FRCPC, MSc
CE208 – Children's Hospital
840 Sherbrook Street
Winnipeg, MB R3A 1S1
Phone: (204) 787-2441

You are being asked to take part in a research study. Please take your time to look over this consent form and talk about any questions you may have with the study staff. You may take your time to decide about taking part in this study. You may talk about it with your friends, family or (if applicable) your doctor before you decide. This consent form might use words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This study will assess the nutritional and health status of Aboriginal women during pregnancy and their babies. This study also hopes to find a possible link between nutrition problems during pregnancy and weak tooth enamel of the baby upper incisors. Women's levels of vitamin D and calcium are of interest.

Problems with nutrition during pregnancy have been found in northern First Nation's communities. Baby bottle tooth decay (early childhood caries) is also common in these areas. Many blame baby bottle tooth decay (early childhood caries) on poor baby-feeding practices. Today, facts suggest that it has more to do with to quality of life factors.

"Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population"

Some facts suggest that baby bottle tooth decay (early childhood caries) might have more to do with low levels of vitamin D during pregnancy instead of certain bacteria found in the mouth. Vitamin D is involved in the body's use of calcium. Vitamin D may be a factor in the making of healthy baby tooth enamel. Weak tooth enamel is less protected against dental decay than healthy tooth enamel.

200 women will take part in this study. Once enough women have agreed to take part, no more will be asked to join.

If you take part in this study, you will have the following procedures

You will be asked to allow a small amount of your blood to be taken during your pregnancy. This one sample will be taken during a prenatal visit with your doctor. This will happen during the second trimester. This is the same time your baby's front top teeth are forming. Your doctor, nurse, or clinic staff will be taking blood samples from you during this session as a regular part of the prenatal process. No extra needles will be needed. Your doctor or nurse will use the same needle to collect the blood for this study. The amount of blood needed is about 15ml or one tablespoon. Your blood will be studied for certain materials. They are calcium, vitamin D (25-hydroxyvitamin D), inorganic phosphorus and alkaline phosphatase. Your blood may be stored and studied at a later date along with other study participants in order to save costs.

You will also be asked to answer a questionnaire. Questions about your health, diet habits and activities will be asked. This may help to determine your risk of having low vitamin D levels during your pregnancy. The blood sample and questionnaire will be completed at your doctor's office during one of your prenatal appointments. The blood sample will not take long. The questionnaire may take 45 minutes to finish. You can refuse to answer any questions that make you feel uncomfortable. Once this is done you will be contacted by the study staff or your doctor's office when your baby nears one year of age.

The last part of this study will look at your child's top front teeth as they grow into the mouth. This will happen around one year of age. A mouth mirror will be used for this checkup. A picture of your child's teeth will also be taken. The picture will be taken in a way so your child can't be recognized. Letters or phone calls may be used to remind you to bring your baby for the dental checkup. Your child's checkup will only take a few minutes at a your community health clinic. If this is not possible the researcher may try to do this at your home or make other arrangements with you. In case you move during this time we may need to contact your doctor's office/health clinic to find out your new address and phone number. You will also be asked to give your Personal Health Information Number to help us reach you in case you move.

Risks/Discomforts and Benefits

We do not expect any harm to you or your child from taking part in this study. Blood samples for this study will be taken at the same time as your regular prenatal blood testing by your doctor, nurse, or staff. You may feel a small initial pinch when the needle is inserted but

no extra sites will require puncture. Minor bruising or discomfort from blood draws may occur. This would not be enhanced with the sample taken for this study.

There may be no direct benefit to you from taking part in this study. We hope that the information we learn will help other women with nutritional problems during pregnancy and young children in the future.

Costs/Payment for Participation

The blood sample, questionnaire and your child's checkup will be provided to you at no cost. You will be paid for taking part in this study. This is to cover out of pocket costs you may have. You will receive $15.00 when you complete the questionnaire and give the blood sample. Another $15.00 will be given when you bring your baby in for the dental checkup.

Confidentiality

Information collected in this study may be published or presented in public forums. Your name and other personal information will not be used or revealed. Even though we will try to keep your personal information confidential, total confidentiality cannot be guaranteed. Your personal information may be revealed if required by law.
Your blood test results will be sent to Dr. Robert J Schroth or Dr. Michael EK Moffatt. All blood tests results will be coded to ensure confidentiality.
The University of Manitoba Health Research Ethics Board may review records related to the study for quality assurance purposes.

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to take part or leave the study at any time. Your decision not to take part or to leave the study will not affect your care at this clinic.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a study participant. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and the study staff: Dr. Robert J Schroth at 975-7764 (cell 981-5041) or Dr. Michael EK Moffatt at 787-2441.

For questions about your rights as a research participant, you may contact The University of Manitoba, Bannatyne Campus Research Ethics Board Office at (204) 789-3389.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

"Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population"

Statement of Consent

I have read this consent form. I have had the chance to discuss this research study with Dr. Robert J Schroth and or his study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to leave at any time. I freely agree to take part in this study.

I understand that information about my personal identity will be kept confidential, but that confidentiality is not guaranteed. I allow the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board, for quality assurance purposes.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to one or more of the following study procedures:

	Yes	No
1. Blood Sample needed for the study	☐	☐
2. Questionnaire	☐	☐
3. Photo of my child's teeth	☐	☐
4. Allowing study staff to contact my doctor's office/clinic to update my address and phone number	☐	☐
5. Allowing study staff to contact me once this study is completed for possible participation in a follow up study of my child's teeth	☐	☐
6. Giving my PHIN PHIN: _____	☐	☐

Participant signature_____ **Date** _____

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____ **Date** _____

Signature: _____

Role in the study: _____

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Robert Schroth

Protocol Reference Number: H2001:126
Date: August 16, 2002

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following is/are approved for use:

- **Annual Approval**

The above was approved by Dr. A. Katz, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated August 13, 2002. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Alan Katz, MB. Ch.B., MSc., CCFP, FCFP.
Chair,
Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary.
Telephone: (204) 789-3255 / **Fax**: (204) 789-3414

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BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
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Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Robert Schroth

Protocol Reference Number: H2001:126
Date: August 15, 2002

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following is/are approved for use:

- **Poster (submitted August 13, 2002)**

The above was approved by Dr. A. Katz, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated August 13, 2002. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Alan Katz, MB. Ch.B., MSc., CCFP, FCFP.
Chair,
Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary.
Telephone: (204) 789-3255 / **Fax**: (204) 789-3414

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Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Robert Schroth

Protocol Reference Number: H2001:126
Date: May 13, 2002

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following are approved for use:

- **Research Participant Information and Consent Form (Version 8, dated May 8, 2002)**

The above was approved by Dr. A. Katz, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated May 7, 2002. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Alan Katz, MB. Ch.B., MSc., CCFP, FCFP.
Chair,
Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary.
Telephone: (204) 789-3883 / **Fax**: (204) 789-3414

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Robert Schroth

Protocol Reference Number: H2001:126
Date: May 30, 2002

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following are approved for use:

- **Questionnaire, Version 8, dated May, 2002**

The above was approved by Dr. A. Katz, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated May 28, 2002. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Alan Katz, MB. Ch.B., MSc., CCFP, FCFP.
Chair,
Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary.
Telephone: (204) 789-3883 / **Fax**: (204) 789-3414

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Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. Robert Schroth

Protocol Reference Number: H2001:126
Date: April 22, 2002

Protocol Title: **Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population**

The following are approved for use:

- **Revisions to protocol per letter dated April 22, 2002**
- **Research Participant Information and Consent Form (Version 6, dated April, 2002)**
- **Questionnaire (Version 7, dated April 2002)**

The above was approved by Dr. A. Katz, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated April 22, 2002. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval should be sought from the relevant institution, if required.

Sincerely yours,

Alan Katz, MB. Ch.B., MSc., CCFP, FCFP.
Chair,
Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary.
Telephone: (204) 789-3883 / **Fax**: (204) 789-3414

Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population – QUESTIONNAIRE

Code: __ __ __ __ __ __ __ Date: _____(dd/mm/yy)
Interviewer: _____
Primary clinic site where prenatal care is being provided:
□ Mount Carmel Clinic □ Health Action Centre □ Women's Hospital

Has informed consent form been explained and signed? □ Yes □ No
Has blood sample been obtained? □ Yes □ No By whom? _____

Participant Profile:
Date of Birth:_____(dd/mm/yy) Province of birth: _____
Where were you born? _____

Current Address: _____Phone: _____
Do you live in Winnipeg? □ Yes □ No

Which Aboriginal category below would best describe your heritage?
□ Status Indian □ Non Status Indian □ Metis □ Inuit □Other_____

Pregnancy & Health Profile:
Is this your first pregnancy? □ Yes □ No
If no, how many children have you had and when did you last have a baby? _____
_____
Did you give any of your babies vitamin D drops (e.g. D-Vi-Sol)? □ Yes □ No

Expected due date: _____(dd/mm/yy)

How would you rate your health during this pregnancy? □ Good □ Average □ Poor
If you rate your health as poor explain: _____

Are you worried about your health during this pregnancy? □ Yes □ No
If yes, explain: _____

Do you think prenatal care is important? □ Yes □ No □ Unsure
If no, explain: _____

Do you regularly come to this medical clinic? □ Yes □ No

How do you get to your doctor's office for prenatal care? □ Walk □ Drive □ Bus □ Taxi □ Friend

Did your doctor recommend that you take vitamins during this pregnancy (Materna, multivitamins)?
□ Yes □ No
If yes, are you taking them? □ Yes □ No
If no, why? _____

If you are taking vitamins during this pregnancy how often are you taking them?
□ Often (once a day or more) □ Sometimes (once a week or more, but less than once a day)
□ Rarely (less than once a week) □ Never

Do you feel that you should take vitamins for good prenatal health? □ Yes □ No □ Unsure

Would you take vitamins if you were not pregnant? □ Yes □ No

Have you ever heard of vitamin D? □ Yes □ No

What is vitamin D important for? _____
What foods have vitamin D in them? _____

Have you ever heard of calcium? □ Yes □ No

What is calcium important for? _____
What foods have calcium in them? _____

Is calcium important during pregnancy for a healthy baby? □ Yes □ No □ Unsure

Do you take calcium supplements? □ Yes □ No □ Unsure

Do you have diabetes? □ Yes □ No □ Unsure
If yes, when did you first find out you were diabetic? _____

Do you suffer bone pain?	□ Yes □ No □ Unsure
Do your arms or legs feel weak?	□ Yes □ No □ Unsure
Do you have trouble walking (e.g. limp)?	□ Yes □ No □ Unsure
Do you have problems standing up?	□ Yes □ No □ Unsure
Do or did you suffer hip problems?	□ Yes □ No □ Unsure

Do you smoke? □ Yes □ No
If yes, number each day? _____, number of years? _____
If no, did you ever smoke? □ Yes □ No
If yes, number each day? _____, number of years? _____
How interested are you in quitting smoking? □ Not at all □ A little □ Very

Do you drink any alcohol? □ Yes □ No If yes how often? _____

Will you bring your baby to this medical clinic for checkups? □ Yes □ No □ Unsure

Nutrition Profile/ Food Security Assessment:
Do you think that the foods you eat are healthy enough for this pregnancy? □ Yes □ No □ Unsure

How have you changed the way you eat since finding out you are pregnant?
□ Better □ Same □ Worse

Do you buy your groceries in your community? □ Yes □ No

How do you get to and from shopping for groceries? □ Walk □ Drive □ Bus □ Taxi □ Friend

Where do you buy most of your family's food?
□ Supermarket(Safeway, IGA, Superstore, etc) □ Convenience store (7-11, Macs)
□ Community grocery store □ Corner store
□ Foodbank □ Family or friends □ Other:_____

Do you only buy what you can carry in one trip? □ Yes □ No

Do you only buy what is available at your nearest food store? □ Yes □ No □ Sometimes

Do you bring your children with you when you shop for food? □ Yes □ No
If yes, does this limit what you can buy during that shopping trip? □ Yes □ No

Are you the main shopper for food in your family? □ Yes □ No

Are you using a foodbank right now? □ Yes □ No
If yes, which foodbank? _____
If no, do you think might use a foodbank during this pregnancy? □ Yes □ No

Are able to buy all the foods you'll need to be healthy during this pregnancy? □ Yes □ No

Do you go to or ever been to a "Healthy Baby" community program (e.g. Healthy Start Mom & Me)? □ Yes □ No

Have you heard about the "Healthy Baby" Manitoba Prenatal Benefit? □ Yes □ No

Are you getting the new "Healthy Baby" Manitoba Prenatal Benefit" from the Province of Manitoba right now? □ Yes □ No

	Never **Rarely** (less than once a week) **Sometimes** (once a week or more, but less than once a day) **Often** (once a day or more)
Do you drink milk?	□ Never □ Rarely □ Sometimes □ Often
Do you cook with milk?	□ Never □ Rarely □ Sometimes □ Often
Do you use milk with cereal?	□ Never □ Rarely □ Sometimes □ Often
Do you use milk in coffee or drinks?	□ Never □ Rarely □ Sometimes □ Often

In your opinion is milk a good source of calcium and vitamin D? □ Yes □ No □ Unsure

What kind of milk do you use at home? □ Homogenized □ 2 % □ 1%
□ Skim □ Buttermilk □ Powdered □ Soy milk (fortified) □ Calcium enriched milk

Does milk or other milk products (e.g. yogurt, cheese) upset your stomach? □ Yes □ No
If yes, does this mean you eat less of them? □Yes □ No

Have you been using more milk and milk products now that you are pregnant? □ Yes □ No

Do you Eat?		**If Never or Rarely, Why (check all that apply)?**	
Never **Rarely** (less than once a week) **Sometimes** (once a week or more, but less than once a day) **Often** (once a day or more)		**Too expensive** **Don't like the taste** **Upsets my stomach** **Not available where I shop** **Other _____**	
Fish (Tuna, Salmon, etc.)	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Liver	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Animal Organ Meats (kidney, heart, etc.)	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Cook with Animal Bones (soup, etc.)	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Eggs	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Margarine	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Milk	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Cheese	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Yogurt	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Sour Cream	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Ice Cream	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop

Green Vegetables	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Broccoli	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop
Calcium Rich Orange Juice	□ Never □ Rarely □ Sometimes □ Often	□ Too expensive □ Upsets my stomach □ Other _____	□ Don't like the taste □ Not available where I shop

If you had low vitamin D and calcium levels would you change your diet and lifestyle to increase these levels? □ Yes □ No □ Unsure

If the following could increase your levels of vitamin D and calcium would you:
Drink or eat more milk or dairy products? □ Yes □ No □ Unsure
Eat more fish or liver? □ Yes □ No □ Unsure
Take vitamins daily or 5-7 times a week? □ Yes □ No □ Unsure
Spend more time outside in the sun? □ Yes □ No □ Unsure
Support ways to add vitamin D and calcium to common food products? □ Yes □ No □ Unsure
Like to know of specific non-dairy foods rich in vitamin D and/or calcium? □ Yes □ No □ Unsure

Early Childhood Caries Profile:
Have you ever heard of early childhood caries (baby bottle tooth decay, nursing decay, tooth rot)?
□ Yes □ No

Who first told you about early childhood caries (baby bottle tooth decay, nursing decay, tooth rot)?
□ Doctor □ Nurse □ Dentist □ TV □ Family Member
□ Other_____ □ This is the first time hearing about it

Do you think that early childhood caries (baby bottle tooth decay, nursing decay, tooth rot) is a normal part of childhood? □ Yes □ No

Have any of your children had early childhood caries (baby bottle tooth decay, nursing decay, tooth rot)? □ Yes □ No
If yes, how did this make you feel? _____

Do you know any children who have had early childhood caries (baby bottle tooth decay, nursing decay, tooth rot)? □ Yes □ No
If yes, how did that make you feel? _____

What do you think causes early childhood caries (baby bottle decay, nursing decay, tooth rot)?
_____

Do you believe that early childhood caries (baby bottle tooth decay, nursing decay, tooth rot) can be prevented? □ Yes □ No

What problems can early childhood caries cause? _____

Do you feel it is important to keep baby teeth healthy? □ Yes □ No
If no, explain: _____

When should you start brushing a baby's teeth? _____

At what age should a child first see the dentist?
□ When first tooth erupts □ ≥1 year of age □ ≥2 years of age
□ ≥3 years of age □ Only after dental pain occurs □ Other _____

Have any of your children needed general anaesthesia for dental surgery? □ Yes □ No
If yes, where did they have their dental surgery (city)? _____
How old were they? _____

How long are you planning to breastfeed? _____

At what age do you think a child should be weaned from breastfeeding? _____

When is it appropriate to breastfeed or bottle-feed your baby?
When hungry □ Yes □ No □ Unsure
When the baby is going to sleep □ Yes □ No □ Unsure
If baby is crying □ Yes □ No □ Unsure

At what age do you think a child should be weaned from the bottle?
□ < 1 year of age □ 1 year to 18 months of age
□ 18 months to 2 years of age □ > 2 years of age

Mom's Oral Health Profile:

Do you think your dental health is important during pregnancy? □ Yes □ No

When did you last see a dentist? □ Past 6 months □ Past year
□ Past 2 years □ 2 – 5 years ago □ > 5 years ago □ Never seen a dentist
If more than 2 years, why? _____

How often do you visit your dentist?
□ Every 6 months □ Every year □ Every few years □ Rarely □ Never

How would you rate your own dental health? □ Good □ Fair □ Poor

Do you have any dental problems? □ Yes □ No
If yes, which best describes your dental problem(s):
□ Cavities/decay □ Abscesses/infections/swellings □ Bleeding Gums
□ Loose teeth □ Pain □ Other _____

Exposure to Sunlight:

During the summer do you like to spend time outside? □ Yes □ No

Do you enjoy being out in the sun? □ Yes □ No
If no, explain: _____

Where do you spend your outside activities? □ Shade □ Sunshine □ Both □ No Difference

In a given week during summer (mid-April to mid-October) how much time would you normally spend outside? □ Everyday □ Most days □ A few days □ Once or twice only □ Never

When you are outside during the summer how long are you outdoors?
□ Less than 15 minutes □ > 15 minutes but < 1 hour
□ 1 hour to 4 hours □ More than 4 hours

What time of day would best reflect the times you normally would spend time outside?
□ Early morning (sunrise to 10:00am) □ Late morning (10:00am to noon)
□ Early afternoon (noon to 3:00pm) □ Late afternoon (3:00pm to 5:00pm)
□ Evening (after 5:00pm)

Do you feel healthier/better when you spend time outside? □ Yes □ No □ No difference

Would you think you'd spend more or less time outside if you were pregnant during the summer months? □ More □ Less □ No difference

When outside do you:

Let the sun reach your skin by wearing short sleeve tops or shorts?	□ Usually □ Now and then □ Never
Wear a hat?	□ Usually □ Now and then □ Never
Use sunscreen?	□ Usually □ Now and then □ Never
Use insect repellant (bug spray)?	□ Usually □ Now and then □ Never

Family & Financial Profile:

Which of the following would best describe you?
□ Single □ Married □ Divorced □ Common-law relationship

Number of people in your household (including yourself): _____

What is the highest level of schooling you have finished? _____

Which source of household income listed below would describe you and your family?
□ Full-time job □ Part-time job(s) □ Government Assistance □ Other: _____

Do you receive financial help from any relatives or friends? □ Yes □ No

Which of the following best describes your yearly income?
□ <$18,000 □ $18,000 to $26,000 □ ≥$26,000

Prenatal Nutritional Deficiency and Early Childhood Caries in an Urban Aboriginal Population - INFANT DENTAL ASSESSMENT

Interview Questions for Mother

Code:__ __ __ __

Date (dd/mm/yy): _____ Date of Photograph (dd/mm/yy): _____

Infant's D.O.B. (dd/mm/yy): _____ Age (months): _____

Infant's Last Name: _____
Infant's First & Middle Names: _____

Sex: ___ M ___ F

Mother's Last Name: _____ Mother's First Name: _____

Mother's Age: _____

Current Address: _____ Phone: _____
Other phone: _____

Child's Physician: _____ Phone: _____

Pregnancy & Delivery Profile:
Child's birthweight: ____lb ___oz OR _____ grams

Did you have diabetes during pregnancy? __ Y __ N

Was your baby premature (less than 37 weeks)? __ Y __ N

How is your child's health?
__ Very Good __ Good __ Fair __ Poor __ Very Poor

Has your child had any serious medical problems? __ Y __ N
If yes, describe _____

Has your child taken sweetened medications? ___ Y ___ N
If yes, for how long? _____

Primary Tooth Profile:
At what age did infant's first tooth come in? _____

Did your baby's teeth look healthy when they came in? ___ Y ___ N
If no, what was wrong with them? _____

Do you believe your child has any dental problems? __ Y __ N
If yes, what do you believe is the problem? _____
Has your child been to the dentist? __ Y __ N
If yes, what was the reason? _____

Have you started to clean your child's teeth? __ Y __ N
If yes, at what age did you start, and how do you clean?
Age: _____
How do you clean? _____
Do you use tooth paste? ___ Y ___ N
Who usually cleans the teeth? ___ Mom ___ Dad ___ Other:_____

Did you give fluoride drops to your infant? ___ Y ___ N

Infant Feeding Profile:
Did you breastfeed your infant? ___ Y ___ N
If yes, are you still breastfeeding? ___ Y ___ N
If no, when did you stop breastfeeding? _____

Did you give your infant vitamin D drops while breastfeeding? ___ Y ___ N
If yes, how often?_____ Starting at age?_____ Until_____
If no, why not? _____

After breastfeeding do/did you clean your child's teeth? ___ Y ___ N

Have you ever breast fed your baby to sleep? ___ Y ___ N
If yes, how often have you done this?
___ Usually ___ Now and then ___ Not often

Does child have breast whenever child wants it? ___ Y ___ N

Did you bottle-feed your infant? ___ Y ___ N
If yes, when did you start using the bottle? _____

If yes, are you still giving your infant the bottle? ___ Y ___ N
If no, when did you stop giving your child the bottle? _____

Have you ever added the following to your child's bottle (check all that apply)?
___ breast milk ___ cow milk ___ water

___ juice ___ pop ___ sugar

___ formula ___ tea ___ cornstarch

___ molasses ___ other: _____

Did you ever add sugar to the bottle? ___ Y ___ N

Have you ever propped the baby's bottle during bottle-feeding? ___ Y ___ N

Have you ever put your baby to bed with the bottle? ___ Y ___ N
If yes, how often have you done this?
___ Usually ___ Now and then ___ Not often

What is most often in your child's bottle? _____

Does child have bottle whenever child wants it? __ Y __ N

After bottle-feeding do/did you clean your child's teeth? ___ Y ___ N

Does/did your child use a tippie (sippy) cup? __ Y __ N
If yes, at what age he/she:
Start _____ Stop _____ Still Uses _____

Common beverages given to infant:_____

Did you give child molasses or cornstarch for constipation? ___ Y ___ N

How much sugar does child receive? _____

Do/did you give your child a soother? __ Y __ N
If yes, do/did you ever dip the soother in sweets like honey or sugar? __ Y __ N

Has your child started to eat solid foods? ___ Y ___ N
If yes, at what age did you introduce solid foods? _____

Family Characteristics Profile:
Number of people in your household (including yourself):_____

Is this your residence or do you live with family or friends: _____

Have you moved during pregnancy or after the baby was born? __ Y __ N
If yes, how many times? _____

Did you see your dentist while your were pregnant? __ Y __ N

Did you have any dental problems during pregnancy or since baby was born? __ Y __ N
If yes, explain: _____

Have you gone to any "Healthy Baby" community programs? __ Y __ N

Did you receive the "Healthy Baby" Prenatal Benefit while pregnant? __ Y __ N

Have you had to use a foodbank since the baby was born? __ Y __ N

Are you receiving any government assistance? __ Y __ N

Which of the following best describes your monthly income?
__ < $1,000/month __ $1,000 to 1,500/month
__ $1,501 to 2,000/ month __ > $2,000/month

Enamel Hypoplasia

Enamel Hypoplasia	
Yes	
No	

Modified DDE Index for use in screening

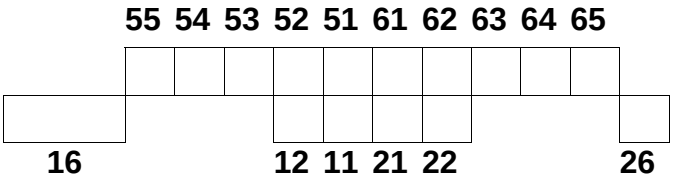
	Code
Normal	0
Demarcated opacity	
White.cream	1
Yellow/brown	2
Diffuse opacity	
Lines	3
Patchy	4
Confluent	5
Confluent/patchy + staining + loss of enamel	6
Hypoplasia	
Pits	7
Missing Enamel	8
Any other defect	9
Combinations	
Demarcated & Diffuse	A
Demarcated & hypoplasia	B
Diffuse & hypoplasia	C
All 3 defects	D

Early Childhood Caries

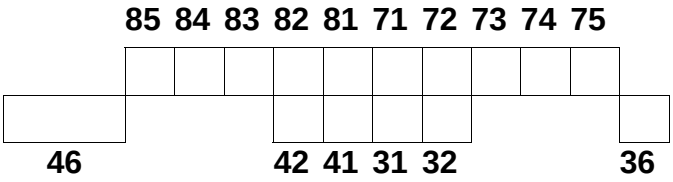
ECC = 1 or more primary teeth with caries

ECC	
Yes	
No	

Upper



Lower



00 = sound
01 = sound with sealant
02 = decayed pit or fissure
03 = decayed smooth surface
04 = decayed BOTH pit and fissure & smooth surface
05 = filled with no other decay in filling/crown
06 = filled & decayed
07 = missing due to caries
08 = lost for reasons other than caries
09 = restored for reasons other than caries
10 = to be lost/extracted due to caries

	Permanent		**Primary**
Condition/Status	Crown	Root	Crown
Sound	0	0	A
white spot lesion (no break in enamel)	1	--	B
enamel lesion (break in enamel, confined to enamel)	2	--	C
dentinal lesion (break in enamel, soft dentin at base)	3	3	D
filled, decay present	4	4	E
filled, no decay present	5	5	F
missing, as a result of caries	6	--	G
missing, other reason	7	--	H
unerupted tooth (crown)	8	8	I
missing data	9	9	9
Sealant	S	--	R
Trauma	T	--	U

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500 University Centre
Winnipeg, Manitoba R3T 2N2

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Address: 507 - 715 McDermot Avenue
Faculty of Dentistry, University of Manitoba
Winnipeg, Manitoba Canada R3E 3P4

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Title of article / book: Determinants of EarlyChildhoodCaries(ECC) in a rural Manitoba communic

Page numbers: 114-120

Title and number of image: Table1RecentStudiesReportingPrevalence&SeverityOfDentalCaries

Publisher and year: American Academy of Pediatric Dentistry 2005

Journal name, issue number: Pediatric Dentistry, Volume 27, Issue 2

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Date: April 28, 2007

Name: Dr. Robert J Schroth Phone:1-204-975-7764 Fax:1-204-789-3913

Address: 507 - 715 McDermot Avenue
Faculty of Dentistry, University of Manitoba
Winnipeg, Manitoba Canada R3E 3P4

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Title of article / book: Caregiver knowledge and attitudes of preschool oral health and ECC

Page numbers: 153-167

Title and number of image: Table1 Previous used terms for ECC among infants & preschoolers

Publisher and year: International Association of Circumpolar Health Publishers

Journal name, issue number: International Journal of Circumpolar Health, 66(2)

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Date: April 16, 2007

Name: Dr. Robert J Schroth Phone: (204) 975-7744 Fax: (204) 789-3919

Address: 507 716 McDermot Avenue

Faculty of Dentistry, University of Manitoba

Winnipeg, Manitoba, Canada R3E 3P4

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Title of article / book: Dietary recommendations for vitamin D: a critical need for functional

Page numbers: 304- 309 end points to establish an estimated average requirement

Title and number of image: Figure 1 - Significance of vitamin D status to chronic disease

Publisher and year: 2006 American Society for Nutritional Sciences

Journal name, issue number: The Journal of Nutrition, 136

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Date: April 16, 2007

Name: Dr. Robert J Schaefer Phone: (204) 975-7764 Fax: (204) 789-3913

Address: 507 - 715 McDermot Avenue

Faculty of Dentistry, University of Manitoba

Winnipeg, Manitoba, Canada R3E 3P4

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Title of article / book: Overview of the proceedings from Experimental Biology 2005 Symposium:

Page numbers: 1114-1116 Optimizing vitamin D intake for populations with special needs:

Title and number of image: Figure 1-Schematic representation of 3 dynamic shifts that have

Publisher and year: 2006American Society for Nutritional Sciences taken place in vitD nutri

Journal name, Issue number: The Journal of Nutrition, 136

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Date: April 16, 2007

Name: Dr. Robert J Schroth Phone: (204) 975-7764 Fax: (204) 789-3913

Address: 507 - 715 McDermot Avenue
Faculty of Dentistry, University of Manitoba
Winnipeg, Manitoba, Canada R3E 3P4

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Title of article / book: Dietary recommendations for vitamin D: A critical need for functional

Page numbers: 304- 309 end points to establish an estimated average requirement

Title and number of image: Figure 2 - Measurement of vitamin D status

Publisher and year: 2005 American Society for Nutritional Sciences

Journal name, issue number: The Journal of Nutrition, 134

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