MATERNAL VITAMIN D:

A SYSTEMATIC REVIEW OF ASSOCIATIONS AND DEFICIENCIES, AN INVESTIGATION OF EARLY CHILDHOOD CARIES IN NORTHERN MANITOBA, AND A PROPOSED STUDY OF PRENATAL VITAMIN D STATUS AND ENAMEL HYPOPLASIA IN INFANTS

By Robert J Schroth

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

MASTER OF SCIENCE

Department of Community Health Sciences
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Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Maternal Vitamin D: A Systematic Review of Associations and Deficiencies, an Investigation of Early Childhood Caries in Northern Manitoba, and a Proposed Study of Prenatal Vitamin D Status and Enamel Hypoplasia in Infants

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Robert J Schroth

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

To my parents

Thank you for encouraging learning
and for providing a nurturing environment.

ABSTRACT

Background:

Vitamin D is essential for calcification of body tissues. It is not only a vitamin but also a hormone that can be endogenously synthesized. Exposure to ultraviolet irradiation of 290-320 nm stimulates this production, our physiological prime source. For those not obtaining adequate sun exposure reliance on dietary intakes to meet vitamin D requirements is essential. Deficiencies of 25(OH)D have been documented in northern Manitoba, due to limited sun exposure and diets that have deviated from the traditional Aboriginal diet.

The developing fetus must rely on its mother for its vitamin D supply for proper development. Mothers who do not meet recommended levels of 25(OH)D during pregnancy have been known to place their infants at risk. For instance, extremely vitamin D deficient mothers can predispose infantile rickets. Problems with inadequate vitamin D may also affect other calcified tissues, including primary teeth. One reported etiology for enamel hypoplasia is low vitamin D. These same hypoplastic defects have been identified as a risk factor in the development of early childhood caries (ECC), a destructive form of tooth decay in young children.

Objectives of this thesis:

This thesis includes several chapters with distinct objectives each linked by the commonality of maternal vitamin D during pregnancy. The principle objective is to better understand the implications of low vitamin D during pregnancy and to appreciate its relationship with enamel hypoplasia, a known risk factor for ECC.

- To review the role of 25(OH)D in health, its sources, and review the problem of vitamin D deficiency in Manitoba.
- To identify those factors associated with maternal 25(OH)D during pregnancy.
- To determine whether vitamin D deficiency has been documented during pregnancy.
- To review the relationship between vitamin D supplementation, enamel hypoplasia and ECC. A community with documented widespread vitamin D deficiency was studied.
- To propose a longitudinal study to identify whether analogous levels of vitamin D during pregnancy exist in an urban Aboriginal population, and to investigate the relationship between prenatal vitamin D status and enamel hypoplasia.

Results:

Strong evidence exists that maternal concentrations are associated with vitamin supplementation during pregnancy, ethnicity, season, and sun exposure. Intermediate evidence supports the association between maternal 25(OH)D and diet, religious practice, stage of pregnancy, and geography, while little or no evidence supports associations with socioeconomic status, maternal age, gravid history, number of fetuses, and antenatal care. There was sufficient evidence to state that maternal-fetal and maternal-infant concentrations are correlated.

Studies from all regions of the globe reported maternal concentrations <35nmol/L, indicating that many women are vitamin D deficient during pregnancy, leaving their developing fetuses at risk of incomplete development.

Vitamin D deficiency has been documented in Garden Hill, Manitoba, a community with a high prevalence of rickets and ECC. A rickets prevention strategy, called modified Stosstherapy (high dose vitamin D supplementation) was initiated during pregnancy and infancy. It was hypothesized that mothers and infants not receiving the supplementation were likely vitamin D deficient, although this was not validated with serum analysis for 25(OH)D while those receiving supplementation had adequate concentrations. Dental surveys revealed a high mean dmft for the study population (N=98), 13.7 ± 3.2, and an ECC prevalence > 90%. Modified Stosstherapy was not associated with reduced enamel hypoplasia and caries rates. However, this examination was limited by the late age of assessment as children should have been examined as teeth were erupting rather than at ages 3,4,and 5. Supplementation was associated with the eruption time of the first primary tooth with those not receiving it having later eruption times, consistent with existing information that vitamin D deficiency is associated with delayed dental eruptions. This gives some promise to the theory that prenatal vitamin D status can impact pediatric dental development.

The use of longitudinal investigations may be promising for uncovering the influence of maternal vitamin D on the developing primary dentition. Such a study has been proposed and is currently under investigation in central Winnipeg. Preliminary data reveal that vitamin D deficiency exists among Aboriginal women in Winnipeg, even in summer months. What remains, is whether enamel hypoplasia can be linked to insufficient vitamin D status of mothers. Should this relationship be confirmed, addressing maternal vitamin D status during pregnancy may become a public health approach in reducing pediatric dental disease.

ACKNOWLEDGEMENTS

Completing this thesis would not have been possible without the support, encouragement, and guidance of my thesis committee. I would especially like to thank my supervisor, Dr. Michael Moffatt for his willingness to serve as my supervisor and for peaking my interest in vitamin D. You have been extremely supportive throughout this process, have provided me with valuable feedback, and have helped me to grow as a researcher. I would like to thank Dr. Chris Lavelle for his constant enthusiasm, gentle prodding, and insight and Dr. Kue Young for his support and pragmatic advice. I am so grateful that each one of you took the time from your busy schedules to serve as my committee. I look forward to continuing to work with you.

This thesis would not be possible without the financial support I received in the form of fellowship support and operating grants. I must thank the Children's Hospital Foundation of Manitoba, Inc. for awarding me with a 2001 Postdoctoral Fellowship and the 2002 Harry Medovy Postdoctoral Fellowship. Operating grant support for the Garden Hill Study was made possible by the Dairy Farmers of Canada. Support for my prospective study was made possible through grants from the Manitoba Medical Service Foundation, Dentistry Canada Fund, and the Faculty of Dentistry, University of Manitoba.

A special word of appreciation goes to the staff of Women's Hospital Outpatient Department, Health Action Centre, and Mount Carmel Clinic for their assistance.

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Chapter 1 – Introduction

1.1 Background

Vitamin D is essential for the calcification of body tissues. It is not only a vitamin but also a hormone that can be endogenously synthesized from sun exposure. For those not obtaining adequate sun exposure reliance on dietary intakes is essential. Vitamin D status is believed to be influenced by many factors including duration outdoors, lifestyle, dietary practice, and use of supplements. While much is known about vitamin D, many questions require further investigation. For instance, is there sufficient evidence that those factors believed to influence vitamin D concentrations affect maternal concentrations during pregnancy? Likewise, how much vitamin D is required for individuals, and do validated threshold values for vitamin D deficiency, insufficiency, and adequacy exist?

Vitamin D deficiencies have been documented among northern Manitoba First Nations peoples [1,2] due to limited sun exposure and diets that have deviated from the traditional Aboriginal diet [3], yet little is known regarding the vitamin D status of urban residents, specifically Aboriginals. The extent of subclinical vitamin D deficiencies for the majority of Canadians is still unknown. In addition, have such deficiencies been documented among other expectant mothers around the globe?

The fetus must rely on its mother for its vitamin D supply for proper development, and maternal vitamin D concentrations are correlated with those of the fetus [4]. Mothers who do not meet recommended levels of vitamin D during pregnancy have been known to place themselves and their infants at risk. For instance, extremely vitamin D deficient mothers may suffer from osteomalacia and may predispose infantile

rickets. Problems with inadequate vitamin D may also affect other calcified tissues, including primary teeth.

Baby bottle tooth decay has now been replaced by the term early childhood caries (ECC) to raise awareness of the multiple factors involved in this disease's etiology, rather than continuing to attribute causation solely to inappropriate feeding practices. ECC is an identified problem among northern First Nations populations [5,6] but is also known to afflict children in urban centres. Many First Nations communities have both widespread vitamin D deficiencies and high prevalence of ECC. One question that warrants investigation is whether vitamin D deficiencies contribute to the development of this pediatric dental disease, as both are common phenomena in many northern communities.

Nutritional deficiencies during amelogenesis may predispose enamel hypoplasia of the primary teeth. Clinically, enamel hypoplasia is identified by the absence of, pitting, grooves, or other irregularities of enamel [7,8]. Note that these defects have been identified as one of several risk factors in the development of ECC [9-11]. Structural defects, in the form of enamel hypoplasia, may place an infant's primary teeth at greater risk for the colonization of cariogenic bacteria, specifically Mutans streptococci, resulting in dental caries [9,12,13] as they provide niches for the microorganisms to colonize and flourish. Calcification of primary maxillary incisors begins during the second trimester, specifically between weeks 13 and 17 in utero. Therefore it is important to investigate the possible etiologies in utero, which can disrupt normal enamel formation. Deficiencies of vitamin D in utero are also believed to be associated with the presence of enamel hypoplasia, because of metabolic insult to ameloblasts [14-16]. This requires investigation, as inappropriate feeding modalities are no longer considered the main

etiology [17]. New research must determine whether nutritional deficiencies of vitamin D play a role in ECC.

Daily intake of vitamin D during pregnancy has been recommended for vitamin D sufficiency and to reduce the development of hypoplastic enamel lesions [14]. The high rate of ECC in Manitoba might be related to low serum vitamin D concentrations reported in the north [1] and among urban Aboriginal women during pregnancy. Perhaps both supplementation and early dental screenings may serve as effective preventive strategies to reduce both enamel hypoplasia and ECC. While the link between high rates of hypovitaminosis D and ECC in Manitoba is plausible, prospective studies are necessary before dietary supplementation can be adopted as a preventive public health strategy for dental health.

1.2 Thesis Objectives

This thesis includes several distinct objectives, each linked by the commonality of maternal vitamin D during pregnancy. The objectives of this study include:

1. Review the role of 25-hydroxyvitamin D (25(OH)D) in health, its sources, and review the problem of vitamin D deficiency in Manitoba.
2. To conduct a systematic review of maternal 25(OH)D during pregnancy. This will identify key associations between maternal concentrations and influencing variables.
3. To identify whether maternal 25(OH)D deficiencies have been previously documented during pregnancy and to review the criteria governing the definition of vitamin D deficiency and insufficiency states.
4. To review the relationship between vitamin D, enamel hypoplasia, and ECC.
5. To analyze data from a study of a community with documented widespread vitamin D deficiency to determine whether Stosstherapy, a rickets prevention strategy, had any effect on enamel hypoplasia and ECC.
6. To propose a longitudinal study to identify the vitamin D status during pregnancy of an urban Aboriginal population, and to investigate the relationship between prenatal vitamin D status and enamel hypoplasia.

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Chapter 2 – An overview of vitamin D metabolism

2.1 Introduction

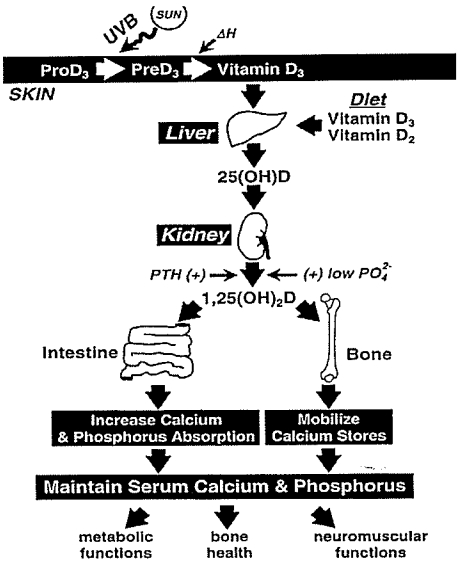
Vitamin D may be defined as a vitamin and a hormone, in that it can be obtained exogenously as a nutrient or synthesized endogenously in the skin on exposure to ultraviolet (UV) radiation of the appropriate wavelength [1,2]. Although initially misclassified solely as a vitamin in the 1920s [3,4], endogenous synthesis is the fundamental (prime) source. As much as 90% of the total vitamin D requirement for humans comes from endogenous synthesis [5]. This relies upon exposure to UV B irradiation [1], with the appropriate wavelength needed to initiate this process ranging between 290 and 320 nm [6-10] although this may vary with skin pigmentation [2]. Significant levels of 7-dehydrocholesterol, a precursor of vitamin D, are concentrated in the deepest layers of the epidermis, the stratum basale and stratum spinosum [2,3]. Upon exposure to solar irradiation it is transformed into previtamin D3, which is then isomerized to vitamin D3 [2,6,9] and is then bound to vitamin D binding protein (DBP) and transported to the liver. It is then metabolized to 25-hydroxyvitamin D (25(OH)D) [1,8] and transported to the kidney for further hydroxylation to 1,25-dihydroxyvitamin D. The serum half life of 25(OH)D is reported to be between 12 to 19 days [11]. 1,25-dihydroxyvitamin D is the most active form of vitamin D [1,2], although 25(OH)D can also be metabolized into 24,25(OH)2D, which may limit osteoclastic activity [2]. 1,25-dihydroxyvitamin D is then bound to DBP and transported to the over 30 target tissues [3], including bone and bone marrow, endocrine glands, skin, and pancreatic cells [2,12] mainly to regulate calcium and phosphorus metabolism [1,2,13] throughout life. Vitamin D is involved in mineral homeostasis ensuring that serum calcium and phosphorus levels

are sufficient for the mineralization of bones [14] and other calcified tissues, including teeth [2], in addition to neuromuscular functions [5].

1,25-dihydroxyvitamin D is the key metabolite in this process and its production is influenced by several factors including circulating levels of calcium, phosphorus, parathyroid hormone (PTH), and calcitonin [2,16]. Deficient concentrations of calcium and phosphorus, or periods of increased need (e.g. pregnancy) stimulate the transformation of 25(OH)D in the kidney to 1,25-dihydroxyvitamin D [13,15] resulting in an increase in serum concentrations. This active metabolite then acts on the epithelial cells lining the intestine to increase uptake of phosphorus and calcium and on osteoclasts to liberate calcium [9,15-17]. Ultimately, a rise in serum concentrations of these two ions to normal levels maintains their important physiological actions [15]. When serum calcium levels are depleted, more PTH is secreted which also stimulates the conversion of 25(OH)D to the active form, 1,25-dihydroxyvitamin D [9,18,19] and assists in calcium absorption [17].

While certain factors can stimulate the hydroxylation to 1,25-dihydroxyvitamin D, feedback mechanisms inhibit further synthesis [18]. For example, normal levels of phosphorus inhibit further synthesis of 1,25(OH)2D through direct feedback mechanisms [2] and once serum calcium concentrations have returned to normal levels, the secretion of PTH is inhibited. This then halts the conversion to 1,25-dihydroxyvitamin D [8,18]. Further production of 1,25(OH)2D is also arrested by high 1,25-dihydroxyvitamin D concentrations, whereas 1,25-dihydroxyvitamin D inhibits PTH secretion [13,14].

Figure 2.1 - Vitamin D Pathway [15]



2.2 Dietary Sources of Vitamin D

Apart from endogenous production of 25(OH)D, the other source of vitamin D for humans comes from exogenous sources, either from diet or dietary supplements, i.e. exogenous nutrients [1,6]. The exogenous form has the same influence as endogenous sources derived from sunshine [20]. Apart from dietary supplements, foods known to contain vitamin D include fatty fish and fish oils [2], liver, eggs [16], vitamin D fortified milk and dairy products [15,21] (e.g. fortified milk products and margarines) in addition to some cereals [2,12,22]. In such cases of complete reliance on dietary sources, 25(OH)D is indeed a vitamin [2]. Exogenous vitamin D can be derived from two forms (a) animals (D3 or cholecalciferol from endogenous origin) frequently contained in fatty fish and fish oils, or (b) from plants (D2 or ergocalciferol) [2,6,9].

While exogenous sources may not be the best method for obtaining vitamin D, if we believe that endogenous synthesis is inherent; it is essential for preserving vitamin D stores in those with barriers to adequate sun exposure or with problems synthesizing 25(OH)D (e.g. renal disease) [17]. Interesting observations reveal that traditional diets of Inuit were high in fish which naturally compensated for their lack of ample UV light while diets of those living in tropical regions were typically low in vitamin D but that their reliance on endogenous synthesis ensured nutritional adequacy [23]. In such situations, vitamin D attainment was maximized.

The use of vitamin D supplements for individuals who are deficient of essential nutrients and who cannot gain sufficient amounts from foods can be scientifically justified [24]. In addition, dietary supplements can be incorporated into the daily routine to ensure that individuals meet targeted levels of vitamin D and are frequently in the form of ergocalciferol (D2) [2] but D3 is more effective in bolstering serum concentrations [11,25]. Some multivitamins contain low quantities of vitamin D2, but it can also be administered in the form of high dose ergocalciferol, called Stosstherapy, either orally or intramuscularly [25]. Dietary supplements vary in dose (e.g. many multivitamin supplements contain 400 IU of vitamin D), although the ideal dosage remains questionable [6,15,26] and depends on demands.

2.3 Vitamin D and Pregnancy

Maintaining vitamin D and calcium adequacy is extremely important, not only for the mother but for the developing fetus, whose calcium demands amount to 30 grams during fetal life [17,27]. This is especially true for women from northern regions, cold climates,

and limited sun exposure [9], where endogenous synthesis is known to be insufficient and are thus almost entirely dependent upon obtaining their requirements from diet and supplementary sources.

Fetal supplies of vitamin D are derived from mother, and maternal and fetal concentrations of 25(OH)D are closely correlated [28,29]. Nutritional needs are significantly heightened as much as 5 times [12] during this formative period as the mother must not only attain sufficient concentrations for both her own needs but also those for the developing fetus. Increased renal clearance of calcium during this period also increases the need for extra calcium [18]. This equilibrium is maintained via increased maternal calcium absorption in the intestines, regulated by the actions of PTH and 1,25-dihydroxyvitamin D [18,26,30]. However, calcium liberation from the maternal skeleton may also contribute to the needs of the fetus [30].

Scientific advancements have helped to uncover how the fetus obtains its vitamin D and calcium. Both 25(OH)D and 1,25-dihydroxyvitamin D cross the placenta [13] although 1,25(OH)2D synthesis occurs in the placenta [17] and fetal tissues [13,18].

Fetal concentrations of 25(OH)D increase during gestation, with concentrations being minimal at the beginning of fetal life [13]. The primary origin of fetal 25(OH)D comes from maternal circulation via placental transfer [13]. While the fetus can synthesize 1,25-dihyroxyvitamin D in the kidney, fetal metabolism is regulated by fetal serum calcium and phosphorus stores [13].

Maternal 1,25-dihydroxyvitamin D rises during pregnancy, whereas 25(OH)D levels remain fairly stable [9,19,27,30]. As PTH falls during pregnancy, the increase in maternal circulation of 1,25(OH)2D likely originates from other sources [9,30], e.g. the

placental transport or the influence of rising calcitonin concentrations on renal synthesis [17,30].

Calcium too is transported from mother to fetus [18] through both active and passive transport [19] and this influence by the vitamin D status of the mother [31]. The fetus has lower serum calcium levels than the mother [30] so that low dietary calcium intakes during prenatal development will lead to the liberation of this ion from maternal skeletal structures and concomitant reductions in fetal mineralization of structures [18]. Supplemental calcium and vitamin D may thus be necessary to reduce this risk [18].

2.4 Vitamin D Supplements

Recommended dietary allowance (RDA) for vitamin D is not deemed to be a valid measure to assess vitamin D intake because of the confounding endogenous production. The use of adequate intake (AI) has therefore been proposed as a superior means to assess vitamin D intake [6,15]. An RDA, is the daily intake amounts needed to meet sufficiency [15] and is based upon estimated average requirements (EAR). This is the amount of nutrient needed to achieve vitamin D sufficiency in half of the population [15]. In addition, an RDA for vitamin D has never been recommended, since this requires some knowledge of the amount of dietary intake needed to attain adequacy in the population and the vitamin D status of populations is often unknown [32].

Previously, the unsubstantiated RDA for vitamin D for expectant and lactating women was 400 IU/day (10 ug/day) [6]. This amount of vitamin D appears in many prenatal supplements, while the recommended AI is 200 IU/day (5 ug/day) [15]. Experts believed that there was insufficient evidence to raise the AI for vitamin D beyond this

amount for expectant women, and proclaimed that they should meet the AI for their respective age category [6,15]. However, the known RDA of 400 IU/day has not been rejected outright [6,15]. Daily intake of 800 IU of vitamin D during pregnancy has been shown to be safe and effective in assuring adequacy during pregnancy [33,34] and may be necessary for women at risk for deficiencies during pregnancy. Once evidence exists for the amount of dietary intake required to attain sufficiency, perhaps a validated RDA for vitamin D can be established [32].

There is little evidence to suggest that calcium requirements beyond age appropriate target levels are required for expectant mothers [26]. As mineralization of the fetal skeleton begins in utero, additional calcium intakes may be required [35]. The current RDA for calcium is 1200 mg daily [35].

2.5 Consquences of low vitamin D for Mothers and Infants

Hypovitaminosis D during pregnancy can influence fetal growth and development through alterations in maternal calcium homeostasis [36]. This ultimately restricts the amount of 1,25-dihydroxyvitamin D that can be produced, resulting in limited intestinal calcium uptake and low serum calcium levels. Increased PTH secretion then triggers calcium liberation from the maternal skeleton [17]. Low concentrations of vitamin D during periods of fetal and early childhood development may trigger hypocalcemia, delayed fetal growth [9] and can lead to nutritional rickets. Other problems may include defects in the deciduous teeth of offspring (enamel hypoplasia), resulting from metabolic injuries to ameloblasts [9,37-42]. This theory was initially proposed by May Mellanby, who identified that vitamin D, phosphorus, calcium, and sunshine influenced the

formation of the primary teeth [23]. Delayed eruption of teeth is also considered a sign of deficient serum 25(OH)D levels [2].

In addition, vitamin D deficient mothers may experience problems including osteomalacia [5], hyperparathyroidism, eventual osteoporosis and problems with neuromuscular function, as heightened fetal demands may necessitate resorption of maternal structures [35]. Hyperparathyroidism can increase the risk of osteomalacia whose symptoms can include bone pain, and muscle fatigue and pain [5]. Problems with low vitamin D may not always be limited to the skeleton and calcified tissues as muscle weakness in the limbs may be associated with vitamin D deficiency [16].

Increasing epidemiological evidence suggests possible associations with autoimmune disorders like MS and Type 1 diabetes, hypertension, and some carcinomas, including colon, breast, and prostate cancers [5,11,25,43]. Hypovitaminosis D during prenatal periods may also predispose to adult onset disorders such as schizophrenia [44] and may reduce immune capabilities to infections [25,45]. This is not surprising considering that vitamin D receptors for 1,25-dihydroxyvitamin D are found in over 30 cell types including those related to tumors and immunity [2].

2.6 How can vitamin D be assessed?

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 [1-3,6,9,11,22,46-48]. Different investigative methods have been used to profile vitamin D status including assaying serum concentrations of 25(OH)D, taking dietary histories and assessments of intake of vitamin

supplements. Others recommend concomitant measurement of other serum metabolites to assist in profiling vitamin D adequacy, including PTH and serum concentrations of calcium, phosphate [17] and alkaline phosphatase as these metabolites have significant influence on calcification. Evidence has shown that elevated levels of alkaline phoshpatase coincide with hypoviatminosis D, and the inverse relationship between PTH and 25(OH)D [6,49] also indicates that PTH may be an appropriate indicator of vitamin D sufficiency [6]. However, a recent Canadian study could not substantiate this relationship between 25(OH)D and PTH [50].

Attempts have been made to determine whether dietary intake reviews can be used to accurately predict 25(OH)D status have been made, although this profiling has not proven to be a successful measure of a person's vitamin D status [25,51]. Others have even attempted to assess vitamin D sufficiency by measuring total sun exposure including both intensity and duration, yet this process is also not without problems [22]. Therefore the most reliable method to determine vitamin D status available is still the simple serum assay. Although there is variation in laboratory methodologies for measuring and reporting 25(OH)D, such assays are accepted for investigative studies [25].

2.7 Variables believed to influence vitamin D

Vitamin D is believed to be affected by a multitude of factors. These factors can generally be categorized as being those that influence endogenous synthesis of vitamin D and those that affect exogenous intake (Table 2.1).

Table 2.1

Factors Influencing Vitamin D Attainment

Factors Influencing Endogenous Production	Factors Influencing Exogenous Attainment
• Season, month, time of day, UV light exposure [7,52-55]	• Dietary restrictions including vegetarian diet, allergies to fish, fortified dairy [20,34,47]
• Climate [15]	• Consumption of fortified milk and dairy & government legislation [9,25]
• Geography – including both latitude and altitude [5,9,25]	• Use of vitamin D supplements and multivitamins [34,63]
• Ethnicity, skin pigmentation [9,56-60]	• Socioeconomic status (SES), purchasing ability, cost of food [64,65]
• Dress – cultural, religious, or clothing to minimize exposure to sunlight [57,61,62]	• Access to nutritious foods, availability [64]
• Air Pollution [44,47]	• Limited knowledge and awareness of vitamin D, and foods containing vitamin D [64]
• Sun screens, insect repellants [2,5,46,47]	• Pregnancy – fetus is dependant on vitamin D transport across placenta, and maternal and fetal concentrations are correlated [28]
• Lifestyles that limit outdoor activity, housebound, and institutionalized [2,47,51]	• Breastfed infants not receiving vitamin D supplementation [28,47,66]
• Problems with endogenous synthesis pathway, including renal disease and liver disease [2,17]	• Malabsorption problems, severe liver failure, Crohn's [2,6]
• Age [2,5]	
• Artificial UV light [25]	

2.8 Vitamin D Research in Manitoba Involving Women and Children

Numerous investigations of vitamin D involving young children, expectant women, and mothers have been conducted in Manitoba. These studies have generally focussed on dietary intake histories, serum analysis of 25(OH)D and related metabolites, or elements of both strategies.

The most recent investigation of vitamin D in Manitoba sampled 25(OH)D concentrations of expectant mothers residing in 3 different northern First Nations communities [67]. In addition to measuring 25(OH)D concentrations, women's levels of calcium, phosphorus, and alkaline phosphatase were also assessed [67]. Participants completed an interviewed questionnaire to elicit information regarding dietary intakes of foods containing vitamin D and calcium, prenatal health, and exposure to sunlight [67]. An overwhelming number of mothers had deficient vitamin D levels, defined as below 25 nmol/L [67]. 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 [67]. Women from Norway House had a statistically greater concentration than those from Island Lake women, which was speculated to be due to a better standard of living, difference in diet, and more sun exposure [67]. Responses to the questionnaire could not overwhelmingly predict maternal 25(OH)D concentration during pregnancy.

Another investigation of vitamin D included women and children from St. Theresa Point and Garden Hill [68]. Interviewed questionnaires and venipunctures of both mothers and infants were performed [68]. Infants and mothers were found to have low concentrations of 25(OH)D, as the mean value for children was 26.2 ± 10.87 nmol/L while that of mothers was 19.8 ± 7.77 nmol/L [68]. Mothers reported infrequent

consumption of milk and multivitamins during pregnancy, while few infants received milk or formula after being weaned from the breast [68]. Not only were vitamin D concentrations low due to intakes of vitamin D, the geographic region of these two communities naturally limited the desired intensity of sunshine required for endogenous synthesis for most of the year.

A retrospective chart audit reviewed the cases of rickets treated at Children's Hospital in Winnipeg between 1972 and 1984 [69]. A total of 48 children were treated, with 83% belonging to the Aboriginal population, 37.5% of who were from the Island Lake community [69]. Chart reviews revealed that PTH and 25(OH)D concentrations were only available for 16 children [69]. Consistent with rickets, the mean concentration for 25(OH)D was 28.2 nmol/L, with the median level being 10.0 nmol/L [69]. Although no maternal concentrations were known during pregnancy, evidence suggested that the majority were not attaining adequate amounts of vitamin D. This further illustrates the magnitude of vitamin D deficiency among the First Nations population in northern Manitoba.

Nutrient intakes of preschool children were assessed in two reports in the communities of Cross Lake, Norway House and Garden Hill Manitoba in the early 1970s [70,71]. The first of these reported vitamin D deficiency in the communities of Cross Lake and Garden Hill and involved clinical examinations, serum assays, and dietary intakes [70]. While this investigation did not measure 25(OH)D levels it did measure both calcium, phosphorus, and alkaline phosphatase concentrations, a psuedo marker for vitamin D adequacy. Many of the children were found to have elevated alkaline phosphatase concentrations, an early indicator of vitamin D inadequacy [70]. Children

were not consuming adequate amounts of vitamin D, phosphorus, and calcium, and the use of supplementation was limited [70]. Considering the northern geographic location of these two communities, children's diets served as the main source for vitamin D. Therefore, the elevated alkaline phosphatase concentrations exhibited should not have been surprising.

The other investigation included nutrient intakes and dietary assessments of preschoolers [71]. Children in the communities of Garden Hill and Cross Lake had more inadequate intakes of vitamin D than any other nutrient under investigation [71]. More than ¾ of children in these two communities had vitamin D intakes below the recommended dietary intake of the era [71]. The main source of vitamin D for these children was determined to originate from fortified dairy as the northern latitude precluded adequate sunshine.

2.9 Vitamin D Challenges for Canadians

Problems related to vitamin D have already been documented in the province of Manitoba, including vitamin D rickets [69] and vitamin D deficiency in expectant mothers in the north [67]. It is hypothesized that similar problems may also be experienced by urban populations residing in southern regions of the province.

There is an obvious awareness that certain populations may face greater challenges in obtaining adequate vitamin D levels. Current reasons for this disparity in vitamin D among populations include differing geography, limited sun exposure, diets low in vitamin D, low SES and inability to secure adequate nutrition [64], cultural and ethnic differences, and dietary restrictions. Further, the vitamin D status of the general

population is not well known and it too may also exhibit vitamin D problems as long Canadian winter seasons with decreased UV B intensity virtually halt endogenous synthesis.

In fact, vitamin D deficiency has been identified in healthy individuals of all age ranges [5,50,52] with many in the general public believed to be vitamin D deficient [25]. Some studies have placed the prevalence of vitamin D deficiency among the normal American adult population between 9 and 13 percent when the cutoff of $\leq$38 nmol/L was used [72]. In addition, there are a significant number of residents of low SES without the capacity to secure nutritious food while others may have little knowledge of what foods are essential. Still others may even report lactose intolerance, and thus limit their fortified dairy intake. Even during summer seasons people may still not be attaining adequate serum concentrations of 25(OH)D as many are using sunscreens, insect repellants, or may be dressing to reduce sun exposure.

Possible explanations why the prevalence of vitamin D is not well understood include that vitamin D serum testing is not a routine serological test requested by medical practitioners, the expense of testing, the sensitivity of the laboratory techniques [22,73], and the necessity of venipuncture.

A recent assessment of the vitamin D status of healthy primarily Caucasian Calgarians found that 34% were found to have insufficient 25(OH)D concentrations, defined as < 40 nmol/L, at least once during the 4 periods of testing [50]. This value is considered low for an otherwise healthy group residing in a developed nation [50] since it was noted that those Canadians typically known to possess low 25(OH)D concentrations have been residents of the north [67], young women [52], and institutionalized seniors.

2.10 Conclusions

Vitamin D deficiency had been identified as a problem in northern Manitoba [74]. Although many indicate that lactose intolerance is widespread, the amount of milk necessary to obtain daily recommended intakes of vitamin D is considered to be tolerable to most. Perhaps some main reasons why milk has not been routinely included in the diet in this population are attributable to cost, cultural diet, and lactose intolerance [69].

There is no knowledge of whether similar deficiencies are exhibited among urban Aboriginals, a population requiring investigation, including expectant mothers who also face many barriers in obtaining adequate vitamin D. Both health care providers and the general population must become informed of the consequences of vitamin D deficiencies on health.

2.11 Issues Requiring Further Exploration

- What is the role of 25(OH)D in human health and how do we obtain it?
- What factors are associated with maternal concentrations of 25(OH)D during pregnancy and is there sufficient scientific information reporting 25(OH)D in pregnancy?
- How common is vitamin D deficiency during pregnancy and what populations are at risk?
- It is hypothesized that if vitamin D deficiency is common among healthy individuals around the world, it may also be an area of concern for expectant mothers of Aboriginal heritage in Canada. As there is evidence of maternal deficiency in the north, do analogous deficiencies exist among urban Aboriginal women in Winnipeg?
- Another hypothesis is that 25(OH)D may also influence the development of the primary dentition, with deficient maternal concentrations during pregnancy predisposing enamel hypoplastic defects. Such defects have already been identified as a risk factor in the development of early childhood caries (ECC). Therefore, hypovitaminosis D may predisopose ECC. Can we demonstrate that vitamin D deficiencies during pregnancy are associated with increased enamel hypoplasia, and thus contribute to ECC?
- What are some of the implications and extent of vitamin D difficulties for pregnant women and their infants in an urban environment?

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Chapter 3 – Vitamin D and Pregnancy in the Literature

3.1 Introduction

To appreciate maternal serum concentrations of vitamin D during pregnancy a **systematic review** was conducted to identify levels of 25-hydroxyvitamin D (25(OH)D) reported for various groups of women during pregnancy in the scientific literature. A search of the Medline electronic database was completed to identify these studies and to understand the factors associated with maternal vitamin D during pregnancy.

3.2 Methods

Inclusion criteria for published studies were as follows:

1. Human participants;
2. Studies reporting the measurement of maternal serum 25(OH)D at some stage of pregnancy, including term, delivery and the hours immediately following childbirth;
3. Clearly reported maternal 25(OH)D levels (mean, median, range) that could be extracted from the published article;
4. No language restrictions enforced;
5. Only those published studies reporting circulating 25(OH)D levels for otherwise healthy pregnancies, without complications (i.e. normal obstetric history, and non-significant medical history).

Exclusion criteria were also established for this exercise and included:

1. Those studies measuring maternal 25(OH)D status other than during pregnancy, delivery, or shortly thereafter.

2. Studies that compared healthy controls with complicated pregnancies, including diabetes, hypertension, or affecting calcium metabolism like parathyroid disorders, bone and gastrointestinal diseases.

A search of Medline was conducted in spring of 2002, with the search strategy including the terms "pregnancy" and "vitamin D" for the years 1966 to 2002.

Information on the number of participants, country of study, characteristics of participants including age and ethnicity, season during which vitamin D levels were assessed, and stage of pregnancy when serum samples were drawn was retrieved from included articles. Of particular interest were mean or median 25(OH)D concentrations for subjects. Those values that were not reported in nmol/L, (i.e. ng/ml) were converted to nmol/L, with 1 nmol/L = 0.374 ng/ml [1], or conversely 1nmol/L = 2.67 ng/ml. [Other researchers use the calculation 1 nmol/L = 2.5 ng/ml [2]].

Studies meeting the inclusion criteria were examined for reported associations among variables and 25(OH)D. Particular inquiries between maternal 25(OH)D and geography, socioeconomic status (SES), ethnicity, season, maternal age, religious dress and practice were made. Further, reported associations between 25(OH)D and vitamin D supplementation during pregnancy, diet, stage of pregnancy, first versus previous pregnancy, single versus multiple fetuses, and antenatal care were assessed. In addition, these same studies were scrutinised to determine if maternal concentrations were correlated with fetal cord levels, or infant concentrations. Associations between maternal 25(OH)D and other metabolites such as calcium, phosphorus, or parathyroid hormone in included studies were not investigated.

A template was created to score associations (Table 3.1). Variables positively associated with 25(OH)D were identified by (+), while negative associations were labelled (--). Those relationships not investigated were left blank, while relationships that were assessed but reported not to be associated were identified as (•). Significant relationships were those with $p \leq 0.05$.

Observational comparisons of pooled mean values from multiple studies were conducted, where possible, with RevMan statistical software (Version 4.1). Weighted mean differences with 95% confidence intervals were calculated, using the random effects model, for studies contrasting different cohorts or for those reporting longitudinal follow-up. For example, studies that reported mean vitamin D values for both supplemented and un-supplemented cohorts were grouped together for comparison of overall effects of supplementation on maternal 25(OH)D. Where stated, standard error units were converted to standard deviations to facilitate this observational meta-analysis. Comparisons that did not state significance were assessed using RevMan to determine whether the mean values being compared differed significantly. Studies only citing median concentrations were excluded from the statistical analysis as the statistical program required both mean and standard deviation.

3.3 Results

A total of 76 studies reporting 25(OH)D concentrations were identified. Three studies were determined to involve the same population and were in fact the same study [3-5]. These 3 articles were combined to develop a profile of the research participants *Brooke et al 1981* in Table 3.1.

All included studies were grouped by geographic region. These regions included Canada, United States, Africa, Middle East, Asia, United Kingdom, Northern Europe and Southern Europe.

All included studies were carefully reviewed to determine whether associations with maternal serum concentrations of 25(OH)D were reported. The following table summarizes the relationships that were found between maternal circulating vitamin D and those variables in the included trials (Table 3.1). Associations and correlations between maternal 25(OH)D concentrations and other variables were scored and appear in Table 3.1. A summary of comparisons of cohorts using RevMan appears in Appendix 3.1. Graphic representations appear in Appendix 3.2

Table 3.1 - Summary of the associations and correlations with maternal 25(OH)D.
+ = Positive association/correlation found
-- = Negative association/correlation found
• = variable assessed; no association/correlation found
Blank = relationship not investigated

Study	Geography	SES	Ethnicity	Season	Age	Religion	Supplementation	Diet	Stage of Pregnancy	First vs previous	Maternal Infant	Single vs Multiple	Maternal – cord	Antenatal care	Sun/hours
Smith 2000	+														
Waiters et al 1999			+				+				+				•
Reddy et al 1983									•			•			
Seeley et al 1997									•						
Okah et al 1996				+								+			
Pittard et al 1992															
Anderson et al 1988							•	•			+		+		
Hollis & Pittard 1984			+										+		
Bikle et al 1984															
Gertner et al 1980													+		
Steichen et al 1980													•		
Fleischman et al 1980											•				
Kumar et al 1979															
Reiter et al 1979									+						
Hillman et al 1978													+		
Weisman et al 1978			•										+		
Hillman & Haddad 1976		•	•	+	•			+	+	•					+
Hillman & Haddad 1974			+				•		•				+		
Feleke et al 1999	+		+												
Sanchez et al 1997									+				•		
Okonofua et al 1986						+									
Mukamel et al 2001				•		+	•								
Ardawi et al 1997									+						
Nehama et al 1987				+							+				
Shany et al 1984			+										+		
Taha et al 1984											+				
Serenius et al 1984		+					+						+	+	
Markestad et al 1984	+		+				+						+		
Lazebnik et al 1983															
Biale et al 1979			+										+		
Goswami et al 2000											+				
Higashi et al 1999									•				+		
Namgung et al 1998				+							+				
Brunvand et al 1998															
Seino et al 1982													+		
Gupta et al 1982													+		
Kuroda et al 1981				+							+		+		
Okonofua et al 1987			+						•						
Ong et al 1983			•												

Study	Geography	SES	Ethnicity	Season	Age	Religion	Supplementation	Diet	Stage of Pregnancy	First vs. Previous	Maternal Infant	Single vs. Multiple	Maternal-cord	Antenatal care	Sun/hours
Verity et al 1981				+			+						+		
Whitehead et al 1981									•						
Bashir et al 1981			+												
Brooke et al 1981			+	•		+	+	+	+				+		
Brown et al 1980													+		
Cockburn et al 1980							+				+		+		
Heckmatt et al 1979													+		
Barragry et al 1978									•						
Dent & Gupta 1975			+					•	•						
Koenig & Elmadfa 2000															
Brunvand et al 1996															
Henrikson et al 1995			+					+							•
Bruinse & van den Berg 1995									•						
Brunvand & Haug 1993			+	•											
Hoogenboezem et al 1989													+		
Axelson & Christensen 1988													+		
Markestad et al 1986				+			+								
Kuoppala et al 1986				+									+		
Markestad et al 1984							•						+		
Kuoppala et al 1984															
Lamberg-Allardt et al 1984				+			+						+		
Bouillon et al 1981				+									+		
Kokot et al 1980				+									+		
Bouillon et al 1977													+		
Zeghoud et al 1997							+				+				
Martinez et al 1991															+
Zeghoud et al 1991	+		+	+							+				+
Zeghoud et al 1988				•			+		+						
Delvin et al 1986							+						+		
Mallet et al 1986							+						+		
Martinez et al 1986				•					•						
Donatelli et al 1984															
Delvin et al 1982													+		
Paunier et al 1978							•						+		
Shimotsuji et al 1979											+		+		

Brooke et al 1981 is a compilation of 3 articles [3-5].

3.3.1 Geography

Four studies found an association between 25(OH)D and geography [6,24,33,69]. Significant differences in circulating median 25(OH)D were found between 3 Island Lakes communities in northern Manitoba [6]. The median value for Garden Hill was significantly lower than that from the community of Norway House Cree Nation [6]. The other community, St. Theresa Point, had a median vitamin D concentration less than Norway House but higher than Garden Hill [6]. Overall, this investigation revealed that women from the Island Lakes region of Manitoba had lower 25(OH)D concentrations during pregnancy than their counterparts from Norway House [6]. Explanations for this geographic difference included less sun exposure in Garden Hill, differences in dietary intakes, and perhaps better educational and income opportunities in Norway House [6].

Another study assessed maternal 25(OH)D concentrations in two distinct populations, Ethiopians and Norwegians [24]. Norwegian women had higher levels of 25(OH)D than Ethiopians in Addis Ababa ($p<0.02$) indicating that women in regions receiving high amounts of ultraviolet light could be clinically, vitamin D deficient [24]. This finding was surprising to the investigators and may possibly be explained by dress, high melanin content of their skin, and the lack of vitamin D fortified in foods in Ethiopia [24].

Markestad et al (1984) compared metabolite concentrations between women in Libya and Norway. This study found that concentrations of 25(OH)D in Libyan women were much lower than expectant mothers in Norway (34 nmol/L vs. 112 nmol/L, $p<0.001$) [33]. Unfortunately, this study only reported median concentrations for the

differing geographic populations, which precluded its inclusion into comparison of means testing

The final study comparing geography investigated differences between women residing in three areas of France: Lyon; Chambery; and Nice [69]. The mean levels from the 3 regions did demonstrate some minor variation as Nice, on the Mediterranean, had higher mean 25(OH)D values than did those from central France, but only during the month of May [69].

Overall, differences in maternal serum 25(OH)D did exist with respect to geography. While some of these regional differences were related to sun exposure and the availability of the necessary UV light, differences in diet and food fortification with vitamin D were likely to have also influenced maternal concentrations.

Only one study [69] allowed itself to be used in comparison of means testing. However, no significant differences were found when mean concentrations were compared between the 3 cities. For instance, the respective p values comparing Lyon with Chambery, Lyon with Nice, and Chambery with Nice were p=0.4, p=0.4, and p=0.7, indicating no statistical significance.

3.3.2 Socioeconomic Status (SES)

Only two studies investigated associations between 25(OH)D levels and SES [22,32]. One study investigated concentrations of women at term in Riyadh, Saudi Arabia [32]. Reported median levels of vitamin D for mothers in the upper class were higher than middle and lower class mothers. This finding was statistically significant and revealed

that there were no cases of extreme vitamin D deficiency ($\leq$3 ng/ml) among those in the upper SES group. There was also no significant difference in 25(OH)D concentrations found between middle and lower class groups [32].

Meanwhile, 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 [22]. While this study attempted to measure the association between social class and serum 25(OH)D they were unable to prove that the relationship was statistically significant.

Limited published evidence exists to support or refute the association between SES and maternal concentrations of 25(OH)D. However, low SES may indirectly influence maternal 25(OH)D as it is known to influence diet, food security, and intake of fortified foods or dietary supplements during pregnancy. The test for overall effect for the comparison of means was not significant (p=0.17) with a weighted mean difference of –5.60 (CI –13.64 to 2.44).

3.3.3 Ethnicity

Thirteen studies found a positive relationship between ethnicity and maternal serum 25(OH)D levels [3,7,13,23,24,30,33,35,43,46,54,56,69] while 4 other studies found no statistically significant relationships or did not report significance [21,22,44,51].

Waiters et al (1999) investigated the differences in serum 25(OH)D concentrations among Caucasians, Inuit, and Native Indians residing in the same region of the Northwest Territories. Non-native mothers had higher serum levels (59.8 ± 29.4

nmol/L) than Inuit and Indian mothers (48.8 ± 14.2 and 52.1 ± 25.9 nmol/L, respectively) at the time of delivery [7]. Further comparisons appear in Appendix 3.1.

Hollis & Pittard (1984) on the other hand, looked at the ethnic differences between Caucasians and Blacks in Ohio. While all mothers took daily vitamin D supplementation of 400 IU, there were distinct differences observed among the two groups [13]. Black mothers had a significantly lower mean serum 25(OH)D concentration than Caucasians [13].

Another study compared term maternal 25(OH)D levels of Blacks and Caucasians [23]. Caucasian women had significantly higher concentrations of 25(OH)D than Black participants, consistent with other studies [13] indicating that maternal vitamin D levels can vary between distinct ethnic groups.

While Feleke et al (1999) contrasted maternal vitamin D concentrations by geography, they also compared two distinct ethnic groups, both Norwegian and Ethiopian women. While the season of collection was different between the two groups, this study found that maternal vitamin D levels were influenced by ethnicity with the Norwegian mothers having higher levels of circulating 25(OH)D than did their Ethiopian counterparts [24].

One study compared the 25(OH)D status between Israeli Bedouins and Jews [30]. While this study involved a small number of expectant mothers, it did reveal that Jews had significantly higher levels than Bedouins (32.8 ± 3.3 ng/ml vs. 15.1 ± 2.6 ng/ml) [30]. Proposed reasons for these differences apart from ethnicity included the dress of Bedouins, which likely precluded endogenous synthesis, and the lack of vitamin D

fortified foods in the Bedouin diet [30]. No seasonal effect could explain this difference as both groups of expectant mothers were tested during the same season [30].

Comparisons between Norwegian and Libyan women revealed that Norwegians had significantly higher concentrations than Libyan mothers (112 nmol/L vs. 34 nmol/L, $p<0.001$) [33]. Possible explanations for this finding included daily vitamin D supplementation of 400 IU among Norwegian mothers during second and third trimesters, when less than half of the Libyan women took vitamin D supplements [33]. It is difficult to prove that season was a factor as Libyan women were tested during the season with the greatest sun intensity [33].

Israeli Jews and Bedouins serum levels were again compared, this time at labour concluding that mothers of Jewish origin had significantly higher concentrations than Bedouin [35], confirmed by a later study [30]. Seasonal effects were controlled for, as all women were enrolled and sampled during the same summer season [35]. It was hypothesized that Bedouins may have had less sun exposure than the Jewish women, and diets of Bedouin's were known to contain phytate, considered an anti-vitamin D agent [35].

The scientific literature is also known to contain comparisons of maternal levels between Caucasians and Asians residing in the same geographic region. A study of 43 Asian mothers and 55 Caucasians found statistically significant differences in maternal mean 25(OH)D between the cohorts in all 3 trimesters of pregnancy and at term ($p<0.002$) indicating that Asians had lower concentrations than their Caucasian counterparts [43]. These findings prompted the authors to recommend that all Asian

women have their 25(OH)D status assessed and supplementation provided to those with low concentrations during pregnancy [43].

Another comparison of Asians and Caucasian during pregnancy also revealed significantly lower 25(OH)D levels in the Asian cohort than in the Caucasian group (14.62 ± 0.92 vs. 44.58 ± 2.55, $p<0.001$) [46].

Differences in the mean maternal 25(OH)D concentrations among specific Asian ethnic groups were also studied, including Indian, Pakistani, Sri Lankan, and Bangladeshi pregnant women residing in England [3]. Significant differences were encountered between some of these ethnic subgroups, as the Bangladeshi group had greater 25(OH)D values than the Indian group ($p<0.05$) and Pakistani group ($p<0.01$) [3]. Similarly, the Sri Lankan cohort had higher values than those of Indian and Pakistani origin ($p<0.01$) [3]. Overall, Pakistani women and Indian women had lower mean concentrations than Sri Lankan and Bangladeshi expectant mothers residing in the same geographic location (10 ± 12 nmol/L, 18 ± 21 nmol/L, 40 ± 24 nmol/L, and 32 ± 15 nmol/L, respectively) [3]. Possible explanations for these findings included religious practice and dietary intake among the different groups, as Sri Lankan and Bangladeshi expectant mothers were known to eat fish while this practice was not common among the other two groups [3].

Another study compared serum values of expectant mothers in both the Norwegian born and Pakistani immigrant populations in Norway [54]. Norwegian women exhibited higher median 25(OH)D concentrations during the second trimester than their Pakistani immigrant counterparts (55 nmol/L vs. 19 nmol/L, $p<0.001$) [54]. Explanations for this finding included the lack of adequate sun exposure among Pakistani

women, lack of vitamin D supplementation, and an overall lack of foods containing vitamin D in their diets [54].

A similar comparison was made, this time involving both Pakistani and Norwegian women at the time of delivery. Consistent with another study's findings [54] Pakistani immigrants had significantly lower median concentrations of 25(OH)D than their Norwegian counterparts at delivery (15.1 nmol/L vs. 43.1 nmol/L, $p<0.0001$) [56]. Proposed reasons for this finding included a Pakistani diet high in phytate and fibre, low in vitamin D, and higher sun exposure among Norwegians [56].

A study in France evaluated maternal vitamin D concentrations at delivery reporting metropolitan women with significantly higher 25(OH)D levels than women of diverse ethnic backgrounds ($p<0.002$) [69]. Caucasian mothers were found to have significantly greater concentrations of 25(OH)D than the ethnically diverse group, which included Southern Europeans, Africans, and Asians [69].

While the majority of the included studies established a distinct ethnic variance with respect to maternal serum 25(OH)D concentrations, four other studies were unable to find statistically significant differences. Both Weisman et al (1978) and Hillman & Haddad (1976) found no difference in maternal serum levels between Caucasians and African Americans. Another study compared Indian, Bangladeshi, and Pakistani expectant mothers residing in Leeds, where mean concentrations did not vary significantly between these Asian subgroups [44].

One study was unique in that it did state that Asian women had lower levels of 25(OH)D during pregnancy than Caucasians, but it did not indicate statistical significance [51], although the difference seemed large enough to be statistically significant. Using

RevMan software this comparison of maternal mean 25(OH)D concentrations at delivery was in fact significant (7.6 ± 1.5 ng/ml vs. 18.3 ng/ml, $p<0.001$) [51].

Significant differences in maternal 25(OH)D concentrations exist between various ethnic groups. For instance, Caucasian women were found to have significantly greater levels of 25(OH)D during pregnancy than Black women ($p<0.001$, WMD 10.46, CI 4.51 to 16.41). Similarly, a significant difference was found between Inuit and Caucasians ($p=0.05$) with Caucasians having the larger mean value (WMD 11.0, CI 0.24 to 21.76). Asian women were also found to have nearly significant lower mean concentrations of 25(OH)D than their Caucasian counterparts ($p=0.07$, WMD 39.56, CI –3.91 to 83.03), while Caucasian women were also found to have significantly greater mean values of 25(OH)D than ethnically diverse mothers in France ($p<0.001$, WMD –13.37, CI –19.26 to –7.49).

Pooling the two studies involving Bedouin and Jewish women demonstrated that Jews did not have significantly greater concentrations of 25(OH)D than Bedouins ($p=0.3$, WMD 22.96, CI –21.92 to 67.83). Significant differences in mean 25(OH)D concentrations were observed between Indians and Sri Lankans ($p=0.02$) with Sri Lankans women having greater concentrations, while there was no difference between Indians and Bangladeshi women ($p=0.17$). Mothers of Sri Lankan origin also had larger mean concentrations than Pakistani women ($p=0.002$), while there was no significant difference between Bangladeshi and Pakistani women ($p=0.19$). The weighted mean difference and 95% confidence intervals for these two comparisons were WMD 30, CI 11.42 to 48.58 and WMD 11.98, CI –5.77 to 29.74.

Conversely, there were no significant differ-ences found between Canadian Aboriginals and Caucasians (p=0.2), and Canadian Aboriginals and Inuit (p=0.5). Similarly, there were no statistically significant differences between Indians and East Africans (p=0.7), Indians and Pakistanis (p=0.3).

There were also no significantly observable variations in maternal mean concentrations of 25(OH)D between Pakistani and East African women (p=0.11), East African and Sri Lankans (p=0.09), East African and Bangladeshi women (p=0.19), Sri Lankan and Bangladeshi mothers (p=0.5), and Asian and West Indian women (p=0.8) during pregnancy.

3.3.4 Season

Twelve studies reported positive associations between 25(OH)D concentrations and season [1,10,22,29,38,42,59,60,63-65,69]. However, 6 studies that investigated seasonal influence could not find or did not report statistically significant associations [3,4,27,56,70,73], two of which were the same study population [3,4].

Okah et al (1996) recorded the dates when samples were collected to investigate the influence of season. Women tested during the summer had statistically higher serum concentrations than spring and autumn groups [10]. The mean concentrations for summer, spring, and autumn were 65 ± 6 ng/ml, 51 ± 9 ng/ml, and 46 ± 3 ng/ml, respectively, a variance that was statistically significant (p=0.01) concluding that summer season is associated with higher maternal 25(OH)D levels than fall and spring [10].

Hillman & Haddad (1976) investigated the effect of season on circulating maternal serum levels of 25(OH)D with the mean concentration for those sampled during

February (15.4 ± 5.9 ng/ml) being significantly less than that of mothers tested in August (42.1 ± 13.9 ng/ml), $p<0.001$. This finding indicated that the summer season, a period of increased sun intensity, increases maternal serum concentrations of 25(OH)D.

Another investigated the seasonal variations in Israel, a country known to have sufficient sunlight throughout the year [29]. The mean spring concentration was significantly less than the fall value [29]. However, the mean value for the September to October group was not stated and precluded its inclusion into statistical testing [29]. Overall, there was a definite variation in maternal 25(OH)D according to season.

Other researchers measured 25(OH)D levels in winter and summer, in order to assess the overall influence of season [38]. Obvious variations existed between the two groupings with women belonging to the winter cohort having a significantly lower 25(OH)D levels (24 ± 13 nmol/L) than the summer cohort (43 ± 18 nmol/L)($p<0.001$) [38].

Further summer and winter comparisons of $25(OH)D_3$ were made [42]. The mean value for mothers sampled during summer was significantly higher than that of the winter group (33.9 ± 12.5 ng/ml vs. 15.8 ± 6.6 ng/ml, $p<0.005$) [42].

While the investigation by Verity et al (1981) explored the effects of supplementation on 25(OH)D, it also grouped participating women into April and September cohorts to determine the effect of season on maternal levels of this same metabolite. In both comparisons of supplemented seasonal groups and unsupplemented seasonal groups there was a definite variance in maternal 25(OH)D attributable to season [1]. In both instances, the mean values were significantly higher in the September groups than the April groups ($p<0.05$) [1].

Markestad et al (1986) also made reference to seasonal variations in maternal 25(OH)D, as they sampled women in their first trimesters, before receiving supplementation. The median value for women sampled during summer was significantly higher than women tested during winter ($p<0.05$) [59]. The median summer concentration being 131 nmol/L while the median value for winter was 80 nmol/L [59]. This validates previous data indicating that season influences serum 25(OH)D during pregnancy [42]. However, this study could not be incorporated into comparison of means testing as only median values were cited.

Kuoppala et al (1986) also found seasonal variations in serum concentrations of 25(OH)D. Statistically significant differences existed between mothers delivering in summer and those delivering in spring (44.3 ± 20.8 nmol/L vs. 26.0 ± 13.0 nmol/L, $p<0.01$) [60]. This investigation concluded that 25(OH)D was affected by season, and proposed that vitamin D supplementation should be considered for pregnant women during winter in Finland to improve serum 25(OH)D status [60].

Lamberg-Allardt et al (1984) measured maternal concentrations at delivery comparing a group of women in summer with a group in autumn. A significant seasonal variation existed ($p<0.001$) in both supplemented and unsupplemented groups with the summer cohorts in the supplemented and unsupplemented arms having significantly greater values than the fall cohorts [63].

Another team conducted a study on the influence of vitamin D binding protein on serum concentrations of vitamin D metabolites, but also found a definite seasonal variation in $25(OH)D_3$ with the mean summer value significantly higher than the mean concentration of other seasons ($p<0.001$) [64]. However, this same study did not report

mean concentrations by month apart from a graph and exact seasonal values could not be extracted.

A comparison between summer-autumn and winter-spring cohorts was conducted to determine whether variations in maternal concentrations existed [65]. The mean concentration for the summer-autumn group was 16.8 ± 2.1 ng/ml while it was 9.3 ± 0.98 ng/ml for the winter-spring mothers [65]. The winter group had a significantly lower concentration than the summer-autumn cohort ($p<0.05$). The winter-spring cohort also had a few mothers with extremely low levels of 25(OH)D.

Significant seasonal variations were found in Lyon, France with women sampled in June having much higher concentrations than those sampled in May ($p<0.03$) [69]. While maternal concentrations in the two cities did show a monthly increase from May through summer, these variations were not found to be statistically significant [69].

While the majority of included studies reported significant monthly or seasonal variations in 25(OH), others could not support this association or did not report an association. For instance, Mukamel et al (2001) did not find a statistically significant seasonal difference in mean concentrations. However, there were more cases of vitamin D insufficiency in winter than in summer among the non-Orthodox Jewish mothers [27]. While there was no seasonal variation, this finding might be explained by the consistent sun exposure in Israel, although another study found seasonal differences in Israel [29].

In addition, Brooke et al (1980) were unable to find variations between winter and summer maternal 25(OH)D levels, even though summer samples were higher than those collected during winter. This study did not report specific data in order to compare mean values [4]. The other publication of this cohort reported the differences in maternal

25(OH)D concentrations between distinct Asian groups and it also compared the seasonal influence on serum concentrations of this same metabolite [3]. The study was spread over several seasons in order to properly assess seasonal effects. While the August-October group had somewhat higher 25(OH)D levels, there was no statistically significant variation in concentration by season [3]. However, there were more cases of low 25(OH)D in the winter months than the summer months [3]. Respective seasonal mean 25(OH)D levels were as follows: November-January 19.8 ± 23 nmol/L, February-April 18.8 ± 18.7 nmol/l, May-July 18.9 ± 14.7 nmol/L, and August-October 28.0 ± 20.0 nmol/L [3].

Similarly, Brunvand & Haug (1993) could not support seasonal variations of 25(OH)D. While no specific data was presented in this article with respect to mean serum concentrations of 25(OH)D by season, this study did report that there was no increase in concentration during spring [56].

Zeghoud et al (1988) investigated the influence of season, however, but did not state maternal values in the article with the exception of March and April values for 9 mothers at 6 months and at term. Neither comparison according to month was statistically significant, as the values for March and April at 6 months gestation were 10.3 ± 1.8 ng/ml and 10.4 ± 0.9 ng/ml, while the values for March and April cohorts in the placebo group were 7.5 ± 1.9 ng/ml and 8.0 ± 0.5 ng/ml at term [70].

The other study that assessed the influence of season on 25(OH)D involved women in Madrid and reported that women tested during September had higher concentrations than those sampled in March [73]. However, it did not report whether this was statistically significant [73]. Mean concentrations from 3rd trimester for September

and March were 33.9 ± 2.7 ng/ml and 19.3 ± 1.6 ng/ml, respectively [73] but when evaluated using RevMan, was found to be significant.

25(OH)D concentrations were associated with season, and varied between different periods of the year. For instance, mean maternal concentrations were significantly greater during summer than winter months (WMD 58.46, p<0.001, CI 42.03 to 74.89). Summer concentrations were also significantly greater than autumn months (p<0.001, WMD 24.39, CI 16.98 to 31.83). Summer values were also higher than spring (p<0.001, WMD 18.30, CI 9.94 to 26.66), while summer/autumn cohort had greater concentrations than winter/spring groups (p=0.001, WMD –20.10, CI –32.20 to –8.00).

Statistically significant differences were also found when maternal concentrations for specific months were contrasted. Mean 25(OH)D levels were far greater during September than April (p<0.001, WMD 23.04, CI 16.72 to 29.36), and far greater in September than March (p<0.001, WMD –39.88, CI –49.84 to –29.92).

While the majority of comparisons found an association with season, comparisons in 2 other studies did not reach statistical significance [3,70]. In addition, comparisons between specific months in the same season, or on the verge of the next season often did not demonstrate variations in 25(OH)D concentrations. For instance, there were no significant variations between May and June (p=0.19), May and July (p=0.3), June and August (p=0.3), and June and July (p=0.18). However, the difference between May and August did approach significance (p=0.06, WMD 21.10, CI –1.05 to 43.25).

No significant differences were found between women tested during November to January compared with those sampled during February to April (p=0.8), November to January and May to July (p=0.8), and November to January and August to October (p=0.2). There were also no significant variations in the data provided in this study for the period February to April and May to July (p=1), February to April and August to October (p=0.15), and May to July and August to October (p=0.15) [3].

3.3.5 Maternal Age

Only one study investigated the relationship between circulating maternal vitamin 25(OH)D levels and maternal age, involving 117 American women sampled during the third trimester [22]. This data was unable to support a statistically significant relationship between the two variables and found that maternal 25(OH)D concentrations were not influenced by maternal age (p=0.10) [22].

Little published evidence exists regarding the association between maternal age and circulating 25(OH)D concentrations during pregnancy. Based upon this one study, maternal 25(OH)D is not likely influenced by age.

3.3.6 Religious Practice

Three studies specifically assessed the relationship between maternal 25(OH)D levels and religious practice [3,26,27]. One study, involved participants from the Moslem community, comparing those practicing purdah (veiling) with those not participating in this practice [26]. An association was found between practicing of purdah and 25(OH)D

status, as Moslem women practicing veiling had a lower mean concentration than the other Moslem women (53 nmol/L vs. 90 nmol/L) [26]. However, this was likely due to religious garb, which limited the endogenous production of 25(OH)D. Unfortunately, standard deviations were not given, which precluded inclusion into the comparison of means testing using RevMan.

Another study involved mothers from the Jewish community in Israel. Women from the non-Orthodox community were compared with Orthodox Jews, demonstrating a statistical association between specific religious practice and maternal circulating vitamin D levels (13.5 ± 7.5 ng/ml for Orthodox Jews vs. 18.5 ± 9.6 ng/ml for non Orthodox Jews, $p<0.002$) [27]. This finding was also likely due to practice differences between these forms of Judaism, including dress.

The final study investigated maternal vitamin D concentrations among Moslem and Hindus from different geographic backgrounds residing in London, England [3]. Indian and East African Moslems had higher maternal vitamin D concentrations than did Indian and East African Hindus [3].

Orthodox Jews were found to have significantly lower 25(OH)D concentrations than other Jewish expectant mothers ($p<0.001$, WMD 13.40, CI 8.56 to 18.24). In addition, Moslem women were found to have greater serum concentrations of 25(OH)D during the third trimester than practicing Hindus, both those of Indian and East African ancestry ($p<0.001$, WMD –22.79, CI –36.31 to –9.28).

Unfortunately, the other study did not cite standard deviations, which precluded a comparison of mean values, although the Moslem mothers had a mean

concentration that was 37 nmol/L higher than those practicing purdah [26]. Variations in maternal 25(OH)D exist during pregnancy based on religion, however, these differences may frequently be attributable to religious dress, which limits their exposure to sunlight, and dietary practice, the two main sources).

3.3.7 Supplementation

Several trials (17) assessed the association between vitamin D supplementation during pregnancy and maternal 25(OH)D. Twelve reported statistically significant relationships [1,4,7,32,33,48,59,63,67,70-72] while the other 5 could not support a relationship [12,23,27,61,76]. The amount and frequency of supplementation varied among the studies.

Waiters et al (1999) investigated the vitamin D status of mothers during pregnancy, and also reported the difference between those receiving vitamin D supplementation and those who did not. Women who received supplementation (87) had a significantly higher mean 25(OH)D concentration than those relying solely on dietary intake to obtain vitamin D (54.3 ± 20.2 nmol/L vs. 46.2 ± 24.8 nmol/L, $p<0.05$) [7].

Median 25(OH)D levels were also significantly lower among those not receiving supplementation compared with those who did (5.25 ng/ml vs. 7.42 ng/ml, $p<0.01$) [32]. The same conclusions were drawn between those receiving supplementation only during the 3rd trimester and those who did not receive this supplementation (7.44 ng/ml vs. 5.80 ng/ml, $p<0.01$) that supplementation resulted in an increase in 25(OH)D [32].

A study primarily investigating the differences in 25(OH)D between Libyan and Norwegian mothers also evaluated the use of supplementation among Libyan women

[33]. The nine Libyan women who received vitamin supplements had a significantly higher median 25(OH)D level than those who did not take supplements (44 nmol/L vs. 24 nmol/L, $p<0.05$) [33]. This finding also supports the evidence that supplementation during pregnancy positively influences maternal 25(OH)D concentrations. As only median values were given this could not be included in comparison of means testing.

Another study investigated the influence of supplementation on maternal concentrations of 25(OH)D during two separate seasons, with serum draws for all participants occurring at the time of delivery [1]. Of the women tested in April (52), 11 were taking supplements, while in the September group (54), nine received supplementation [1]. In both groupings, mean serum concentrations were greater among the supplemented than the unsupplemented (April 22.4 ± 4.1 ng/ml vs. 16.7 ± 4.7 ng/ml, $p<0.01$; September 33.0 ± 10.6 ng/ml vs. 25.1 ± 7.0 ng/ml, $p<0.01$) [1], further contributing to the evidence that supplementation raises circulating levels of 25(OH)D.

A randomized, double-blinded, placebo controlled study involving Asian women residing in southern England reported that the placebo group had a mean concentration of 16.2 ± 2.7 nmol/L while the group receiving 1000 IU of vitamin D daily during the third trimester had a mean 25(OH)D value of 168 ± 12.5 nmol/L, a difference that statistically favoured vitamin D supplementation, although the p value was never reported [4]. Comparison of means using RevMan 4.1 software confirmed that this was significant, $p<0.001$. The good study design enhances the findings of this study.

Another placebo-controlled study investigated the influence of vitamin D supplementation on maternal levels with the cohort receiving supplementation taking daily supplements, 400 IU vitamin D, from the 12^{th} week of pregnancy [48]. Significant

variation was found between the cohorts at 24 weeks, 34 weeks, and at delivery, demonstrating that supplementation did result in higher 25(OH)D levels during pregnancy [48]. The respective concentrations were 39.0 nmol/L vs. 32.5 nmol/L ($p<0.01$) at 24 weeks, 44.5 nmol/L vs. 38.5 nmol/L at 34 weeks ($p<0.01$), and 42.8 nmol/L vs. 32.5 nmol/L ($p<0.001$) at delivery [48].

Similarly, a study designed to measure the influence of vitamin D supplementation compared a group not supplemented with a cohort receiving 12.5 ug D_3 during the 2nd and 3rd trimesters [63]. Findings were consistent with other studies as supplementary vitamin D increased circulating 25(OH)D concentrations (87 ± 38 nmol/L vs. 59 ± 29 nmol/L, $p<0.001$) [63].

Another randomized trial of vitamin D supplementation was conducted in France, and randomly assigned women to a control group or to a supplementation group, receiving 1000 IU vitamin D daily during the last trimester [71]. At 230 days gestation and at delivery, the supplemented group had significantly higher mean 25(OH)D concentrations than the unsupplemented group ($p<0.0005$) [71]. The respective mean values for supplemented and unsupplemented groups at 230 days and at delivery were 22 ± 4 ng/ml vs. 11 ± 4 ng/ml, and 26 ± 7 ng/ml vs. 13 ± 8 ng/ml, respectively [71].

Mallet and colleagues (1996) followed women from the 3rd trimester of pregnancy and assigned them to receive no supplementation, 100 000 IU daily in the last trimester, or a dose of 200 000 IU in the 7th month [72]. The two groups that received supplementation had significantly higher mean concentrations than unsupplemented women, and supplemented values approached those normally recorded during peak summer months [72]. Their respective mean values were 9.4 ± 4.9 nmol/L, 25.3 ± 7.7

nmol/L, and 26.0 ± 6.4 nmol/L, and both supplementation strategies resulted in significantly greater levels of 25(OH)D than the control group ($p<0.001$) when data were compared using RevMan 4.1 software.

Zeghoud et al (1997) also investigated the influence of vitamin D supplementation on 22 mothers given 100000 IU in either the 6^{th} or 7^{th} month and testing occurred at delivery. The mean value for supplemented group was 49.3 ± 19 nmol/l, higher than the mean for the entire study population, 36 ± 19 nmol/L [67]. However, this study did not report a mean concentration for the unsupplemented mothers. Rather it presented this data according to set categories of 25(OH)D concentrations, which precluded this study from being included in the comparison of means statistical testing [67].

An earlier study by Zeghoud et al (1988) looked at vitamin D supplementation during winter, and two groups received a single dose of 100000 IU D3 at the 6^{th} or 7^{th} month and a third group received a placebo. Those receiving supplementation had significantly greater 25(OH)D concentrations than the placebo group, although the p value was not reported [70]. Statistical comparisons using RevMan 4.1 software revealed that these differences were significant ($p<0.05$).

Anderson et al (1988) were unable to find significant differences in 25(OH)D concentrations with supplementation. Some mothers participating in this study were given a prescription of 400 IU vitamin D2 to be used daily, but differences could not be assessed as the mean values for supplemented and unsupplemented groups, were not cited [12].

Hillman and Haddad (1974) found that mothers who took multivitamins during pregnancy did not have a significantly different mean 25(OH)D value than unsupplemented mothers (16 ± 8.02 ng/ml vs. 12.5 ± 6.49 ng/ml). Similarly, Mukamel et al (2001) found no significant difference in 25(OH)D concentration between supplemented and unsupplemented mothers.

Finally, a study comparing a cohort that regularly took 500 IU vitamin D with a cohort who did not take supplements during pregnancy found that mean values for supplemented and unsupplemented mothers were 11.1 ± 1.3 ng/ml vs. 9.1 ± 1.5 ng/ml, ($p>0.05$); a difference that was not statistically significant [76].

Two studies were unique in that they followed women prospectively and compared their pre and post intervention 25(OH)D values. One study compared pre-supplementation levels of 25(OH)D in the 1st trimester with measured 25(OH)D levels later on during pregnancy in the same expectant mothers [59]. This increase was found to be statistically significant ($p<0.02$) demonstrating that supplementation increased maternal circulating levels of 25(OH)D [59].

The other study measured expectant mothers vitamin D concentrations prior to initiating supplementation and then compared this mean value with the mean concentration at time of delivery following 400 IU daily during the last 20 to 30 weeks of pregnancy [61]. The increase in concentrations was not significant (36.9 ± 17.8 vs. 30.8 ± 14.2 ng/ml, $p>0.05$) [61].

Significant published information exists to extol the benefits of supplementation on 25(OH)D during pregnancy. Expectant mothers who were supplemented during pregnancy had consistently higher 25(OH)D concentrations

than those not receiving supplementation ($p<0.001$, WMD 23.64, CI 16.17 to 31.11). Similarly, the weighted mean difference for those studies sampling maternal concentrations at term or delivery was also significant ($p<0.001$, WMD 23.36, CI 15.48 to 31.23), and was also significant for those studies sampling mothers during their third trimester of pregnancy ($p=0.04$, WMD 30.37, CI 0.74 to 60.00).

3.3.8 Diet

Six studies reportedly investigated possible effects of maternal dietary practice, exclusive of supplementation on circulating levels of 25(OH)D. Four found statistically significant associations with dietary intake [3,4,22,54], while two others were unable to find a significant association [12,51].

Hillman and Haddad (1976) investigated the factors influencing late gestational human serum 25(OH)D including dietary intake of vitamin D. While some mothers took vitamin D supplements, this study also recorded dietary intakes of mothers to assess their intake of vitamin D [22]. While dietary intake and supplement intake were combined together, this study did demonstrated that maternal serum 25(OH) was correlated with total vitamin D intake in the winter cohort ($p<0.02$), and was related to ethnicity [22].

Another assessed the effects of maternal dietary practice comparing vegetarian and non-vegetarian cohorts [3]. The 29 vegetarians had a mean concentration at 28-32 weeks gestation of 6.6 ± 9.2 nmol/L, while the 86 non-vegetarians had a mean concentration of 24.8 ± 21.1 nmol/L, a statistically significant difference ($p<0.001$) [3].

Brooke et al (1980) also published results from a double-blind study comparing the effects of supplementation with placebo on maternal 25(OH)D concentrations during

pregnancy. There were more cases of undetectable 25(OH)D levels among women who were vegetarians than those who were not vegetarians [4]. While this finding was statistically significant ($p<0.01$) it did not report mean concentrations for the vegetarian and non-vegetarian cohorts [4] and precluded its entry into the comparisons of means testing.

Henrikson et al (1995) compared Norwegian women and Pakistani immigrant women residing in Oslo. This publication did attest to the difference in dietary intake of foods known to contain vitamin D but it did not compare women with two distinct diets. Rather this study found significant differences in the dietary intake of vitamin D between Norwegians and Pakistani immigrants ($p<0.05$) and found that the Pakistani women's mean serum 25(OH)D concentration was correlated with fortified margarine intake, their main source of vitamin D ($r=0.48$, $p<0.01$). The diet of Norwegian mothers was significantly higher ($p<0.05$) in daily vitamin D than that of the women of Pakistani origin [54].

Further comparisons between Asian vegetarians and non-vegetarians were made [51]. The mean concentrations ± SE for vegetarians ($n=23$) and non-vegetarians ($n=16$) at 28-32 weeks gestation was 9.18 ± 2.4 ng/ml and 10.13 ± 1.6 ng/ml, respectively [51], a finding that did not support conclusions of another study [3], that significant variations among vegetarians and non-vegetarians existed.

Another study investigated whether maternal concentrations of 25(OH)D were associated with dietary intake of vitamin D during the last trimester of pregnancy [12]. This investigation concluded that there was no association between maternal dietary

history exclusive of supplementation and maternal concentrations of 25(OH)D [12], and no values were presented for this comparison.

Only two studies allowed themselves to be used in comparison of means testing. Overall, this demonstrated that expectant mothers partaking in a vegetarian diet did not have significantly lower 25(OH)D concentrations than non vegetarians (p=0.12, WMD –12.01, CI –27.01 to 3.00) when the random effects model was used. Vegetarian lifestyle with high fibre intake has been associated with reduced calcium and vitamin D absorption [78].

3.3.9 Stage of Pregnancy

Several studies (15) looked at variations in 25(OH)D concentrations with stage of gestation. A majority (9) supported the belief that serum 25(OH)D did not change considerably during pregnancy [8,9,23,37,43,45,50,51,55,73], while 5 studies did find significant alterations in maternal 25(OH)D concentrations during pregnancy [4,19,22,25,28]. Another study reported values for the placebo group both at 6 months and at term and this was included in the statistical analysis to see whether concentrations changed during gestation [70].

Reddy et al (1983) investigated women with both single and multiple gestations. Vitamin D levels were measured during the first, second, and third trimesters, and at delivery. Mean concentrations for each period were calculated and contrasted, and no significant differences existed in both single and twin pregnancies with respect to stage of pregnancy [8]. For instance, the mean concentration for single gestations in all four stages was 16 ± 7 ng/ml, 18 ± 1.8 ng/ml, 16 ± 1.6 ng/ml, and 16.4 ± 2.4 ng/ml [8].

Consistent mean values were found in each distinct period of pregnancy indicating that 25(OH)D concentrations remained relatively constant throughout pregnancy.

A study of 23 women measured 25(OH)D levels along with other metabolites during second and third trimesters and postpartum [9]. The mean concentration for the second and third trimesters, were 45 ± 3 ng/ml and 47 ± 5 ng/ml, demonstrating there was no difference in these values [9]. This indicated that 25(OH)D values were constant and did not change significantly during pregnancy, further supporting this hypothesis.

Another study also found relatively consistent maternal values of 25(OH)D during specific periods of pregnancy. Hillman & Haddad (1974) evaluated serum levels of 18 healthy women, evaluating some at months 5 and 6, and at month 7, 8, and 9. The mean concentration for 5-6 months of gestation was 22.3 ± 1.5 ng/ml, while the values for months 7, 8, and 9 were 25.5 ± 8.3 ng/ml, 25.0 ± 8.1 ng/ml, and 24.4 ± 8.0 ng/ml, respectively [23]. The 25(OH)D levels of expectant mothers measured at different periods of gestation did not statistically differ, indicating that 25(OH)D may be relatively consistent throughout second and third trimesters.

One published article involved different participants including pregnant women and measured their vitamin D concentrations during first, second, and third trimesters, and at delivery [37]. The mean concentration for the second trimester was 52.8 ± 15.5 nmol/L, higher than all of the other trimesters and delivery, but this difference was not statistically significant [37]. Therefore, this study also indicated concentrations of 25(OH)D remain relatively stable during pregnancy.

Okonofua et al (1987) studied Asian and Caucasian women, measuring their 25(OH)D concentrations at 4 distinct periods of pregnancy. Median values were

determined for weeks 10-20, weeks 21-30, weeks 31-40, and during labour [43]. While this study did determine that Asians were found to have significantly lower concentrations of 25(OH)D than Caucasians, the median 25(OH)D concentrations for each of these 2 ethnic groups did not vary significantly during the course of pregnancy [43]. As median concentrations were given, this data was not included in the statistical analysis.

Whitehead et al (1981) grouped participants into four cohorts each having their 25(OH)D concentrations sampled at a different stage of pregnancy (10-12 weeks, 20-22 weeks, 30-32 weeks, and 36-40 weeks). While the mean concentration for pregnant women at 10-12 weeks and 20-22 weeks were higher than concentrations of those sampled during the second half of pregnancy, the difference was not statistically significant [45]. The respective mean values for the periods of weeks 10-12, weeks 20-22, weeks 30-32, and weeks 36-40 were 13.2 ng/L, 13.5 ng/L, 8.9 ng/L, and 8.5 ng/L, respectively [45]. Unfortunately, standard deviations were not cited which precluded this study from being incorporated in comparison of means testing.

Another study involved 37 women of Asian and Caucasian heritage, and found that 25(OH)D concentrations decreased during pregnancy as samples were drawn during the first, second, and third trimesters [50]. The mean values for each trimester were 99.2 $\pm$ 29.0 nmol/L (S.E.), 80.2 $\pm$ 11.4 nmol/L, and 59.0 $\pm$ 8.3 nmol/L [50]. Although this data demonstrated an observable decrease in mean concentration as pregnancy progressed, no overall conclusions could be reached as no statistical evidence was provided. However, this study could not be included into the overall statistical testing to

determine whether maternal 25(OH)D was influenced by stage of pregnancy as the number of women sampled during each of the 3 trimesters could not be ascertained.

Three ethnic groups were observed in another study that collected serum samples from expectant women in order to assess their circulating levels of 25(OH)D [51]. Serum collection occurred at 10-26 weeks, 28-32 weeks, and at 33-40 weeks, and in each ethnic group, maternal mean concentrations showed little variation over the various periods [51]. No significant changes were observed in maternal 25(OH)D in any of the ethnic groups as pregnancy progressed [51]. 25(OH)D concentrations remained relatively constant during pregnancy in all 3 ethnic groups.

Bruinse and van den Berg (1995) investigated the change in certain vitamin levels during pregnancy, sampling subjects at 16 weeks, 28 weeks, 34 weeks, and even postpartum. While specific mean values were not cited, apart from a graph, this text did state that 25(OH)D remained constant for the most part throughout pregnancy [55]. However, this study could not be included in comparison of means testing.

Martinez et al (1986) investigated effects of season and stage of pregnancy on maternal concentrations of 25(OH)D. Women were grouped into March and September cohorts with the March group including 10 women in their first trimester, 11 in their second trimester, and 11 in their third trimester [73]. The September group included 14 women in their first trimester, 19 in their second, and 12 in their third trimester of pregnancy [73]. No stage of pregnancy variations in either seasonal group, were noticed [73]. The mean (± S.E.) for the first, second, and third trimesters in the September group were 29.2 ± 3.2 ng/ml, 32.6 ± 2.8 ng/ml, and 33.9 ± 2.7 ng/ml, while the March group values were 16.4 ± 2.3 ng/ml, 16.1 ± 1.1 ng/ml, and 19.3 ± 1.6 ng/ml, respectively [73].

While no significant variations were observed, the authors proposed that decreases in 25(OH)D during pregnancy were not found because of the high sun exposure women in this region of Europe received [73].

Sanchez et al (1997) enrolled pregnant Nigerian women and grouped them according to trimesters, with each group consisting of 10 women. Maternal concentrations were two to three times higher between the first and third trimesters, demonstrating an increasing concentration with progressing gestation [25]. The respective mean concentrations for first, second, and third trimesters were 9.69 ± 3.23 ng/ml, 17.2 ± 7.92 ng/ml, and 29.8 ± 10.5 ng/ml [25]. This was statistically significant ($p<0.001$) [25].

Another study involving Saudi Arabian women followed 40 participants throughout pregnancy, finding that mean 25(OH)D concentrations decreased significantly from the first trimester to the third trimester (54 ± 10 nmol/L vs. 33 ± 8 nmol/L, $p<0.001$) [28]. Levels near the end of pregnancy were significantly lower than those at the beginning period of gestation. Maternal mean concentration at term was similar to that of the 3rd trimester (33 ± 8 nmol/L), while the mean value at term was also considerably less than the recorded concentration of the first trimester ($p<0.001$) [28].

Women participating in another study had serum concentrations assessed at 28-32 weeks of pregnancy and were randomized into either a control group or treatment with supplemented vitamin D [4]. The control group was used to assess the possible variation in maternal 25(OH)D with stage of pregnancy. The control group's mean concentration at term was compared with the total participant value at 28 weeks when all participants

were enrolled. The mean 25(OH)D concentration at 28 weeks and at term were 20.1 ± 1.9 and 16.2 ± 2.7 nmol/L, which did not differ significantly [4].

Hillman & Haddad (1976) did not compare measured 25(OH)D levels at two distinct periods of gestation, but did determine whether the week of pregnancy (weeks 29-42) was correlated with 25(OH)D concentration. 25(OH)D was found to be positively correlated with the length of gestation at the time of serum testing ($p<0.05$) [22].

The final study that considered the association between stage of pregnancy and 25(OH)D involved women in southern California [19]. 94 women had their serum 25(OH)D levels sampled at various periods of pregnancy, and were tested at 0-10 weeks, 11-20 weeks, 21-30 weeks, and 31-40 weeks [19]. Women measured in the first period of pregnancy had a significantly higher mean concentration than those tested during weeks 31-40 (24.3 ± 2.6 vs. 16.3 ± 1.3 ng/ml, $p<0.006$) [19]. Likewise, the mean concentration in the first quarter of pregnancy was also significantly greater than the second quarter (24.3 ± 2.6 ng/ml vs. 18.2 ± 1.3 ng/ml, $p<0.04$) indicating a decrease in 25(OH)D as pregnancy progressed, but no significant differences were found between the later stages of gestation [19].

The placebo arm of the study by Zeghoud et al (1988) was also included to assess the variation of serum 25(OH)D between the second trimester and term. Maternal values for women belonging to the March and April subgroups of the placebo cohort were used to determine whether levels of 25(OH)D were statistically significant for these periods. While both subgroups demonstrated higher values during the second trimester, only the April subgroup demonstrated statistical significance when analyzed using RevMan [70].

Variations did not exist for concentrations of 25(OH)D during certain stages of pregnancy. For instance, there were no significant differences in concentrations between first and third trimesters (p=0.4, WMD 9.75, CI –14.93 to 34.44). Similarly, significant differences were not observed between first and second trimesters (p=0.08, WMD 7.70, CI -0.80 to 16.19). These findings may be confounded by supplementation that may have been introduced in some of the studies or by sun exposure.

No significant differences in mean 25(OH)D concentrations were also observed between first trimester and term (p=0.3, WMD –10.73, CI –30.09 to –8.63) and second and third trimester (p=0.4). However, a significant difference in mean concentrations was found between second trimester and term/delivery (p=0.004) favouring second trimester values, but not third trimester and term (p=0.5).

No significant differences were observed between 5^{th} and 6^{th}, 7^{th}, 8^{th} or 9^{th} month of pregnancy (5^{th} and 6^{th} month vs. 7^{th} month (p=0.5), 5^{th} and 6^{th} month vs. 8^{th} months (p=0.5), and 5^{th} and 6^{th} month vs. 9^{th} month (p=0.5). 7^{th} vs. 8^{th} month, 7^{th} vs. 9^{th} month, and 8^{th} vs. 9^{th} month were p=0.9, p=0.8, and p=0.9, respectively).

Likewise, two other studies compared specific weeks of gestation. No significant differences in maternal concentrations of 25(OH)D were found between weeks 10-26 and weeks 28-32 (p=0.7), 10-26 weeks and 33-40 weeks (p=0.9), 28-32 weeks and 33-40 weeks (p=0.9) [51]. However, another study demonstrated significant differences in values of 25(OH)D between 0-10 weeks gestation and 11-20 weeks (p=0.04, WMD –16.29, CI –31.50 to –1.08), and 0-10 weeks and 31-40 week

period (p=0.006, WMD –21.36, CI –36.57 to –6.15), with both comparisons demonstrating higher values at 0-10 weeks.

Therefore, differences in maternal 25(OH)D are not likely to occur between first stages of gestation and those mid to late stages of gestation. In addition, no significant differences are likely to be experienced from start of the second trimester until term and delivery according to the literature. Some conflicting evidence does exist and this needs to be resolved, perhaps through new investigations.

3.3.10 Gravid History

Only one study assessed whether previous gestations had any impact on circulating concentrations of 25(OH)D [22]. Mothers who had previous children were not more likely to have lower vitamin D concentrations than those reporting their first pregnancy as no statistical difference in serum concentration between women who were primigravid or multigravid existed [22]. Unfortunately, the mean serum concentrations were not cited in the text, which precluded a statistical comparison of mean values.

Limited information exists regarding the influence of gravid history on maternal levels of 25(OH)D.

3.3.11 Single vs. Multiple Fetuses

Two studies investigated the relationship between the number of fetuses and maternal 25(OH)D concentration. One study did not find a significant relationship between the number of fetuses and maternal concentration of 25(OH)D [8], while the other did [10]. The study by Okah et al (1996) involved 17 expectant mothers carrying multiple fetuses

and 30 women carrying a single fetus. The mean 25(OH)D for singleton pregnancies was 39 ± 2 ng/ml while the mean concentration among multiple gestations was 61 ± 5 ng/ml, a finding that was statistically significant ($p<0.001$) [10]. The other investigated the variations in mean maternal vitamin D concentrations between single and multiple gestations, demonstrating that mean values among the multiple gestation group, were slightly less than those of the single pregnancy group [8]. However, it did not indicate if this difference was statistically significant [8]. RevMan software revealed that the values for single and multiple gestations were not statistically different from each other.

Limited published evidence exists to determine the exact relationship between the number of gestations and maternal 25(OH)D concentrations. Comparison of means calculated a weighted mean difference of 25.30 (CI –37.49 to 88.10) which was not statistically significant, p=0.4. Comparisons between single and multiple gestations in the second trimester and at delivery were also not statistically significant with p=0.3 and p=0.1, respectively. Therefore, maternal 25(OH) concentrations may not be associated with number of fetuses.

3.3.12 Antenatal Care

Only one study sought to determine the relationship between antenatal care and maternal vitamin D levels concluding that concentrations of vitamin D were associated with antenatal care [32]. Specifically, mothers who did not receive antenatal care had significantly lower median 25(OH)D concentrations (6.4 ng/ml) than those women who received antenatal care during their pregnancy (7 ng/ml), even if it only entailed one visit during pregnancy ($p<0.05$) [32]. An explanation proposed for this finding associates

antenatal care with SES, with those women from the higher SES group all receiving antenatal care, while less than one third in the lower SES group received this same attention [32]. As only median values for 25(OH)D were cited, this precluded comparison of means testing.

Limited information currently exists to ascertain the relationship between maternal serum concentrations of 25(OH)D and antenatal care, although this one study did find a difference, likely related to socioeconomic factors.

3.3.13 Sun Exposure/Hours Exposed

Two studies were unable to find an association between sun exposure and maternal vitamin D concentration [7,54], but another three studies did find that maternal concentrations were significantly higher among women with increased sun exposure [22,68,69]. Other studies that investigated religious practice and dress are indirectly related to sun exposure as religious dress or practice may have prohibited endogenous synthesis.

A study of Canadian women residing in the Northwest Territories investigated whether hours of bright sunshine influenced maternal concentrations of 25(OH)D [7]. Maternal concentrations were measured at the time of delivery, and mean concentrations of 25(OH)D for each month were compared with the amount of hours of bright sunshine in each month [7]. While a significant relationship was not found, a negative correlation nearing significance was found between 25(OH)D level and hours of sunshine ($p=0.07$) [7]. Maternal 25(OH)D concentrations were found to be near their lowest when hours of sunshine a month were the highest [7]. The low concentration measured at delivery

during May was believed to reflect the insufficient endogenous production during the winter and spring months [7].

Another study investigated vitamin D levels in Pakistani immigrants and Norwegians [54]. In addition to comparing differences between the ethnic groups, this study also investigated the effect of sunshine on serum levels of 25(OH)D [54]. Many Pakistanis were not exposed to frequent sunshine likely attributable to dress yet those who covered their skin and had less sunshine exposure did not have a statistically different median 25(OH)D concentration than the remaining Pakistanis ($p=0.3$, 17 nmol/L vs. 21 nmol/L) [54]. While this study did not find a significant influence on maternal 25(OH)D, the authors speculated that this was likely due to the limited amount of sunshine at that latitude, and the Pakistani cultural practice of limiting sun exposure [54].

A study of African Americans and Caucasians in the United States considered the influence of many variables on maternal 25(OH)D concentrations, including the number of hours spent outside [22]. Results indicated that no significant correlations existed between these variables for women belonging to the summer cohort [22]. Among the winter cohort, there was also no significant correlation between maternal 25(OH)D levels and hours spent outside for Caucasian women, but a statistically significant correlation was found among African Americans belonging to the winter group ($p<0.01$).

Martinez et al (1991) investigated both diabetic and healthy pregnant women during pregnancy and their levels of 25(OH)D, and sampled women during all 3 trimesters of pregnancy. Mean serum concentrations for each trimester were presented according to the minimum and maximum sunlight periods [68]. Mean values for the

healthy women were higher in all three trimesters during the period of maximum solar irradiation [68]. First, second, and third trimester mean concentrations for the healthy women in the maximum and minimum solar irradiation groupings were 29.2 ± 11.5 ng/ml vs. 15.4 ± 7.5 ng/ml, 32.6 ± 11.8 ng/ml vs. 17.2 ± 7.9 ng/ml, and 33.9 ± 8.9 ng/ml vs. 19.5 ng/ml [68]. In all situations, mean concentrations were higher during the period of greatest sunlight [68].

Zeghoud et al (1991) contrasted maternal mean values of 25(OH)D between those exposed and unexposed to sunlight during the last trimester of pregnancy. The mean values for exposed and unexposed were 18.9 ± 7.7 ng/ml and 9.8 ± 4.9 ng/ml, a difference that was statistically significant, $p<0.0001$ [69].

Significant variations in maternal 25(OH)D were observed between periods of maximum and minimum sunshine, when measured in both first and second trimester of pregnancy ($p<0.001$, WMD 13.80, CI 6.94 to 20.66) and ($p<0.001$, WMD 15.40, CI 9.19 to 21.61), respectively. A maximum amount of sunshine was associated with higher serum concentrations. Greater hours spent outdoors in summer compared with winter also demonstrated significance, with greater concentrations of 25(OH)D in summer hours ($p<0.001$, WMD 26.70, CI 22.88 to 30.52). Similarly, mothers exposed to sunlight had greater concentrations of 25(OH)D than those not exposed ($p<0.001$, WMD –24.30, CI –34.39 to –14.21).

3.3.14 Correlation of Maternal and Cord(Fetal) 25(OH)D

Thirty-six studies investigated the relationship between maternal 25(OH)D and cord levels. All studies with the exception of two found a positive correlation between

maternal and cord concentrations, with the level found in the cord being lower than in the mother [1,3,4,12,13,15,20,21,23,30,32,33,35,37,40-42,47-49,57,58,60,61,63-66,71,72,75-77]. Only two studies were unable to corroborate this finding, and found no correlation between measured levels of maternal 25(OH)D and cord concentrations [16,25].

3.3.15 Correlation of Maternal and Infant 25(OH)D

Twelve studies assessed the association between maternal 25(OH)D and infant levels. Positive correlations were found in 11 studies indicating that infant concentrations are correlated with those of the mother [7,12,29,31,36,38,42,48,67,69,77], while one found no correlation [17].

3. 4 Discussion

This exercise identified studies reporting maternal concentrations of 25(OH)D during pregnancy, and reviewed those factors associated or correlated with maternal concentrations in healthy expectant mothers.

Ample evidence was found concluding that 25(OH)D status during pregnancy was associated with the explanatory variables supplementation, ethnicity, season, and sun exposure. Women who received vitamin D supplementation during pregnancy were known to have higher concentrations than unsupplemented women, and this was confirmed via comparison of means testing. This finding is not surprising since an increase in exogenous intake will naturally cause levels of 25(OH)D to rise.

Concentrations were also found to vary depending on the ethnic background. Caucasians had greater concentrations than Asians and African Americans, and this may

be attributable to skin pigmentation or differences in factors influencing endogenous and exogenous 25(OH)D attainment between these groups [79,80]. Statistical analysis revealed that the differences in 25(OH)D between Jews and Bedouins, and between certain Asian subgroups were insignificant. On an interesting note, no significant variations existed between Caucasians and Aboriginals residing in Canada's north [7].

Considering that endogenous production of 25(OH)D is the physiological prime source for humans, it was not surprising that season and sun exposure were associated with maternal concentrations during pregnancy. Summer seasons and periods of increased sun exposure were known to produce significantly higher concentrations.

Intermediate evidence exists supporting relationships between maternal diet, religious practice, stage of pregnancy, and geography with 25(OH)D. Few studies specifically examined maternal diets, with the exception of those that contrasted the vegetarian lifestyle with non-vegetarians. While individual studies reported differences, when results were combined, variations between the two dietary practices did not exist. Some evidence existed that there were differences in 25(OH)D depending on religious practice, but this is likely confounded by religious dress, which may have impeded endogenous synthesis. More evidence is required before accurate conclusions can be assembled. While several studies indicated geographical differences in 25(OH)D the fact that only one study could be included in comparison of mean testing limits a confident prediction that geography is associated with 25(OH)D levels during pregnancy.

Conflicting evidence exists surrounding the variability of 25(OH)D throughout pregnancy and requires additional investigation. Knowing that 25(OH)D may be

generally consistent throughout pregnancy validates the comparisons made in this systematic review.

Very limited or no evidence exists to formally assess the influence of SES, age, gravid history, number of fetuses, and antenatal care on 25(OH) concentrations during pregnancy. Future 25(OH)D studies may shed more light on these relationships.

While this systematic review has uncovered interesting evidence, certain limitations exist. Only one electronic database was searched, which is a recognizable limitation of this study since other studies may have been retrieved through searches of other databases and the grey literature. In addition, a more refined and robust search strategy may also have helped to uncover additional information. However, given that the random effects model was used for the statistical analysis this strategy was considered sufficient. Publication bias may also exist, in that studies that were not able to demonstrate significant associations with maternal 25(OH)D may not have been published. Methodological study design also varied between studies with many being either cross sectional, or longitudinal investigations. Only a few were randomized controlled trials.

Comparison of means revealed frequent heterogeneity when multiple studies were pooled to evaluate the variance of maternal 25(OH)D. This is likely due to differences in study design (i.e. cross sectional vs. intervention studies) and populations (i.e. ethnicity, geography, etc.).

One of the most significant limitations was that multiple regression analyses were not undertaken in most included studies, rather relying on less robust analysis of variance or other bivariate statistical tests. The advantage of multiple regression is its ability to

identifying those explanatory variables most genuinely associated with the continuous outcome variable, in this instance mean 25(OH) D concentration, while controlling for the other variables.

Knowing that the sun exposure and vitamin D supplementation are strongly associated with higher serum concentrations of 25(OH)D, they represent the best modalities for improving 25(OH)D status during pregnancy. However, considering that women in Winnipeg can only endogenously produce 25(OH)D for just over half the year, vitamin D supplementation during pregnancy may be essential to ensuring sufficiency in parts of Canada.

More research is needed to clarify ambiguities surrounding the relationships between possible explanatory variables and maternal 25(OH)D. Ultimately, should future studies measure 25(OH)D levels during pregnancy, the associations between 25(OH)D and the multitude of proposed explanatory variables may be quantifiable. Should much of this uncertainty be explained, the development a patient scorecard, or a risk assessment tool, could eventually serve as a proxy measure for health professionals to gauge the 25(OH)D status of their patients, since the costs of serum assays for 25(OH)D are prohibitive.

3.5 Conclusions

There is strong and convincing evidence that the following variables are associated with maternal serum 25(OH)D:

- Vitamin D supplementation during pregnancy
- Ethnicity
- Season
- Sun exposure

There is intermediate evidence supporting the association between maternal serum 25(OH)D and:

- Maternal diet
- Religious practice
- Stage of pregnancy
- Geography

There is little or no evidence to support the association between maternal serum 25(OH)D and:

- SES
- Maternal age
- Gravid history
- Single vs. multiple fetuses
- Antenatal care

In addition, there is ample evidence to support that:

- Maternal and fetal concentrations of 25(OH)D are correlated
- Maternal and infant concentrations of 25(OH)D are correlated

Appendix 3.1 – Summary of Comparisons ($p \leq 0.05$ is statistically significant)

Comparison	Weighted Mean Difference ± 95% C.I.	n	Favours	p value
3.3.1 Geography				
Lyon, France vs. Chambery, France	5.24 ± 11.57	29	Neither	0.4
Lyon, France vs Nice, France	6.71 ± 15.91	32	Neither	0.4
Chambery, France vs. Nice, France	2.65 ± 13.05	25	Neither	0.7
3.3.2 SES				
Lower SES vs. Upper SES	-5.60 ± 8.04	54	Neither	0.17
3.3.3 Ethnicity				
Black vs. Caucasian	10.46 ± 5.95	245	Caucasian	<0.005
Native Indian vs. Caucasian	7.70 ± 13.05	70	Neither	0.2
Inuit vs. Caucasian	11.00 ± 10.76	84	Caucasian	0.05
Native Indian vs. Inuit	-3.30 ± 9.21	88	Neither	0.5
Bedouin vs. Jew	22.96 ± 44.87	71	Neither	0.3
Indian vs. Pakistani	-3.73 ± 7.37	120	Neither	0.3
Indian vs. East African	3.00 ± 13.37	82	Neither	0.7
Indian vs. Sri Lankan	22.00 ± 18.5	72	Sri Lankan	<0.05
Indian vs. Bangladeshi	7.15 ± 10.25	101	Neither	0.17
Pakistani vs. East African	11.00 ± 13.49	36	Neither	0.11
Pakistani vs. Sri Lankan	30.00 ± 18.58	26	Sri Lankan	<0.005
Pakistani vs. Bangladeshi	11.98 ± 17.76	53	Neither	0.19
East African vs. Sri Lankan	19.00 ± 21.65	24	Neither	0.09
East African vs. Bangladeshi	11.00 ± 16.62	24	Neither	0.19

Comparison	Weighted Mean Difference ± 95% C.I.	n	Favours	p value
(3.3.3 Ethnicity Continued)				
Sri Lankan vs. Bangladeshi	-8.00 ± 20.97	14	Neither	0.5
Asian vs. Caucasian	39.56 ± 43.47	184	Neither	0.07
Asian vs. West Indian	2.11 ± 13.32	45	Neither	0.8
Diverse Ethnicity vs. Caucasian	13.37 ± 5.88	60	Caucasian	<0.005
3.3.4 Season				
Summer vs. Winter	58.46 ± 16.43	217	Summer	<0.005
Summer vs. Autumn	24.39 ± 7.42	237	Summer	<0.005
Summer vs. Spring	18.30 ± 8.36	68	Summer	<0.005
Summer/Autumn vs. Winter/Spring	20.10 ± 12.1	45	Summer/ Autumn	<0.005
April vs. September	23.04 ± 6.32	106	September	<0.005
May vs. June	6.62 ± 9.87	43	Neither	0.19
May vs. August	21.10 ± 22.15	12	Neither	0.06
May vs. July	10.42 ± 18.25	15	Neither	0.3
June vs. August	13.62 ± 25.41	13	Neither	0.3
June vs. July	12.55 ± 18.25	19	Neither	0.18
November-January vs. February/April	-1.00 ± 9.2	79	Neither	0.8
November/January vs. May/July	-0.90 ± 9.13	66	Neither	0.8
November/January vs. August/October	8.20 ± 12.86	56	Neither	0.2
February/April vs. May/July	0.10 ± 8.57	59	Neither	1

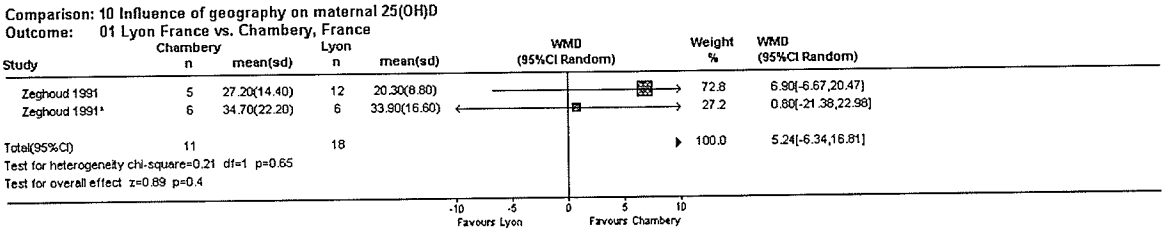
Comparison	Weighted Mean Difference ± 95% C.I.	n	Favours	p value
(3.3.4. Season Continued)				
February/April vs. August/October	9.20 ± 12.47	49	Neither	0.15
May/July vs. August/October	9.10 ± 12.42	36	Neither	0.15
September vs. March	39.88 ± 9.96	77	September	<0.005
March vs. April	0.30 ± 10.53	9	Neither	1
3.3.5 Maternal Age				
no comparisons available				
3.3.6 Religious Practice				
Orthodox Jew vs. Non Orthodox Jew	13.40 ± 4.84	341	Non Orthodox	<0.005
Moslem vs. Hindu	22.79 ± 13.52	82	Moslem	<0.005
3.3.7 Supplementation				
No supplementation vs. Supplementation	23.64 ± 7.47	919	Supplementation	<0.005
No supplementation vs. Supplementation (Term/Delivery)	23.36 ± 7.87	893	Supplementation	<0.005
No supplementation vs. Supplementation (Third Trimester)	30.37 ± 29.63	200	Supplementation	<0.05
3.3.8 Diet				
Non-vegetarian vs. Vegetarian	-12.01 ± 15.00	154	Neither	0.12
3.3.9 Stage of Pregnancy				
First Trimester vs. Third Trimester	9.75 ± 24.69	189	Neither	0.4
First Trimester vs. Second Trimester	7.70 ± 8.49	115	Neither	0.08
First Trimester vs. Term	-10.73 ± 19.36	110	Neither	0.3

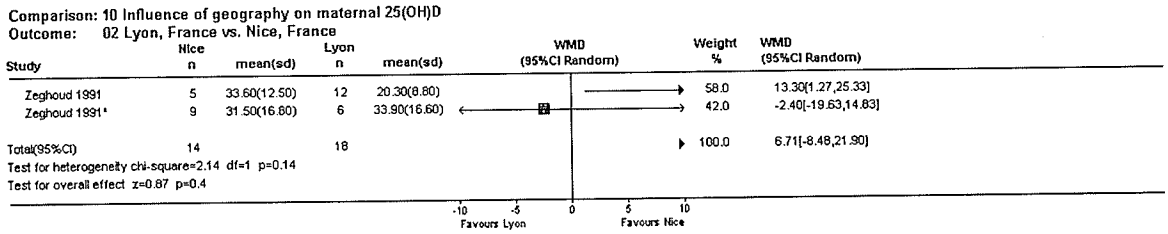
Comparison	Weighted Mean Difference ± 95% C.I.	n	Favours	p value
(3.3.9 Stage of Pregnancy Continued)				
Second Trimester vs. Third Trimester	4.45 ± 9.99	191	Neither	0.4
Second Trimester vs. Term/Delivery	6.80 ± 4.59	70	Second Trimester	<0.005
Third Trimester vs. Term	-1.66 ± 4.77	276	Neither	0.5
5^{th} & 6^{th} Month vs. 7^{th} Month	8.55 ± 22.19	7	Neither	0.5
5^{th} & 6^{th} Month vs. 8^{th} Month	7.21 ± 21.68	7	Neither	0.5
5^{th} & 6^{th} Month vs. 9^{th} Month	5.61 ± 16.46	10	Neither	0.5
7^{th} vs. 8^{th} Month	-1.34 ± 30.35	8	Neither	0.9
7^{th} vs. 9^{th} Month	-2.94 ± 26.87	11	Neither	0.8
8^{th} vs. 9^{th} Month	-1.39 ± 26.45	11	Neither	0.9
10-26 weeks vs. 28-32 weeks	-2.12 ± 9.32	112	Neither	0.7
10-26 weeks vs. 33-40 weeks	0.29 ± 8.79	112	Neither	0.9
28-32 weeks vs. 33-40 weeks	-0.43 ± 7.30	112	Neither	0.9
0-10 weeks vs. 11-20 weeks	-16.29 ± 15.21	44	0-10 weeks	<0.05
0-10 weeks vs. 21-30 weeks	-12.01 ± 16.26	44	Neither	0.15
0-10 weeks vs. 31-40 weeks	-21.36 ± 15.21	44	0-10 weeks	<0.01
11-20 weeks vs. 21-30 weeks	4.28 ± 11.20	50	Neither	0.5
11-20 weeks vs. 31-40 weeks	-5.07 ± 9.62	50	Neither	0.3
21-30 weeks vs. 31-40 weeks	-9.35 ± 11.2	50	Neither	0.1
3.3.10 Gravid History				
no comparisons available				

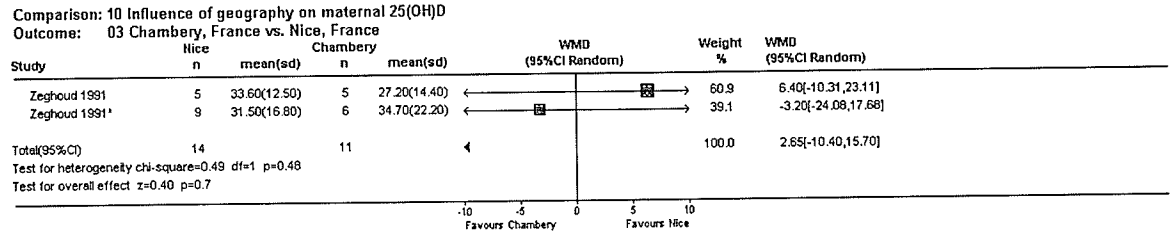
Comparison	Weighted Mean Difference ± 95% C.I.	n	Favours	p value
3.3.11 Single vs. Multiple Fetuses				
Single vs. Multiple Gestations Third Trimester	25.30 ± 62.8	88	Neither	0.4
Single vs. Multiple Gestations Second Trimester	-8.01 ± 15.29	31	Neither	0.3
Single vs. Multiple Gestations Delivery	-14.42 ± 16.97	21	Neither	0.1
3.3.12 Antenatal Care				
no comparisons available				
3.3.13 Sun Exposure/Hours Exposed				
Minimum Solar Irradiation vs. Maximum Solar Irradiation – First Trimester	13.80 ± 6.86	34	Maximum	<0.005
Minimum Solar Irradiation vs. Maximum Solar Irradiation – Second Trimester	15.40 ± 6.21	42	Maximum	<0.005
Hours Outside Winter vs. Hours Outside Summer	26.70 ± 3.82	117	Hours Outside Summer	<0.005
No Sun Exposure vs. Sun Exposure	24.30 ± 10.09	60	Sun Exposure	<0.005

Appendix 3.2

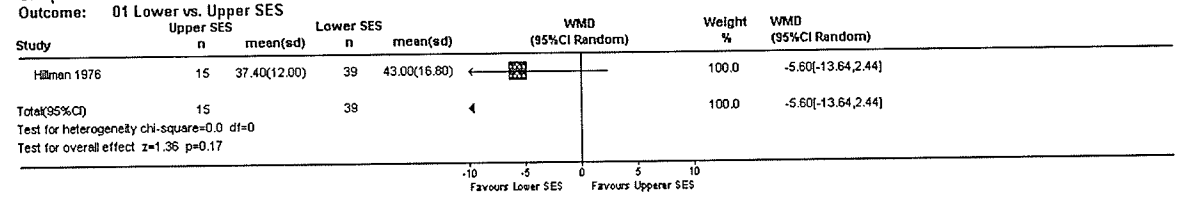
3.3.1 Geography





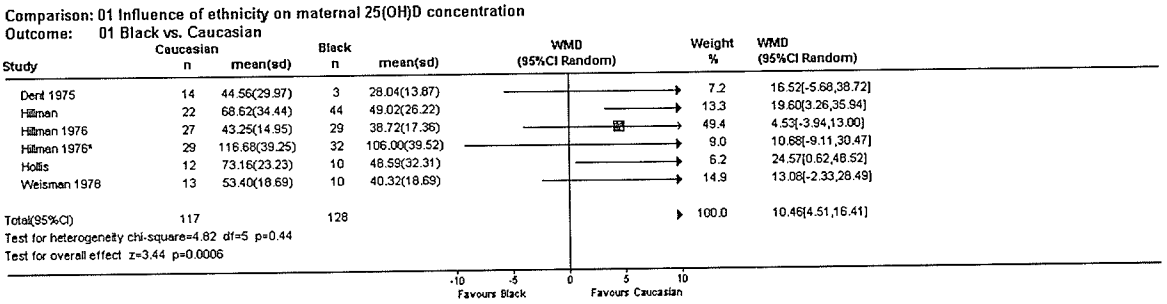


3.3.2 SES

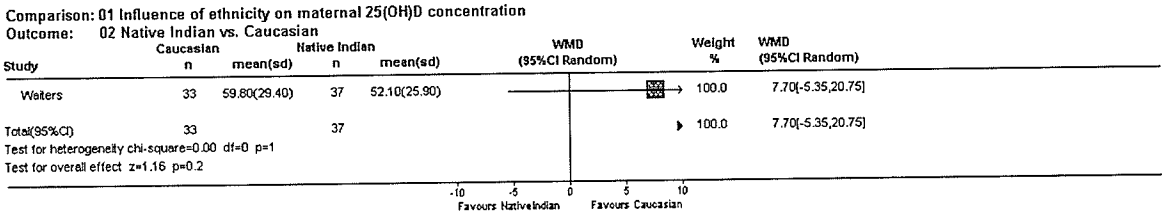


3.3.3 Ethnicity

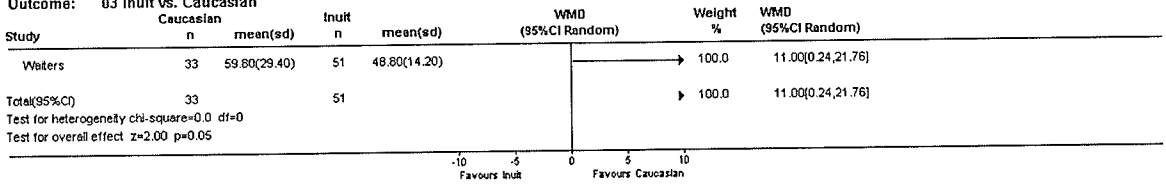
Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 01 Black vs. Caucasian



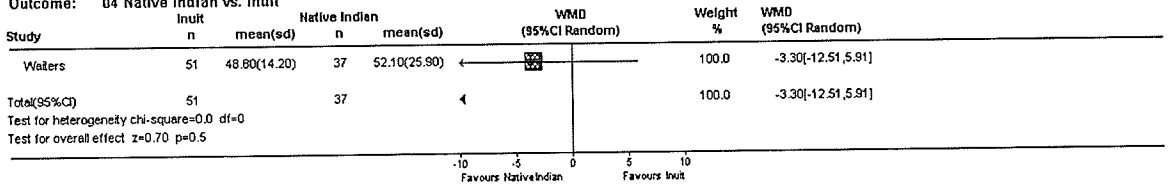
Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 02 Native Indian vs. Caucasian



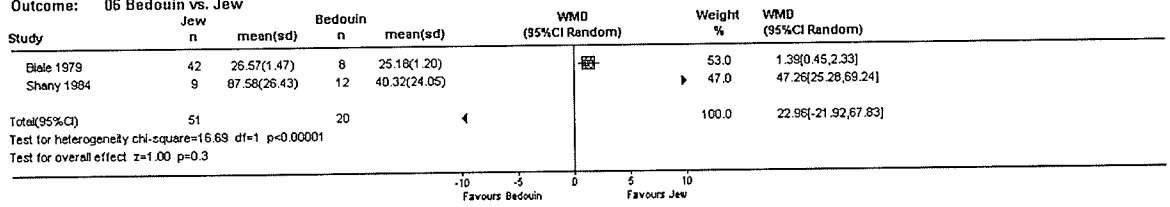
Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 03 Inuit vs. Caucasian



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 04 Native Indian vs. Inuit



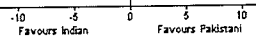
Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 06 Bedouin vs. Jew



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 07 Indian vs. Pakistani

Study	Pakistani n	Pakistani mean(sd)	Indian n	Indian mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	19	10.00(12.00)	65	18.00(21.00)		42.0	-8.00[-15.43,-0.57]
Ong 1983	17	10.15(6.01)	19	10.79(8.22)		58.0	-0.64[-5.31,4.03]
Total(95%CI)	36		84			100.0	-3.73[-10.85,3.39]

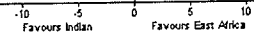
Test for heterogeneity chi-square=2.70 df=1 p=0.1
Test for overall effect z=1.03 p=0.3



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 08 Indian vs. East African

Study	East African n	East African mean(sd)	Indian n	Indian mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	17	21.00(26.00)	65	18.00(21.00)		100.0	3.00[-10.37,16.37]
Total(95%CI)	17		65			100.0	3.00[-10.37,16.37]

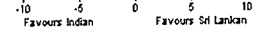
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=0.44 p=0.7



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 09 Indian vs. Sri Lankan

Study	Sri Lankan n	Sri Lankan mean(sd)	Indian n	Indian mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	40.00(24.00)	65	18.00(21.00)		100.0	22.00[3.50,40.50]
Total(95%CI)	7		65			100.0	22.00[3.50,40.50]

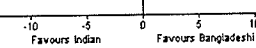
Test for heterogeneity chi-square=0.00 df=0 p=1
Test for overall effect z=2.33 p=0.02



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 10 Indian vs. Bangladeshi

Study	Bangladeshi n	Bangladeshi mean(sd)	Indian n	Indian mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	32.00(15.00)	65	18.00(21.00)		36.8	14.00[1.77,26.23]
Ong 1983	10	13.94(6.59)	19	10.79(8.22)		63.2	3.15[-2.36,8.66]
Total(95%CI)	17		84			100.0	7.15[-3.11,17.40]

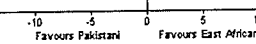
Test for heterogeneity chi-square=2.51 df=1 p=0.11
Test for overall effect z=1.37 p=0.17



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 11 Pakistani vs. East African

Study	East African n	East African mean(sd)	Pakistani n	Pakistani mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	17	21.00(26.00)	19	10.00(12.00)		100.0	11.00[-2.49,24.49]
Total(95%CI)	17		19			100.0	11.00[-2.49,24.49]

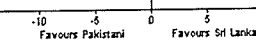
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.60 p=0.11



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 12 Pakistani vs. Sri Lankan

Study	Sri Lankan n	Sri Lankan mean(sd)	Pakistani n	Pakistani mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	40.00(24.00)	19	10.00(12.00)		100.0	30.00[11.42,48.58]
Total(95%CI)	7		19			100.0	30.00[11.42,48.58]

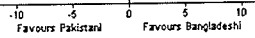
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=3.16 p=0.002



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 13 Pakistani vs. Bangladeshi

Study	Bangladeshi n	Bangladeshi mean(sd)	Pakistani n	Pakistani mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	32.00(15.00)	19	10.00(12.00)		45.0	22.00[9.65,34.35]
Ong 1983	10	13.94(6.59)	17	10.15(6.01)		55.0	3.79[-1.19,8.77]
Total(95%CI)	17		36			100.0	11.98[-5.77,29.74]

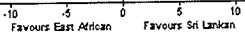
Test for heterogeneity chi-square=7.18 df=1 p=0.0074
Test for overall effect z=1.32 p=0.19



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 14 East African vs. Sri Lankan

Study	Sri Lankan n	Sri Lankan mean(sd)	East African n	East African mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	40.00(24.00)	17	21.00(26.00)		100.0	19.00[-2.65,40.65]
Total(95%CI)	7		17			100.0	19.00[-2.65,40.65]

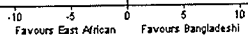
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.72 p=0.09



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 15 East African vs. Bangladeshi

Study	Bangladeshi n	Bangladeshi mean(sd)	East African n	East African mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	32.00(15.00)	17	21.00(26.00)		100.0	11.00[-5.62,27.62]
Total(95%CI)	7		17			100.0	11.00[-5.62,27.62]

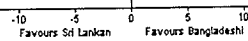
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.30 p=0.19



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 16 Sri Lankan vs. Bangladeshi

Study	Bangladeshi n	Bangladeshi mean(sd)	Sri Lankan n	Sri Lankan mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	7	32.00(15.00)	7	40.00(24.00)		100.0	-8.00[-28.97,12.97]
Total(95%CI)	7		7			100.0	-8.00[-28.97,12.97]

Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=0.75 p=0.5



Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 17 Asian vs. Caucasian

Study	Caucasian n	Caucasian mean(sd)	Asian n	Asian mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Bashir 1981	59	119.03(52.31)	58	39.04(18.72)		34.0	79.99[65.80,94.18]
Dent 1975	14	44.56(29.97)	16	27.05(17.09)		33.3	17.51[-0.28,35.30]
Dent 1975*	14	44.56(29.97)	23	24.51(30.73)		32.8	20.05[-0.05,40.15]
Total(95%CI)	87		97			100.0	39.56[-3.91,83.03]

Test for heterogeneity chi-square=38.21 df=2 p<0.00001
Test for overall effect z=1.78 p=0.07

Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 18 Asian vs. West Indian

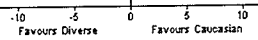
Study	West Indian n	West Indian mean(sd)	Asian n	Asian mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Dent 1975	3	28.04(13.87)	16	27.05(17.09)		56.1	0.99[-16.80,18.78]
Dent 1975*	3	28.04(13.87)	23	24.51(30.73)		43.9	3.53[-16.57,23.63]
Total(95%CI)	6		39			100.0	2.11[-11.22,15.43]

Test for heterogeneity chi-square=0.03 df=1 p=0.85
Test for overall effect z=0.31 p=0.8

Comparison: 01 Influence of ethnicity on maternal 25(OH)D concentration
Outcome: 19 Diverse Ethnic (Southern Europe, African, Middle East) vs. Caucasian

Study	Caucasian n	Caucasian mean(sd)	Diverse Ethnic n	Diverse Ethnic mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991	11	57.20(21.36)	8	38.98(12.82)		14.5	18.22[2.78,33.66]
Zeghoud 1991 a	24	31.24(14.15)	17	18.69(6.14)		85.5	12.55[6.18,18.92]
Total(95%CI)	35		25			100.0	13.37[7.49,19.26]

Test for heterogeneity chi-square=0.44 df=1 p=0.51
Test for overall effect z=4.45 p=0.00001

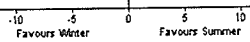


3.3.4 Season

Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 01 Summer vs. Winter

Study	Summer n	Summer mean(sd)	Winter n	Winter mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Hillman 1976	61	112.41(37.11)	56	41.12(15.75)		41.1	71.29[61.10,81.48]
Kuroda	17	90.51(33.38)	12	42.19(17.62)		29.9	48.32[29.58,67.06]
Namgung	37	114.81(48.06)	34	64.08(34.71)		29.1	50.73[31.34,70.12]
Total(95%CI)	115		102			100.0	58.46[42.03,74.89]

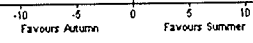
Test for heterogeneity chi-square=6.43 df=2 p=0.04
Test for overall effect z=6.97 p<0.00001



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 02 Summer vs. Autumn

Study	Summer n	Summer mean(sd)	Autumn n	Autumn mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Kokot 1980	19	44.86(24.43)	26	24.83(13.35)		37.4	20.03[7.91,32.15]
Lamberg-Allardt	30	75.00(31.00)	63	52.00(24.00)		34.8	23.00[10.42,35.58]
Lamberg-Allardt a	45	105.00(36.00)	54	73.00(35.00)		27.8	32.00[17.94,46.06]
Total(95%CI)	94		143			100.0	24.39[16.98,31.81]

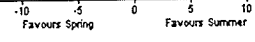
Test for heterogeneity chi-square=1.67 df=2 p=0.43
Test for overall effect z=6.45 p<0.00001



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 04 Summer vs. Spring

Study	Summer n	Summer mean(sd)	Spring n	Spring mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Kuoppala	32	44.30(20.80)	36	26.00(13.00)		100.0	18.30[9.94,26.66]
Total(95%CI)	32		36			100.0	18.30[9.94,26.66]

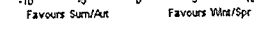
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=4.29 p=0.00002



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 16 Summer/Autumn vs. Winter/Spring

Study	Winter/Spring n	Winter/Spring mean(sd)	Summer/Autumn n	Summer/Autumn mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Kokot 1980	26	24.80(13.30)	19	44.90(24.40)	←	100.0	-20.10[-32.20,-8.00]
Total(95%CI)	26		19		◀	100.0	-20.10[-32.20,-8.00]

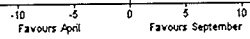
Test for heterogeneity chi-square=0.00 df=0 p=1
Test for overall effect z=3.25 p=0.001



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 03 April vs. September

Study	September n	September mean(sd)	April n	April mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Verity	9	88.11(28.30)	11	59.81(10.95)		10.4	28.30[8.71,47.89]
Verity *	45	67.02(18.69)	41	44.59(12.55)		89.6	22.43[15.75,29.11]
Total(95%CI)	54		52			100.0	23.04[16.72,29.36]

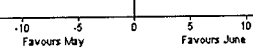
Test for heterogeneity chi-square=0.31 df=1 p=0.58
Test for overall effect z=7.15 p<0.00001



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 05 May vs. June

Study	June n	June mean(sd)	May n	May mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991	6	33.91(16.55)	12	20.29(8.81)		43.6	13.62[-0.53,27.77]
Zeghoud 1991 *	6	34.71(22.16)	5	27.23(14.42)		19.6	7.48[-14.30,29.26]
Zeghoud 1991 **	9	31.51(16.82)	5	33.64(12.55)		36.8	-2.13[-17.68,13.42]
Total(95%CI)	21		22			100.0	6.62[-3.24,16.49]

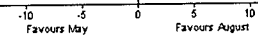
Test for heterogeneity chi-square=2.16 df=2 p=0.34
Test for overall effect z=1.32 p=0.19



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 06 May vs. August

Study	August n	August mean(sd)	May n	May mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991 *	7	48.33(24.56)	5	27.23(14.42)		100.0	21.10[-1.05,43.25]
Total(95%CI)	7		5			100.0	21.10[-1.05,43.25]

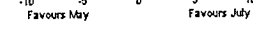
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.87 p=0.06



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 07 May vs. July

Study	July n	July mean(sd)	May n	May mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991 **	10	44.06(23.50)	5	33.64(12.55)		100.0	10.42[-7.83,28.67]
Total(95%CI)	10		5			100.0	10.42[-7.83,28.67]

Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.12 p=0.3



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 08 June vs. August

Study	August n	August mean(sd)	June n	June mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991 *	7	48.33(24.56)	6	34.71(22.16)		100.0	13.62[-11.79,39.03]
Total(95%CI)	7		6			100.0	13.62[-11.79,39.03]

Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.05 p=0.3

Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 09 June vs. July

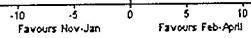
Study	July n	July mean(sd)	June n	June mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991 **	10	44.06(23.50)	9	31.51(16.82)		100.0	12.55[-5.70,30.80]
Total(95%CI)	10		9			100.0	12.55[-5.70,30.80]

Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.35 p=0.18

Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 10 November-January vs. February-April

Study	February-April n	February-April mean(sd)	November-January n	November-January mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	36	18.80(18.70)	43	19.80(23.00)		100.0	-1.00[-10.20,8.20]
Total(95%CI)	36		43			100.0	-1.00[-10.20,8.20]

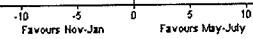
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=0.21 p=0.8



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 11 November-January vs. May-July

Study	May-July n	May-July mean(sd)	November-January n	November-January mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	23	18.90(14.70)	43	19.80(23.00)		100.0	-0.90[-10.03,8.23]
Total(95%CI)	23		43			100.0	-0.90[-10.03,8.23]

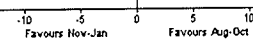
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=0.19 p=0.8



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 12 November-January vs. August-October

Study	August-October n	August-October mean(sd)	November-January n	November-January mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	13	28.00(20.00)	43	19.80(23.00)		100.0	8.20[-4.66,21.06]
Total(95%CI)	13		43			100.0	8.20[-4.66,21.06]

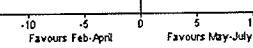
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.25 p=0.2



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 13 February-April vs. May-July

Study	May-July n	May-July mean(sd)	February-April n	February-April mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	23	18.90(14.70)	36	18.80(18.70)		100.0	0.10[-8.47,8.67]
Total(95%CI)	23		36			100.0	0.10[-8.47,8.67]

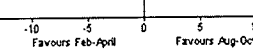
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=0.02 p=1



Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 14 February-April vs. August-October

Study	August-October n	August-October mean(sd)	February-April n	February-April mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	13	28.00(20.00)	36	18.80(18.70)		100.0	9.20[-3.27,21.67]
Total(95%CI)	13		36			100.0	9.20[-3.27,21.67]

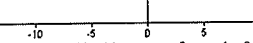
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.45 p=0.15

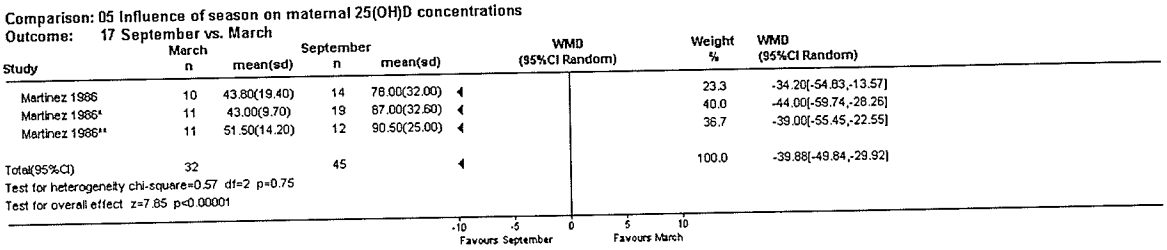


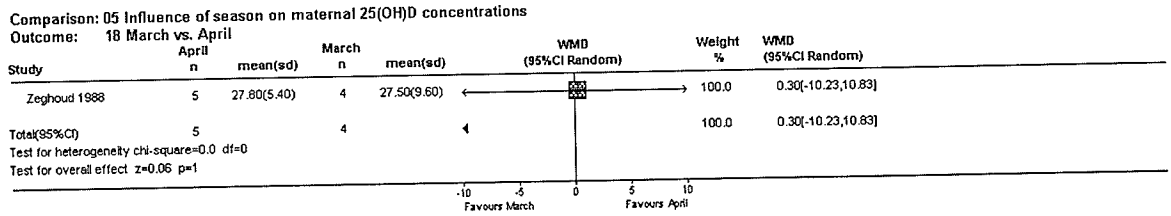
Comparison: 05 Influence of season on maternal 25(OH)D concentrations
Outcome: 15 May-July vs. August-October

Study	August-October n	August-October mean(sd)	May-July n	May-July mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Brooke 1981	13	28.00(20.00)	23	18.90(14.70)		100.0	9.10[-3.32,21.52]
Total(95%CI)	13		23			100.0	9.10[-3.32,21.52]

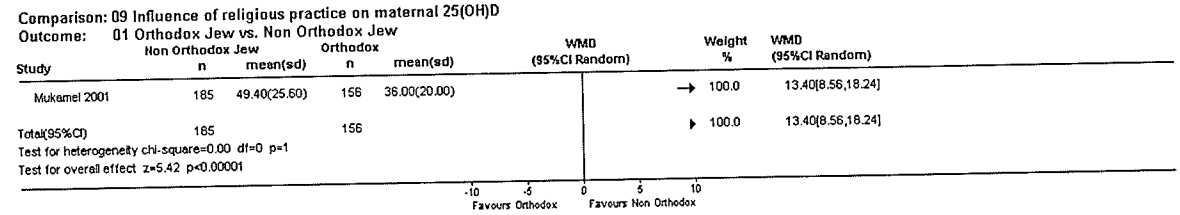
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.44 p=0.15

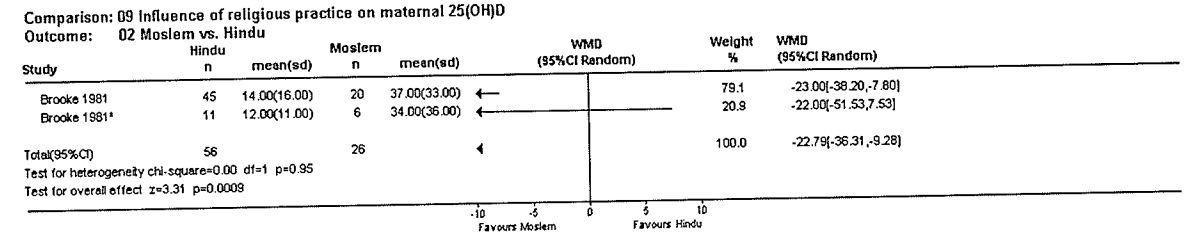




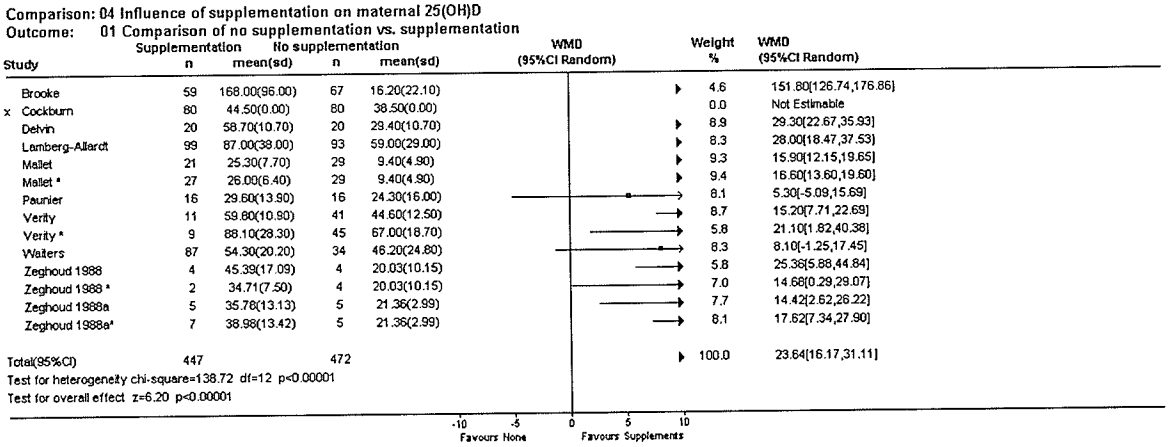


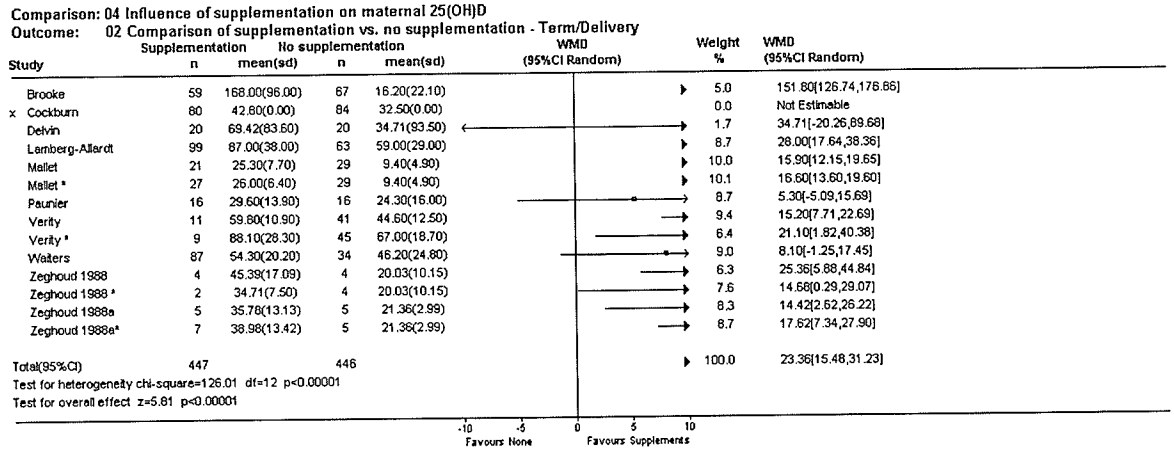
3.3.6 Religious Practice

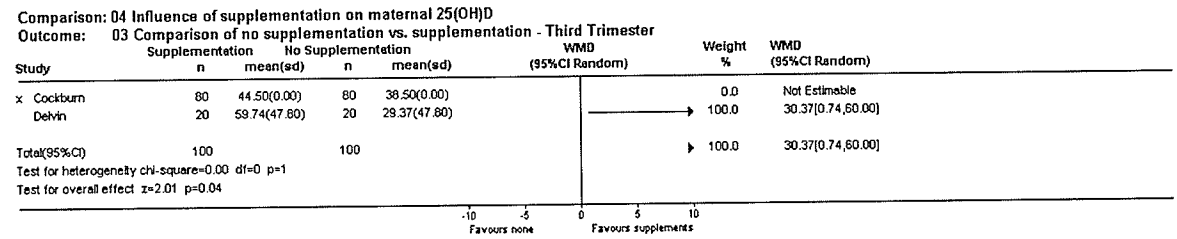




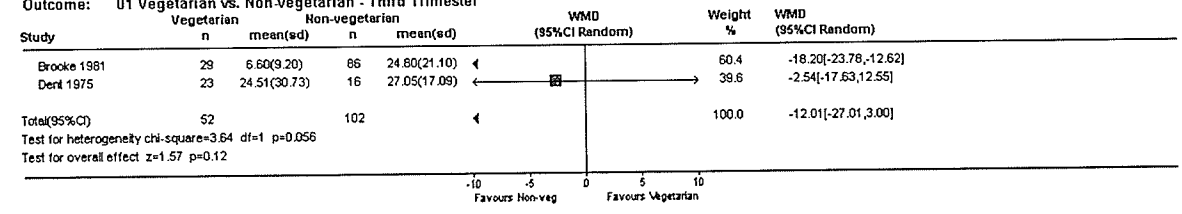
3.3.7 Supplementation







3.3.8 Diet

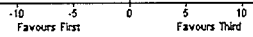


3.3.9 Stage of Pregnancy

Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 01 First Trimester vs. Third trimester

Study	Third Trimester n	Third Trimester mean(sd)	First Trimester n	First Trimester mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Ardawi 1997	40	33.00(8.00)	40	54.00(10.00)		21.5	-21.00[-24.97,-17.03]
Martinez 1986	12	90.51(24.97)	14	77.96(31.97)		18.5	12.55[-9.36,34.46]
Martinez 1986*	11	51.53(14.17)	10	43.79(19.42)		20.1	7.74[-6.92,22.40]
Reddy 1983	22	42.72(20.04)	20	42.72(20.30)		20.5	0.00[-12.22,12.22]
Sanchez 1997	10	79.57(28.04)	10	25.87(8.62)		19.4	53.70[35.52,71.88]
Total(95%CI)	95		94			100.0	9.75[-14.93,34.44]

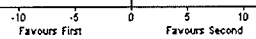
Test for heterogeneity chi-square=64.34 df=4 p<0.00001
Test for overall effect z=0.77 p=0.4



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 02 First Trimester vs. Second Trimester

Study	Second Trimester n	Second Trimester mean(sd)	First Trimester n	First Trimester mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Martinez 1986	19	87.04(32.59)	14	77.96(31.97)		12.0	9.08[-13.17,31.33]
Martinez 1986*	11	42.99(9.74)	10	43.79(19.42)		25.5	-0.80[-14.14,12.54]
Reddy 1983	21	48.06(2.02)	20	42.72(20.30)		38.9	5.34[-3.60,14.28]
Sanchez 1997	10	45.92(21.15)	10	25.87(8.62)		23.6	20.05[5.89,34.21]
Total(95%CI)	61		54			100.0	7.70[-0.80,16.19]

Test for heterogeneity chi-square=4.74 df=3 p=0.19
Test for overall effect z=1.78 p=0.08



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 03 First Trimester vs. Term

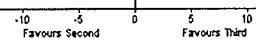
Study	Term n	Term mean(sd)	First Trimester n	First Trimester mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Ardawi 1997	40	35.00(11.00)	40	54.00(10.00)		58.8	-19.00[-23.61,-14.39]
Reddy 1983	10	43.79(23.64)	20	42.72(20.30)		41.2	1.07[-16.07,18.21]
Total(95%CI)	50		60			100.0	-10.73[-30.09,8.63]

Test for heterogeneity chi-square=4.91 df=1 p=0.027
Test for overall effect z=1.09 p=0.3

Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 04 Second Trimester vs. Third Trimester

Study	Third Trimester n	Third Trimester mean(sd)	Second Trimester n	Second Trimester mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Martinez 1986	12	90.51(24.97)	19	87.04(32.59)		13.7	3.47[-16.89,23.83]
Martinez 1986*	11	51.53(14.17)	11	42.99(9.74)		23.9	8.54[-1.62,18.70]
Reddy 1983	22	42.72(20.04)	21	48.06(22.02)		21.1	-5.34[-17.94,7.26]
Reddy 1983*	19	37.38(10.47)	10	40.05(19.42)		20.7	-2.67[-15.59,10.25]
Sanchez 1997	10	79.57(28.04)	10	45.92(21.15)		12.7	33.65[11.88,55.42]
Seely 1997	23	112.14(64.02)	23	120.15(38.42)		8.0	-8.01[-38.52,22.50]
Total(95%CI)	97		94			100.0	4.45[-5.54,14.44]

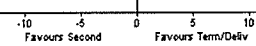
Test for heterogeneity chi-square=11.62 df=5 p=0.04
Test for overall effect z=0.87 p=0.4



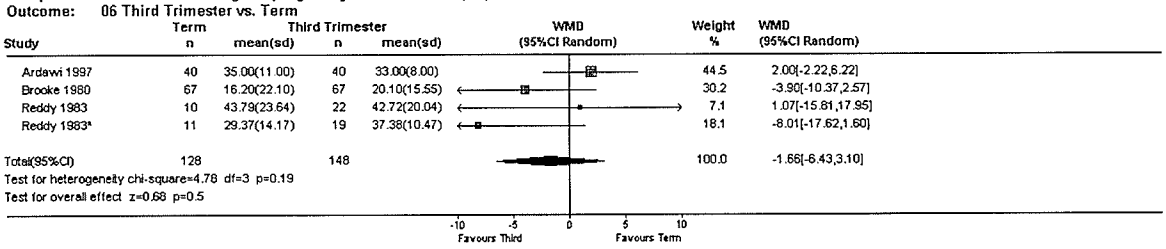
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 05 Second Trimester vs. Term/Delivery

Study	Term/Delivery n	Term/Delivery mean(sd)	Second Trimester n	Second Trimester mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Reddy 1983	10	43.79(23.64)	21	48.06(22.02)		7.0	-4.27[-21.69,13.15]
Reddy 1983*	11	29.37(14.17)	10	40.05(19.42)		9.8	-10.68[-25.34,3.98]
Zeghoud 1988	4	20.00(10.10)	4	27.50(9.60)		11.3	-7.50[-21.16,6.16]
Zeghoud 1988 *	5	21.40(3.00)	5	27.80(5.40)		71.9	-6.40[-11.81,-0.99]
Total(95%CI)	30		40			100.0	-6.80[-11.39,-2.20]

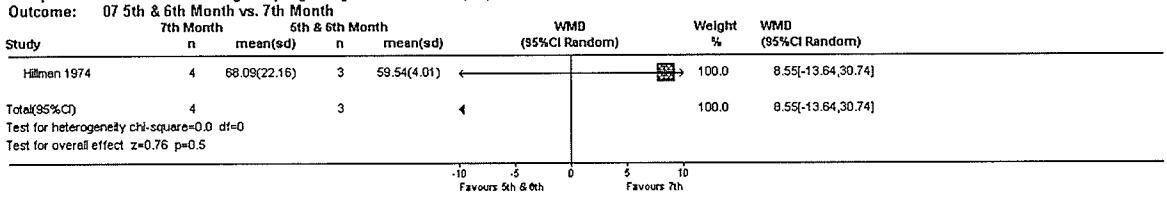
Test for heterogeneity chi-square=0.38 df=3 p=0.94
Test for overall effect z=2.90 p=0.004



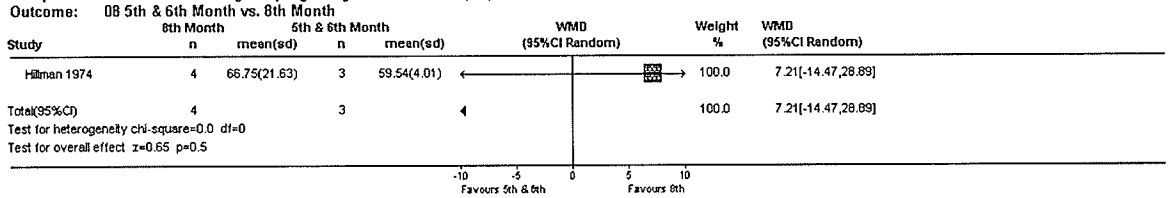
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 06 Third Trimester vs. Term



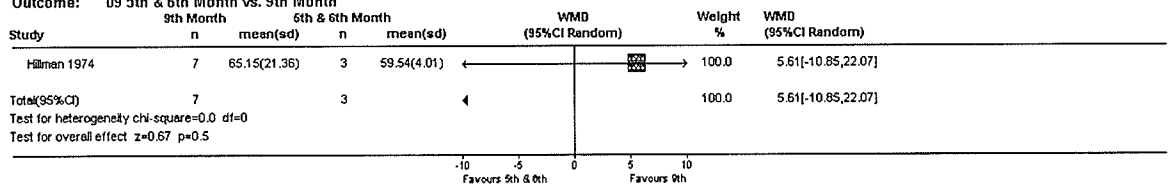
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 07 5th & 6th Month vs. 7th Month



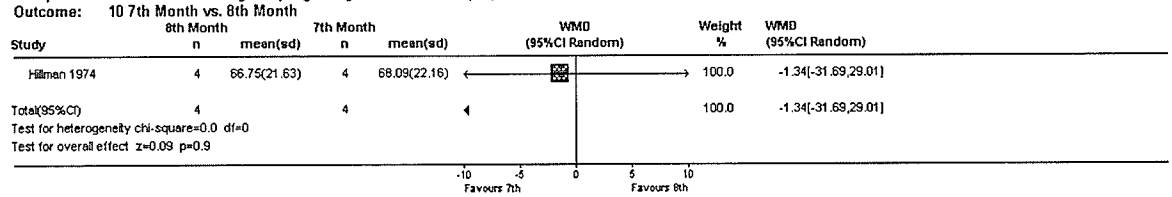
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 08 5th & 6th Month vs. 8th Month



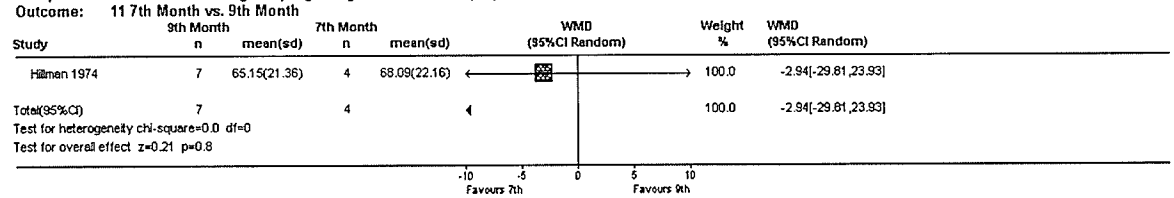
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 09 5th & 6th Month vs. 9th Month



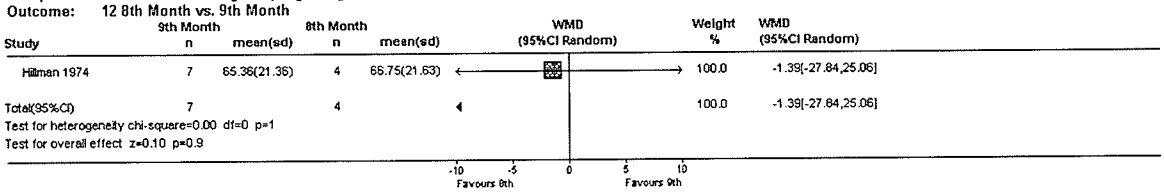
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 10 7th Month vs. 8th Month



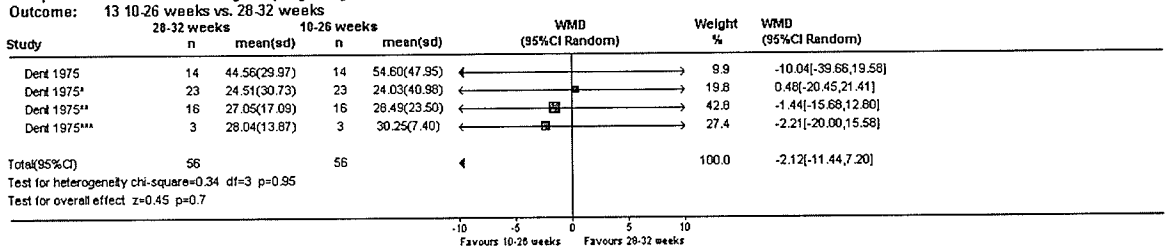
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 11 7th Month vs. 9th Month



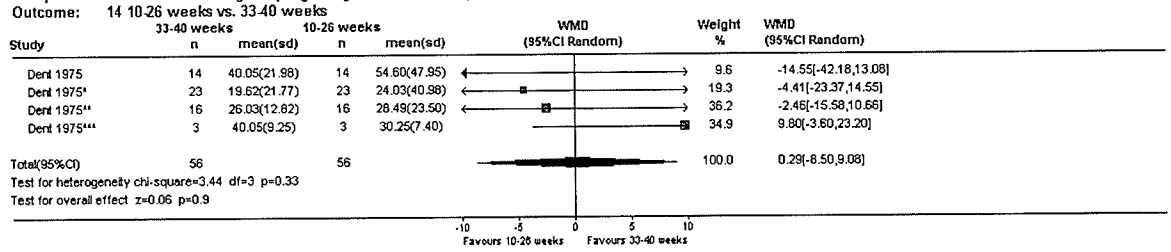
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 12 8th Month vs. 9th Month



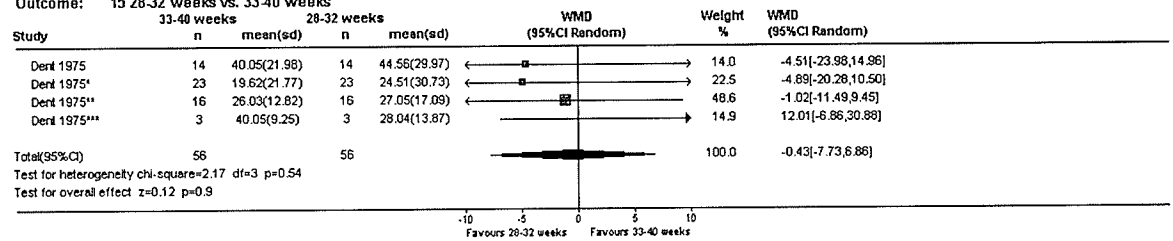
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 13 10-26 weeks vs. 28-32 weeks



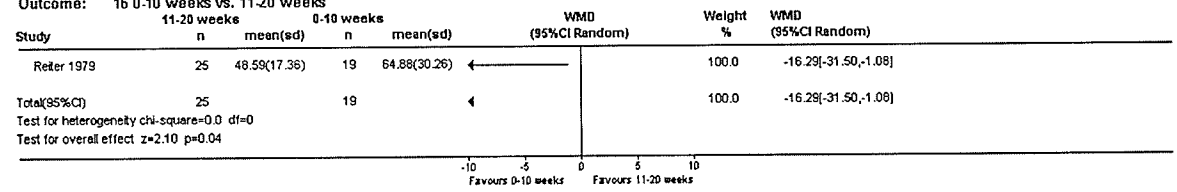
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 14 10-26 weeks vs. 33-40 weeks



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 15 28-32 weeks vs. 33-40 weeks



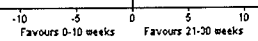
Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 16 0-10 weeks vs. 11-20 weeks



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 17 0-10 weeks vs. 21-30 weeks

Study	21-30 weeks n	21-30 weeks mean(sd)	0-10 weeks n	0-10 weeks mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Reiter 1979	25	52.87(22.70)	19	64.88(30.26)		100.0	-12.01[-28.27,4.25]
Total(95%CI)	25		19			100.0	-12.01[-28.27,4.25]

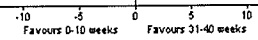
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.45 p=0.15



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 18 0-10 weeks vs. 31-40 weeks

Study	31-40 weeks n	31-40 weeks mean(sd)	0-10 weeks n	0-10 weeks mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Reiter 1979	25	43.52(17.36)	19	64.88(30.26)		100.0	-21.36[-36.57,-6.15]
Total(95%CI)	25		19			100.0	-21.36[-36.57,-6.15]

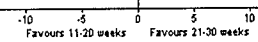
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=2.75 p=0.006



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 19 11-20 weeks vs 21-30 weeks

Study	21-30 weeks n	21-30 weeks mean(sd)	11-20 weeks n	11-20 weeks mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Reiter 1979	25	52.87(22.70)	25	48.59(17.36)		100.0	4.28[-6.92,15.48]
Total(95%CI)	25		25			100.0	4.28[-6.92,15.48]

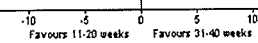
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=0.75 p=0.5



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 20 11-20 weeks vs. 31-40 weeks

Study	31-40 weeks n	31-40 weeks mean(sd)	11-20 weeks n	11-20 weeks mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Reiter 1979	25	43.52(17.36)	25	48.59(17.36)		100.0	-5.07[-14.69,4.55]
Total(95%CI)	25		25			100.0	-5.07[-14.69,4.55]

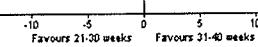
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.03 p=0.3



Comparison: 07 Influence of stage of pregnancy on maternal 25(OH)D
Outcome: 21 21-30 weeks vs. 31-40 weeks

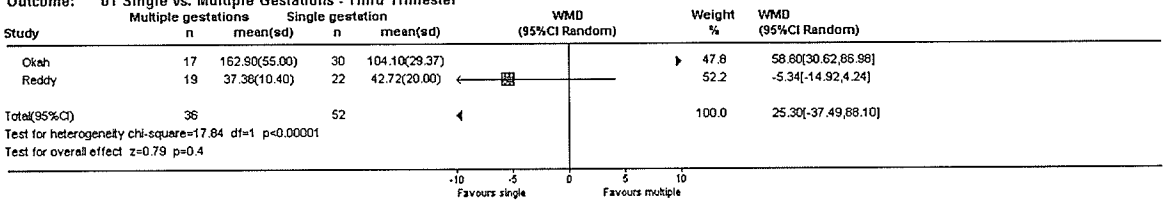
Study	31-40 weeks n	31-40 weeks mean(sd)	21-30 weeks n	21-30 weeks mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Reiter 1979	25	43.52(17.36)	25	52.87(22.70)		100.0	-9.35[-20.55,1.85]
Total(95%CI)	25		25			100.0	-9.35[-20.55,1.85]

Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=1.64 p=0.10

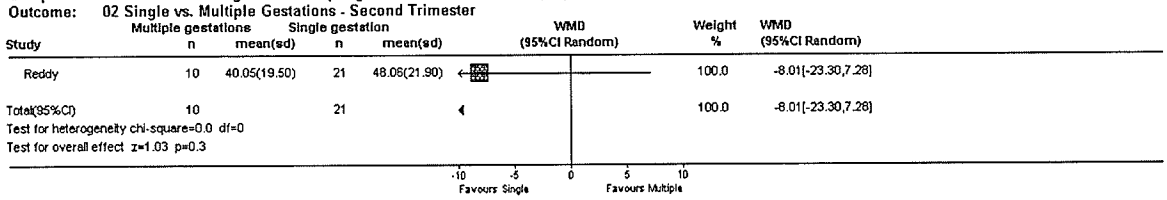


3.3.11 Single vs. Multiple Fetuses

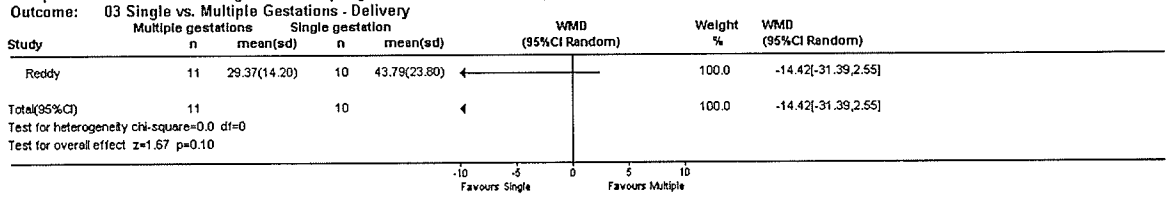
Comparison: 02 Influence of single and multiple gestations on maternal 25(OH)D
Outcome: 01 Single vs. Multiple Gestations - Third Trimester



Comparison: 02 Influence of single and multiple gestations on maternal 25(OH)D
Outcome: 02 Single vs. Multiple Gestations - Second Trimester

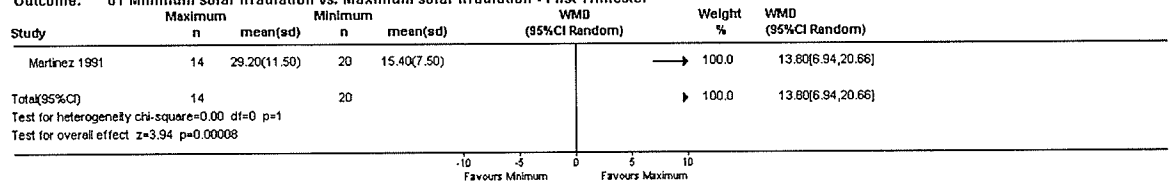


Comparison: 02 Influence of single and multiple gestations on maternal 25(OH)D
Outcome: 03 Single vs. Multiple Gestations - Delivery

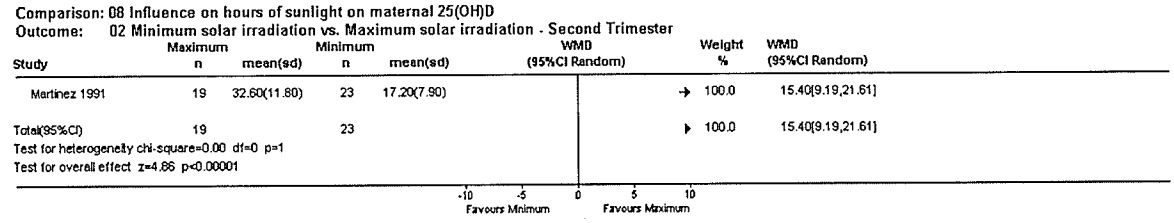


3.3.13 Sun Exposure/Hours Exposed

Comparison: 08 Influence on hours of sunlight on maternal 25(OH)D
Outcome: 01 Minimum solar irradiation vs. Maximum solar irradiation - First Trimester



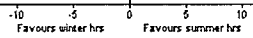
Comparison: 08 Influence on hours of sunlight on maternal 25(OH)D
Outcome: 02 Minimum solar irradiation vs. Maximum solar irradiation - Second Trimester



Comparison: 08 Influence on hours of sunlight on maternal 25(OH)D
Outcome: 03 Hours outside in Winter vs. Hours outside in Summer

Study	19±14 hours-summer n	mean(sd)	10±12.1 hours-winter n	mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Hillman 1976	61	42.10(13.90)	56	15.40(5.90)		100.0	26.70[22.88,30.52]
Total(95%CI)	61		56			100.0	26.70[22.88,30.52]

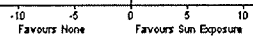
Test for heterogeneity chi-square=0.0 df=0
Test for overall effect z=13.72 p<0.00001



Comparison: 08 Influence on hours of sunlight on maternal 25(OH)D
Outcome: 04 No Sun Exposure vs. Sun Exposure during Third Trimester

Study	Sun Exposure n	mean(sd)	No Sun Exposure n	mean(sd)	WMD (95%CI Random)	Weight %	WMD (95%CI Random)
Zeghoud 1991	19	50.50(20.60)	41	26.20(13.10)		100.0	24.30[14.21,34.39]
Total(95%CI)	19		41			100.0	24.30[14.21,34.39]

Test for heterogeneity chi-square=0.00 df=0 p=1
Test for overall effect z=4.72 p<0.00001



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Chapter 4 – Vitamin D Deficiency in the Literature

4.1 Introduction

Three possible situations can explain vitamin D deficiencies in humans; a) inadequate skin irradiation, b) limited dietary intake of vitamin D, and c) vitamin D pathway impairment [1]. The extent of such vitamin D deficiencies are unknown but have been documented in several populations, including Asian immigrants to northern European climates, dark skinned individuals, and medical inpatients [2-7]. Further, low concentrations of vitamin D are also often found in northern populations during winter seasons, as this period of insufficient sunshine cannot stimulate endogenous synthesis of 25-hydroxyvitamin D (25(OH)D) [8,9]. Recent research further confirms that vitamin D deficiency also occurs among women during pregnancy [10-12].

25(OH)D is the accepted metabolite to measure in serum in order to assess an individual's vitamin D status [13-15] as it reflects the body's vitamin D reserves [16]. The difficulty in describing vitamin D deficiency though is often challenging as differing definitions of what constitutes deficiency or low levels exist [14,16-18]. For instance, a survey of laboratories in various countries revealed that the lower limits of normal for 25(OH)D ranged from 8 to 45 nmol/L depending on the country and institution [17]. Therefore, authors need to clearly define their respective lower limit of normal when identifying participants as vitamin D deficient.

25(OH)D concentrations below 25 nmol/L are considered to be deficient by some, while others have indicated that values equal or less than 35 nmol/L are below normal (Department of Clinical Chemistry, Health Sciences Centre, Winnipeg, MB). Concentrations of 25(OH)D below 40 nmol/L may be indicative of insufficient vitamin D stores [18], still others go further to state that concentrations below this threshold are

synonymous with deficiency, and those possessing concentrations below 25 nmol/L could be considered to have rickets or osteomalacia [16]. The term hypovitaminosis D, low vitamin D, is also used to define those with concentrations of 12 ng/ml (30 nmol/L) or less [19]. However, this term too is debatable as others assign this to concentrations between 20 and 37 nmol/L, with those above 37 nmol/L having adequate concentrations [7]. Yet, others go further and even propose that higher serum concentrations of 25(OH)D are desirable, beyond 80 nmol/L [18], as values below this threshold more accurately reflect hypovitaminosis D [20]. 25(OH)D adequacy may in fact be represented by values exceeding 100 nmol/L [20].

Relying on reported nutritional intakes to infer 25(OH)D adequacy is often problematic and does not give insight into actual circulating levels [7,8,21]. Therefore, assessing dietary intakes may not be the best predictor of 25(OH)D status. Rather, the best method to assess vitamin D stores is still to measure serum 25(OH)D concentrations [8]. In instances where vitamin D cannot be easily measured, serum concentrations of alkaline phosphatase may give some insight into 25(OH)D status. For instance, elevations in serum alkaline phosphatase might be an indicator of low serum 25(OH)D [22,23]. Still others call for simultaneous parathyroid hormone testing to confirm a physiological deficiency of vitamin D as parathyroid hormone concentrations are inversely related to 25(OH)D levels [7].

Persons with deficient levels of vitamin D may be at risk as vitamin D is involved in the body's use of calcium and bone integrity. Low levels of vitamin D may lead to rickets, hypocalcemia, delayed bone ossification, and abnormal enamel development in infants and children, while adults might be predisposed to increased bone turnover,

osteoporosis, osteomalacia, and bone fractures [6,13,24]. Active metabolites of vitamin D help to increase intestinal absorption of calcium and phosphate ensuring bone mineralization. Periods of low vitamin D translate into bone demineralization as decreased amounts of phosphate and calcium are absorbed from the intestines [23]. Concern is also great for expectant mothers who have deficient concentrations of 25(OH)D as mothers serve as the prime source of 25(OH)D for their developing fetus [25,26]. As maternal 25(OH)D levels have already been shown to be correlated with fetal and infant concentrations [25,27-31], with the infant having a lower concentration than its mother, it is obvious that a deficient mother would leave the developing fetus with inadequate vitamin D for proper development [6,32]. This risk would likely continue for infants of deficient women breastfeeding without simultaneously administering vitamin D drops to their infants [25].

Specific references to vitamin D deficiency and its sequelae have been cited among certain populations in Manitoba, specifically those of the Island Lakes region of the province [10,33-35]. Some of these studies involved vitamin D assays while others involved dietary assessments. These studies identified clinical vitamin D deficiency or dietary inadequacies of vitamin D among the populations under investigation in Manitoba [10,22,33]. Knowing that deficiencies have been documented in northern Manitoba, it is important to establish whether these same deficiencies exist in southern regions of the province. A review of the literature would also benefit health professionals in knowing whether deficiencies do exist in urban women of childbearing age, even those from developed nations and those regions known to have ample sunlight.

4.2 Methods

The existing literature was searched to identify those studies reporting maternal serum 25(OH)D concentrations in the deficient range. The source of this literature was limited to those included studies reviewed to identify those studies reporting maternal concentrations of 25(OH)D in Chapter 3, either during pregnancy, at term, delivery, or shortly thereafter. Included published articles were carefully scrutinized to identify those with maternal values considered to be in the deficient range, with the definition selected for the purposes of this review being concentrations < 35 nmol/L. This is the lower limit of normal for 25(OH)D testing performed at Winnipeg's Health Sciences, Clinical Chemistry Department. Those studies reporting serum concentrations in units other than nmol/L were converted to these units for the purposes of this exercise (1nmol/L = 2.67 ng/ml).

4.3 Results

A total of 76 included studies were identified using the search strategy mentioned in Chapter 3. Of these, thirty-five were found to report mean or median maternal serum 25(OH)D concentrations < 35 nmol/L. These studies reporting maternal values in the range of deficiency were ordered in descending chronological order of publication (Table 4.1).

As shown in Table 4.1, low 25(OH)D concentrations were not restricted to only certain regions of the world. In fact low vitamin D concentrations were reported in different ethnic groups in many regions of the world, even those residing in regions known to receive ample sunshine. While 3 studies were actually from the same original

investigation, each was reported separately [27,36,37] and each presented unique yet important data of deficiencies among the study groups.

Table 4.1 – Included Studies Reporting 25(OH)D in the Deficient Range (<35nmol/L)

Included Study	**Population and Details**	**25(OH)D (Mean ± SD, or Median) (nmol/L)**
Goswami R et al (2000)	Dehli, India, Pregnancy Summer	21.9 ± 10.73 nmol/L
Koenig J & Elmadfa (2000)	Austria, Pregnancy	17.0 ± 33.1 nmol/L
Smith PJ (2000)	Canadian First Nations, Pregnancy Garden Hill, Manitoba St. Theresa Point, Manitoba Norway House, Manitoba	 18 nmol/L median 21 nmol/L median 24 nmol/L median
Feleke Y et al (1999)	Addis Ababa, Ethiopian Pregnancy August-September Group	 25 nmol/L median
Brunvand L et al (1998)	Karachi, Pakistan Primiparous women, Delivery	19 nmol/L median
Namgung R et al (1998)	South Korean, Pregnancy Winter Season	25.6 ± 14.2 nmol/L
Sanchez PA et al (1997)	Maiduguri, Nigeria First Trimester	25.87 ± 8.62 nmol/L
Brunvand L et al (1996)	Oslo, Norway Pakistani Immigrants – Delivery	14 nmol/L median
Bruinse HW & van den Berg (1995)	Netherlands – Late Winter End of Pregnancy	32 ± 9 nmol/L
Henrikson C et al (1995)	Oslo, Norway Pakistani Immigrants – Second Trimester	19 nmol/L median
Brunvand L & Haug (1993)	Oslo, Norway Pakistani Immigrants Delivery	15.1 nmol/L median

Zeghoud F et al (1991)	France –Delivery	
	Lyon:	
	May	20.3 ± 8.8 nmol/L
	June	33.9 ± 16.6 nmol/L
	Chambery:	
	May	27.2 ± 14.4 nmol/L
	June	34.7 ± 22.2 nmol/L
	Nice:	
	May	33.6 ± 12.5 nmol/L
	June	31.5 ± 16.8 nmol/L
Zeghoud F et al (1988)	France	
	Placebo Group	
	March: 6 months	27.5 ± 9.6 nmol/L
	Delivery	20.0 ± 10.1 nmol/L
	April: 6 months	27.8 ± 5.4 nmol/L
	Delivery	21.4 ± 3.0 nmol/L
	100000 IU at 6 month	
	April: 6 month (pre)	31.5 ± 17.3 nmol/L
	100000 IU at 7 months	
	March: 6 months	21.4 ± 11.2 nmol/L
	Term	34.7 ± 7.5 nmol/L
	April: 6 months	26.7 ± 11.3 nmol/L
Okonofua F et al (1987)	London, Asians	
	First Trimester	6.2 nmol/L median
	Second Trimester	12.5 nmol/L median
	Third Trimester	7.8 nmol/L median
	Term	13.1 nmol/L median
	Caucasian	
	First Trimester	21.7 nmol/L median
	Third Trimester	26.5 nmol/L median
	Term	23.5 nmol/L median
Delvin EE et al (1986)	Lyon, France	
	Control Group	
	Third Trimester	29.4 ± 47.8 nmol/L
	Delivery	34.7 ± 95.5 nmol/L
Kuoppala T et al (1986)	Tampere, Finland	
	Spring – Delivery	26.0 ± 13.0 nmol/L

Mallet E et al (1986)	France, Winter – Term	
	Control Group	9.4 ± 4.9 nmol/L
	1000 IU daily 3rd Trimester	25.3 ± 7.7 nmol/L
	200000 IU 7th Month	26.0 ± 6.4 nmol/L
Kuoppala T et al (1984)	Tampere, Finland	
	Delivery	13.8 ± 9.8 nmol/L
Markestad T et al (1984)	Benghazi, Libya	
	Delivery	34 nmol/L median
Serenius F et al (1984)	Riyadh, Saudi Arabia	
	Measured at Term	
	Supplemented during pregnancy	19.81 nmol/L median
	Unsupplemented	14.02 nmol/L median
	Supplemented during Third pregnancy	19.86 nmol/L median
	Unsupplemented	15.49 nmol/L median
Taha SA et al (1984)	Riyadh, Saudi Arabia	
	Winter, Delivery	35.5 ± 38.2 nmol/L
Ong SP et al (1983)	Leeds, England	
	Pregnancy	
	November – January	
	All	11.53 ± 7.05 nmol/L
	Pakistani	10.15 ± 6.01 nmol/L
	Indian	10.79 ± 8.22 nmol/L
	Bangladeshi	13.94 ± 6.59 nmol/L
Reddy GS et al (1983)	Montreal, Canada	
	Twin Pregnancy, Third Trimester	29.4 ± 18.6 nmol/L
Gupta MM et al (1982)	Pune, India	
	March-October – Delivery	31.32 ± 17.68 nmol/L

Brooke OG et al (1981)	London, Third Trimester	
	Indian	18 ± 21 nmol/L
	Pakistani	10 ± 12 nmol/L
	East African	21 ± 26 nmol/L
	Bangladeshi	32 ± 15 nmol/L
	Nov-January	19.8 ± 23.0 nmol/L
	February – April	18.8 ± 18.7 nmol/L
	May – July	18.9 ± 14.7 nmol/L
	August – October	28.0 ± 20.0 nmol/L
Maxwell JD et al (1981)	London, Asians	
	Third Trimester	20.0 nmol/L
Whitehead JD et al (1981)	London, May – July	
	30-32 weeks	21.4 nmol/L
	36-40 weeks (term)	20.4 nmol/L
Brooke OG et al (1980)	London	
	Placebo Group – Term	16.2 ±22.1 nmol/L
Brown IRF et al (1980)	London	
	All pre-28 weeks	20.1 ± 20.2 nmol/L
	Placebo Group – Term	16.0 ± 19.7 nmol/L
Cockburn F et al (1980)	Edinborough	
	Placebo Group – 24weeks	32.5 nmol/L
	Placebo Group – Delivery	32.5 nmol/L
Kokot F et al (1980)	Poland – Winter-Spring	
	Third Trimester	24.83 ± 13.3 nmol/L
Biale Y et al (1979)	Beersheva, Israel	
	Bedouin during Labour	20.72 ± 19.97 nmol/L
Heckmatt JZ et al (1979)	Leeds	
	Asians, September – October, Delivery	14.2 ± 20.3 nmol/L
Paunier L et al (1978)	Switzerland, Delivery	
	January – February	
	Control Group	24.3 ± 16.0 nmol/L
	500 IU vitamin D in multivitamin	29.6 ± 13.9 nmol/L

Dent CE & Gupta (1975)	London, July-August	
	Asians at Delivery	20.3 ± 13.3 nmol/L
	Vegetarian Asian	
	10-26 weeks	24.0 ± 41.0 nmol/L
	28-32 weeks	24.5 ± 30.7 nmol/L
	33-40 weeks	19.6 ± 21.8 nmol/L
	Non-vegetarian Asian	
	10-26 weeks	28.5 ± 23.5 nmol/L
	28-32 weeks	27.0 ± 17.1 nmol/L
	33-40 weeks	26.0 ± 12.8 nmol/L
	West Indian	
	10-26 weeks	30.3 ± 7.4 nmol/L
	28-32 weeks	28.0 ± 13.9 nmol/L

Low maternal serum concentrations of 25(OH)D were reported for various regions of the world including North America, Europe, the United Kingdom, Africa, the Middle East, and Asia.

4.4 Discussion

Vitamin D deficiency among expectant women is not limited to only certain ethnic groups or regions [6] but in fact has been reported across the globe. While many studies reporting levels below 35 nmol/L involved Asian immigrants and those of Asian extraction residing in northern Europe and the United Kingdom [2-4,27,49,57], there is also mounting evidence that low levels of 25(OH)D are exhibited among pregnant women residing in regions with ample sunshine. For instance, expectant mothers were found to be have serum 25(OH)D concentrations at or below 25 nmol/L during summer in Delhi [11], at delivery in Pakistan [39], and in Addis Ababa, Ethiopia [38]. Even

mothers in the south of France were found to have low levels when measured during May and June [42].

What is more alarming is that low levels of 25(OH)D have also been identified among expectant mothers in Canada, a developed nation. However, it is perhaps less surprising when one reflects upon deficiencies identified among northern Aboriginals in Manitoba. The low socioeconomic status (SES) of this region and the abandonment and lack of a traditional diet, along with the northern latitude preclude proper attainment of vitamin D [60].

While the majority of studies reporting mean or median maternal values below 35nmol/L are from Europe and the United Kingdom, this is likely due to the fact that the majority of studies measuring maternal serum concentrations have been conducted on the European continent and were therefore more likely to identify existing deficiencies. It is likely that more women are deficient during pregnancy but that limited research does not identify these populations. Vitamin D metabolite assays are not routine medical tests and the cost of performing serum testing for 25(OH)D is indeed costly and is therefore seldom performed [61]. This is probably one of the main reasons limited research has been conducted to determine the prevalence and extent of vitamin D deficiencies around the world.

While this review demonstrates many populations with low concentrations of 25(OH)D, it also indicates that supplementation alone is not sufficient to produce maternal serum concentrations above 35 nmol/L in all circumstances [28,43]. Mothers receiving 100 000 IU of vitamin D at 7 months gestation had a mean concentration just shy of 35 nmol/L when measured at term [43], while mothers receiving differing

supplementation strategies were found to have term levels below 25 nmol/L [28]. Further evidence indicates that regular intake of multivitamins and dietary intake of fortified foods may not be sufficient to produce dramatic increases to 25(OH)D levels above the 40 nmol/L threshold [7].

Comparisons of maternal 25(OH)D concentrations between different published studies can be difficult as laboratory techniques may vary depending on the institution as radioimmunoassay generally yields higher concentrations of 25(OH)D than high performance liquid chromatography (HPLC) [19]. However, these comparisons are still needed and are still compelling nonetheless.

Those health care providers providing ambulatory care to expectant mothers should be cognizant that mothers and their developing fetuses may be at risk of having insufficient serum concentrations of 25(OH)D. In Canada, this is especially true for northern residents, as their latitude and less than nutritious diet can result in deficiency. However, this may also be true for those residing in southern regions of Canada, especially during winter seasons [8] or those of low SES who are unable to secure foods naturally containing or fortified with vitamin D.

Recommendations have now surfaced for all expectant mothers with dark complexion or limited sun exposure to be routinely assessed for 25(OH)D status during their pregnancies [6]. What is now needed is for future research to identify those optimal concentrations that would provide the maximum effectiveness in maternal, fetal, and infant health [6].

4.5 Conclusions

Findings of this review reveal that many groups of expectant mothers have been shown to possess mean serum 25(OH)D concentrations near or below 35 nmol/L, nearly half of the studies identified in Chapter 3. These findings are of concern as the developing fetus acquires its 25(OH)D across the placenta. Therefore, offspring of mothers with low levels of 25(OH)D will also have low concentrations, which ultimately leaves them vulnerable to potential rickets, hypocalcemia, and possible enamel defects.

Expectant mothers in Canada have even been found to possess concentrations below 35 nmol/L [10,50]. Considering the latitudes at which Canadians live that limit endogenous synthesis, obviously dietary intakes may be insufficient to raise serum 25(OH)D to normal concentrations. Attempts to guarantee sufficient vitamin D concentrations should be endorsed given the sequelae/consequences that can affect both mother and infant. In addition, future epidemiological investigations are welcomed to resolve the discourse and debate surrounding ambiguous threshold concentrations for vitamin D deficiencies and insufficiencies.

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Chapter 5 – Vitamin D, Enamel Hypoplasia and Early Childhood Caries: a Review

5.1 Early Childhood Caries

Dental caries disproportionately affects members from the North American Aboriginal population, especially children. Several Canadian studies have revealed that the prevalence of decay among Aboriginal children and other children living in the north is high when compared with the general population [1-5]. Reasons for this discrepancy are generally attributed to the lower socioeconomic status (SES) of these groups [3] and their limited access to dental care [1,5].

Baby bottle tooth decay has now been replaced by the term early childhood caries (ECC). ECC was adopted in an attempt to raise awareness of the multiple factors involved in this disease's etiology, rather than continuing to attribute causation solely to inappropriate feeding practices. Prevention efforts to curb improper feeding methods alone have had limited success in reducing ECC [6]. Further, associations between ECC and improper feeding practices are not consistent [7]. It is no longer considered to be the principle etiology [7].

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 [8,9]. Others have classified ECC according to 3 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 [10]. Whether these 3 patterns are discrete or represent sequential stages of a single disease process remains obscure.

The traditional etiological triad model for dental caries includes the susceptibility of the host (tooth enamel), diet (fermentable sugars), and cariogenic bacteria over time [11,12]. ECC too, is multifactorial in origin, including these same factors of sugar consumption, enamel integrity, cariogenic microorganisms, and time [13]. However, additional factors have also been associated with ECC, including SES [6,14,15], psychosocial issues, and child rearing practices [10,14]. ECC is frequently found in lower socioeconomic households.

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 [16]. Bottle- use for nutritive purposes must be distinguished from pacification, where inappropriate bottle use increases the risk for caries [17]. Other evidence concludes that "at-will breastfeeding" and enamel hypoplasia may predispose a child to ECC [18]. Preventive efforts to address the issue of bottle-use have not had tremendous impact in decreasing ECC in the pediatric population [6] and so are clearly insufficient. A recent systematic review suggests that there is also insufficient scientific evidence that a strong association between breastfeeding and ECC exists [19].

5.2 Enamel Hypoplasia

To understand the biological mechanisms of ECC, the integrity of the primary tooth enamel is inherently significant. Enamel hypoplasia results from defective amelogenesis and is clinically identified by absences of, pitting, grooves, or other irregularities of enamel [20-22]. 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 [11]. 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 [23-25]. 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 [25].

Primary tooth enamel defects have been correlated with several factors ranging from genetic disorders to difficulties arising during prenatal and early postnatal periods [11]. Such prenatal and perinatal disorders include low birth weight, malnourishment, prematurity, and metabolic difficulties [11]. Deficiencies of vitamin D in utero are also believed to be associated with the presence of enamel hypoplasia, because of metabolic insult to ameloblasts [26-32]. This theory was first proposed by the work of May Mellanby.

The general prevalence of hypoplastic defects in primary teeth ranges from 13-39% and can approximate 62% among premature infants [11]. Enamel hypoplasia has also been found to be more prevalent among children of low SES [11,23,33-36].

5.3 Enamel Hypoplasia and Early Childhood Caries

Controversy with the prenatal nutritional hypothesis primarily relates to the lack of demonstrated associations between nutritional deficiencies in utero and ECC [24], although children with neonatal tetany resulting from maternal deficiencies demonstrate

enamel defects [26], 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 [37,38]. Evidence suggests that the incidence of hypoplasia in the adult upper incisors may be reduced through vitamin supplementation both pre and post-natally, over several years [15,37]. Likewise, children who's mothers received 400 IU of vitamin D during pregnancy had a lower incidence of hypoplastic defects [39]. Therefore, vitamin supplementation may be necessary to eliminate these defects.

If enamel hypoplasia results from nutritional inadequacies and knowing it provides refuge for cariogenic bacteria [40-42] that can increase the risk of caries, then an association between enamel hypoplasia and caries is reasonable [37,43-45]. Hypoplasia is associated with caries and dramatically increases the tooth's susceptibility to caries [15,20,21,44,46-50]. However, there needs to be more clarity regarding research on ECC and enamel hypoplasia [6].

5.4 The Role of Vitamin D in Enamel Hypoplasia and Early Childhood Caries

Although nutritional supplementation can lead to a decrease in the incidence of enamel hypoplasia, these assessments have been primarily conducted in permanent dentitions of adolescents [15,37] with the exception of one prenatal supplementation study that included hypoplasia as an outcome [39]. To date, direct evidence that nutritional inadequacies place a child at increased risk for dental decay [51] 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 [34]. Current literature is skewed to those factors influencing decay on erupted teeth rather than those factors that act before eruption [7].

Vitamin D plays an important role in calcium and phosphorus homeostasis [discussed in Chapter 2, 52,53] controlling intestinal calcium and phosphorus absorption [54,55]. The main source of 25(OH)D is from endogenous synthesis. For many residing in northern regions, exposure to adequate ultraviolet (UV) irradiation is often insufficient, necessitating reliance on exogenous sources [53,56]. Furthermore, food sources containing vitamin D including fish, fortified milk and soy products, eggs and liver [53] 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.

Traditionally, research into ECC has focussed on oral hygiene practices, nursing practices and sugar consumption; however, there is a stronger association between low SES and ECC [6,7]. Poor prenatal care can translate into low birth weight yet the influence this may have on the primary dentition requires investigation [57]. For example, low SES influences food security, which in turn affects the nutritional status of expectant mothers during periods of critical fetal tooth formation [58]. 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 [58]. Episodes of newborn malnutrition, during tooth formation, increase the risk of caries in the primary dentition [51].

Brief episodes of nutritional insufficiency of calcium can increase the likelihood of enamel hypoplasia, although the impact that prenatal vitamin D deficiencies on the

incidence of enamel hypoplasia has yet to be evaluated prospectively [59]. Both vitamin D and vitamin A deficiencies are primary systemic factors associated with enamel hypoplasia [60]. Others have confirmed that enamel hypoplasia is a predisposing factor for ECC [61]. Enamel hypoplasia is an important public health concern as it is remarkably prevalent among poorly nourished children, while afflicted teeth have increased vulnerability to caries attack [28,43,62].

Much of the initial focus on the role of vitamin D in enamel hypoplasia and caries occurred during the 1910s, 1920s, and 1930s. This work 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 [63]. Additional human investigations demonstrated that the use of vitamin D resulted in significant reduction of caries in children who received irradiated ergosterol [64,65] and that these hypoplastic defects were associated with increased caries activity [65]. Historical evidence exists documenting the beneficial effects of vitamin D supplementation in children to reduce dental caries [64-67].

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, and that preventive approaches to positively improve dental mineralization and increase host resistance was through modifications in maternal prenatal diet; since their formation is dependent on nutritional availability of the mother [68,69].

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 [70]. Prenatal and postnatal nutrition must emerge as the focus for new primary prevention efforts against ECC [25] since deficiencies during these stages are believed to place the newborn at risk for an assortment of diseases including caries [71].

Adequate serum concentrations of 25(OH)D during pregnancy are essential for the calcification of body structures. Considering Manitoba's geographic location, many residents are believed to have insufficient 25(OH)D concentrations [72,73]. Thus, dietary intakes are essential in maintaining 25(OH)D adequacy for northern populations and especially expectant mothers [73]. However, many Aboriginal expectant women are at risk for not attaining adequate nutrition as many face obstacles in securing food, especially cost [59]. Lactose intolerance among Aboriginals is also believed to be significant and may influence the consumption of vitamin D fortified dairy products.

It is well known that nutritional deficiencies exist among Aboriginals in Canada [59]. Recent research places the prevalence of vitamin D deficiency during pregnancy in 3 northern communities in Manitoba in excess of 80% [74]. 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 [59]. For instance, 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 [75]. Such prices obviously deter many families from purchasing, and consuming milk.

Such nutritional stressors during prenatal development may affect the integrity of the developing primary tooth enamel thus limiting the ability of the tooth to resist bacterial invasion and caries attack, and may explain the high prevalence of ECC in

northern Manitoba [7,12,57]. Enamel hypoplasia has already been identified as an additional risk factor in ECC development [10,11,33,76]. Episodes of malnutrition or deficiencies during enamel formation can predispose teeth to enamel hypoplasia [7,24,62]. 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 socioeconomic populations [11,23,33,34].

New evidence is now uncovering the role of vitamin D in human immunology indicating that deficiency states may reduce immunologic responses of the host towards microbial infections [77]. Therefore, it is also possible that deficiencies of vitamin D may also reduce host resistance to cariogenic bacteria in addition to predisposing enamel hypoplastic defects, further facilitating the development of ECC.

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 [26]. Current discussions have also raised the issue of whether biological identifiers of ECC in the preclinical state can be evaluated in the infant population under 12 months of age [8]. Perhaps both supplementation and early dental screenings may serve as effective preventive strategies to reduce both enamel hypoplasia and ECC.

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Chapter 6 - The Garden Hill Experience

6.1 Introduction

Although assays of 25(OH)D are the best predictor of vitamin D status, they are prohibitive and not routine in isolated northern communities. Many have considered nutritional intakes as a means to profile an individual's vitamin D status. However, this is often problematic and difficult to achieve [1]. Lactose intolerance is a known phenomenon among some Aboriginals, and given the high costs of dairy products in Canada's north [2], vitamin supplements containing vitamin D may be a better method of improving the vitamin D status of these populations [3] rather than relying solely on intake of fortified dairy products. However, it is also known that multivitamins are often not enough to sustain improved 25(OH)D concentrations in the desired range [4]. Daily intakes of multivitamin preparations also pose another issue, compliance. Thus it is necessary to consider alternatives, including the use of high dose vitamin D preparations, or modified Stosstherapy, as a means of achieving satisfactory levels of 25(OH)D for northern residents.

To combat the known and documented problem of rickets and vitamin D deficiency among northern Aboriginals, preventive approaches like the use of high dose vitamin D, Stosstherapy, had been initiated in several northern Manitoba communities [5]. One such community was Garden Hill First Nation.

6.2 Purpose & Hypothesis

The intent of this investigation was to study the effect of modified Stosstherapy on the primary dentition and to assess the prevalence of childhood decay in Garden Hill First

Nation. Vitamin D supplementation during pregnancy has been shown to be associated with greater maternal serum 25(OH)D concentrations [6,7]. Further, a placebo-controlled trial of maternal vitamin D supplementation of 400 IU/day during pregnancy demonstrated a statistically significant reduction in primary enamel defects ($p<0.01$) in the primary teeth [7].

Children born in Garden Hill First Nation, Manitoba after 1994 were included in a program to reduce the very high risk of rickets in this community. A modified form of Stosstherapy was used where mothers were offered 100,000 IU of oral vitamin D (ergocalciferol) at diagnosis of pregnancy and in the third trimester, and their babies received the same dose at 6 weeks of age. 100,000 IU of ergocalciferol is comparable to 400 IU of vitamin D daily during pregnancy.

The hypothesis was that this rickets prevention strategy would also reduce the amount of enamel hypoplasia in the primary teeth, thus reducing caries activity. Recall that enamel hypoplasia is a risk factor in the development of early childhood caries (ECC) [8,9]. Mothers were interviewed regarding their prenatal health and nutrition, infant feeding practices, and oral hygiene and dental care of their children. While maternal 25(OH)D concentrations were not assessed via serum assays, it was assumed that the high doses of ergocalciferol would help normalize circulating maternal concentrations.

6.3 Methods

Beginning in 1999 a pediatric dental survey was conducted in Garden Hill First Nation, Manitoba, a community, situated on the north shore of Island Lake, 610 km northeast of Winnipeg [10]. Population statistics for 1999 reveal that there were 2241 on reserve residents [11].

A cross sectional and retrospective cohort study design investigated the oral health of young children and investigated the influence of modified Stosstherapy. According to band documentation, there were 179 eligible children one year prior to and one year after the introduction of Stosstherapy, of the appropriate ages, to participate. Participant Information and Consent Forms were utilized in the recruitment of study volunteers (Appendix 6.1). Logistics were arranged with input from the community leadership. The University of Manitoba's Health Research Ethics Board approved this research. Parents or guardians were approached to consent to have their child undergo a thorough dental examination. In addition, chart reviews were performed and mothers were asked to participate in a structured interview conducted by the study coordinator. The dental examiner was blind to the interview and chart review data. The study should have examined children at age 3 but logistical problems delayed this. There also were varying degrees of intake of the ergocalciferol among the study population.

The dental examination was limited to the primary dentition and collected information relating to dental caries, missing or extracted teeth, enamel hypoplasia, existing restorative care, and dental malocclusion traits. The interview questionnaire focused on oral hygiene practices and previous dental care of the child, maternal health and nutrition, and mother and infant exposure to vitamin D. Chart reviews were

performed to confirm whether women received modified Stosstherapy during pregnancy and at what stage of pregnancy and whether young infants also received this same supplementation at 6 weeks of age.

The interview and dental exam results were coded for anonymity and mother and child components were combined. Study data was analyzed utilizing both SAS (Version 7) and NCSS 6.0 statistical software.

6.4 Results

A total of 98 (54.8%) of 179 eligible children participated in this study. The mean age for children was 46.4 ± 6.3 months (≈3.8 years) while the mean age of the mothers was 27.1 ± 5.1 years. 57 (58.2%) of the children were male. Mother's highest level of education ranged from grade 3 to12, with the mean grade being 9.4 ± 1.9.

Chart reviews revealed whether mothers had received vitamin D supplementation during pregnancy and the specific stage of pregnancy when given. Table 6.1 details maternal supplementation. Further, the majority of mothers took prenatal multivitamins during their pregnancy, although the frequency varied (Table 6.1). Several infants received modified Stosstherapy at their clinic visit at 6 weeks of age, while less than 1/3 of children received vitamin D drops from their mothers (Table 6.2). On average, vitamin D drops began at 5.4 ± 4.0 months and ceased at 19.4 ± 11.0 months of age.

Table 6.1 - Maternal Stosstherapy and Prenatal Multivitamin Intake

	n	*Percent*	*95% C.I.*
Mother received Stosstherapy during pregnancy			
Yes	41	41.8	9.8
No	30	30.6	9.1
Unsure	27	27.6	8.9
When mothers received Stosstherapy			
At one of first prenatal appointments	23	60.5	15.5
7 months of pregnancy	14	36.8	15.3
Unsure	1	2.6	5.1
Mother took prenatal multivitamins			
Yes	66	75.9	9.0
No	21	24.1	9.0
Frequency of prenatal multivitamin intake			
Almost daily since pregnancy confirmed	24	36.4	11.6
A few times weekly since pregnancy confirmed	8	12.1	7.9
Almost daily during late pregnancy only	8	12.1	7.9
A few times weekly during late pregnancy only	8	12.1	7.9
Rarely	18	27.3	10.7

Table 6.2 - Infant Stosstherapy and Vitamin D Drop Intake

		n	*Percent*	*95% Confidence Interval*
Infant received Stosstherapy at 6 weeks of age				
	Yes	32	32.7	9.3
	No	57	58.2	9.8
	Unsure	9	9.2	5.7
Mother gave child vitamin D drops				
	No	57	66.3	10.0
	Yes	26	30.2	9.7
	Unsure	3	3.5	3.9
Frequency of vitamin D drop administration				
	Almost daily	21	42.9	13.9
	A few times each week	19	38.8	13.6
	Not very often	9	18.4	10.8

As this study was conducted to assess the influence of vitamin D supplementation on the primary dentition of the child, analysis of variance was performed to determine if vitamin D supplementation was associated with the presence of enamel hypoplasia and caries experience. While 50% of the children had enamel hypoplasia, maternal supplementation did not lead to a decrease in enamel hypoplasia, as hypothesized (Table 6.3). In addition, vitamin D supplementation was not associated with fewer decayed teeth (Table 6.4). No statistically significant differences were found among supplemented and unsupplemented groups in either comparison ($p>0.9$ and $p>0.5$,

respectively). Logistic regression analysis found no statistically significant associations with the presence of enamel hypoplasia.

The mean deft was high (13.7 ± 3.2), with only one child having a deft score of zero. According to the American Academy of Pediatric Dentistry definition, a minimum of one primary tooth affected by caries in children under 72 months [9,12], 98.9% of children had ECC. Mean scores for d,e,f, and the overall deft appear in Table 6.5 and Figure 6.1. Many children who had undergone previous dental treatment for ECC also had high rates of active decay (d).

Table 6.3

Maternal Vitamin D Supplementation	**N**	**Mean # Teeth with Hypoplasia (± S.D.)**
Yes	41	1.7 ± 2.5
No	30	1.6 ± 2.2
Unkown	27	1.7 ± 2.0

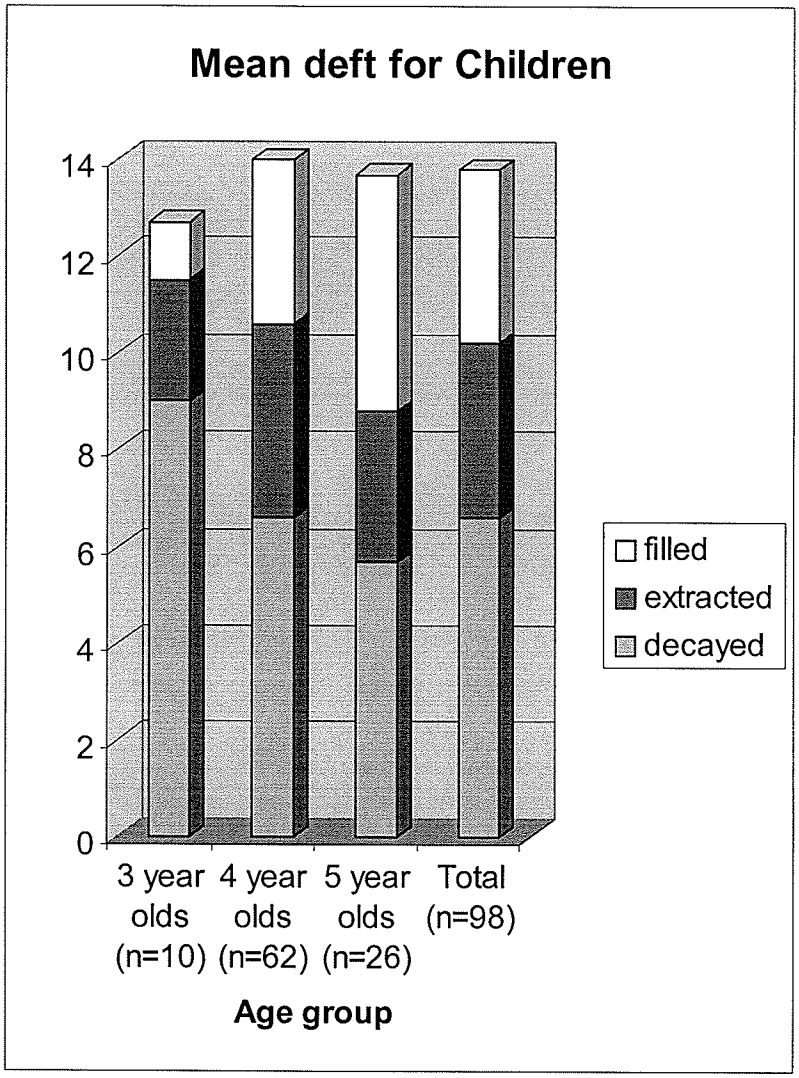
Table 6.4

Maternal Vitamin D Supplementation	**N**	**Mean deft (± S.D.)**
Yes	41	14.1 ± 2.3
No	30	13.3 ± 3.5
Unknown	27	13.7 ± 3.9

Table 6.5

	Mean ± S.D.	**3 year-old (n=10)**	**4 year-old (n=62)**	**5 year-old (n=26)**
d score	**6.6 ± 5.4**	9.0 ± 6.0	6.6 ± 5.0	5.7 ± 5.9
e score	**3.6 ± 2.6**	2.5 ± 2.7	4.0 ± 2.6	3.1 ± 2.5
f score	**3.6 ± 4.5**	1.2 ± 3.2	3.4 ± 4.3	4.9 ± 5.0
deft score	**13.7 ± 3.2**	12.7 ± 5.6	13.9 ± 2.8	13.7 ± 2.9

Figure 6.1



Various child oral hygiene and preventive practices, such as tooth brushing, use of toothpaste, and infant fluoride drops were assessed (Table 6.6). The mean age for initiating brushing was 2.1 ± 0.9 years.

Table 6.6

Child's Oral Hygiene & Preventive Practices	**Percent**	**95 % C.I.**
Brushed independently	54.4	10.3
Brushed a few times a week or less	70.3	11.2
Brushed daily	29.6	11.2
Brushed before bed	29.4	12.5
Used toothpaste	95.4	5.1
Brushed for 1 minute or more	50.8	12.4
Used Fluoride drops	8.1	5.8

The interview posed questions relating to infant feeding methods and interesting child feeding practices (Table 6.7). Breast-feeding was common with 57 respondents (62.0%) reporting that they breastfed their child. The mean age for weaning was 18.6 ± 13.8 months. Eighty women indicated that they gave their child a bottle at some stage of the child's infancy and of the data collected women initiated bottle-feeding at 9.1 ± 17.2 months and ceased to do so at 29.5 ± 17.1 months. Many mothers routinely practiced high-risk caries behaviours with respect to bottle use. These included putting their child to bed with the bottle or giving the bottle on demand, which are generally known to promote decay [13]. Bottle contents also varied and included such things as formula, canned milk, whole milk, tea, juice, koolaid, or soft drinks. Formula, canned milk and

whole milk were the most common contents in the bottle with the following percentages 32.5%, 25.0%, and 23.8%.

Table 6.7

Child Feeding Practices	Percent	95% C.I.
Breast-fed	62.0	10.0
Bottle-fed	87.9	6.7
Put child to bed with bottle	86.1	7.6
Bottle given on demand	67.7	10.3
Added sugar to bottle	65.4	10.6

The mean age for the eruption of the first tooth was 6.1 ± 3.7 months, the average child's first dental visit occurred at 2.3 ± 1.0 years, while the mean age when first diagnosed with caries by a dentist or dental therapist was 2.3 ± 0.8 years. Forty percent of children were diagnosed with caries by 2 years of age (CI 28.5 – 51.5%), 41.3% (CI 31.2 – 51.4%) travelled to Winnipeg for treatment, while 39.1% (CI 28.8 – 49.4%) required general anaesthesia for dental treatment.

This study also posed questions to mothers about their health and nutrition. Five (5.8%) respondents were diabetic while 12 (13.2%) reported gestational diabetes. Responses to women's dietary intakes of specific food items appear in Table 6.8.

Table 6.8

Dietary Intakes of Mothers	Percent	95% C.I.
Drank milk on a daily basis	34.5	10.0
Milk with cereal a few times a week or more	71.6	9.4
Consumed cheese a few times per week or more	73.9	9.2
Ate fish a few times per week or more	35.2	10.0
Ate animal organ meats (e.g. liver) ≥a few times a week	13.6	7.2
Ate soup from animal or fish stock ≥a few times a week	40.9	10.3

Further analysis of variance testing was performed to determine if other relationships existed between variables in this study. No gender or age differences were apparent. Children having dental treatment in Winnipeg had higher deft scores ($p<0.05$), 14.7 ± 2.6 compared with 13.2 ± 2.8. This is likely due to the fact that the most severe cases of ECC were sent to Winnipeg for treatment.

While analysis of variance did not demonstrate a significant relationship between prenatal Stosstherapy and hypoplasia or caries, it did show an association with the eruption time of the child's first tooth. Children whose mothers did not receive prenatal vitamin D supplementation had reported eruption times later than those from supplemented mothers ($p<0.15$) (Table 6.9). When the unsure category was removed from this analysis the difference in eruption times between supplemented and unsupplemented groups became statistically significant ($p<0.05$).

Table 6.9

Maternal Vitamin D Supplementation	N	Eruption Time (months) (± S.D.)
Yes	23	5.0 ± 2.2
No	18	7.2 ± 4.6
Unsure	18	6.4 ± 4.1

Analysis of variance was finally performed to assess whether caries experience was associated with those behaviours known to be risk factors for ECC. Mean deft was found to be associated with adding sugar to the child's bottle. This relationship was significant with those with sugar added to their bottles demonstrating higher deft scores than those whose mothers did not add sugar to their bottles (mean deft 14.3 ± 2.8 compared to 13.1 ± 2.5, $p<0.06$). A higher mean deft score (15.1 ± 2.6 compared to 13.6 ± 2.8, $p<0.05$) was also associated with households not equipped with refrigerators, perhaps serving as a proxy measure of household socioeconomic status or of milk consumption by household. Multiple regression analysis also confirmed that these 2 variables were significantly associated with caries rates ($p<0.05$).

6.5 Discussion

Less than one half of mothers were found to have received the modified Stosstherapy during pregnancy, with the majority receiving it during one of their initial prenatal visits. In addition, 75.9% of mothers stated that they used prenatal multivitamins during gestation, but only 36.4% took them almost daily from the confirmation of their

pregnancy indicating that compliance was not consistent. Meanwhile, only 1/3 of infants received the modified Stosstherapy at 6 weeks of age.

The majority of mothers did not give their children vitamin D drops, but of those who did, 80.7% gave them a few times weekly or more. These variations in vitamin D and prenatal vitamin supplementation surrounding pregnancy and infancy are of some concern, especially in remote northern populations where diet and sun exposure are often insufficient to maintain recommended circulating serum levels.

One of the main objectives of this study was to test whether high dose vitamin D supplementation during pregnancy and/or during infancy could reduce ECC and enamel hypoplasia in the primary dentition. This study of high dose vitamin D at specific times during pregnancy or in early infancy did not support this hypothesis at this time. One of the largest limitations of the study is that the children were beyond the desirable ages to assess enamel hypoplasia and ECC in the primary maxillary incisors. This precluded a proper assessment of prevalence. However, the findings of this study provide information on the oral health status of children residing in this northern First Nation community and the nutritional practices of their mothers and infant feeding practices.

Caries experience was high for the population under investigation. The mean deft of 13.7 ± 3.2 is far larger than deft scores observed in other regions of Canada's North [14,15]. For instance, deft scores for 3 to 5 year olds from the Keewatin and from Churchill, Manitoba were 8.12 ± 5.49 and approximately 2.0 respectively [14-16]. Recent unpublished data regarding the oral health status of children from Churchill reported a mean deft for Treaty status Aboriginals ages 4 and 5 years of 1.60 ± 3.58 teeth [17].

When reviewing the mean d, e, and f scores among the pediatric population, these children exhibited a great deal of active decay, d = 6.6 ± 5.4 teeth. We must also be cautious in interpreting the results of the e score (mean = 3.6 ± 2.6 teeth), as this was likely enlarged due to the practice of pediatric dentists to extract the primary maxillary incisors rather than restoring these teeth.

While half of the children demonstrated the presence of enamel hypoplasia in their primary teeth, logistic regression analysis was unable to identify any variables significantly associated with the presence of these dental defects.

Statistical analysis of the data revealed that high dose vitamin D supplementation was associated with the eruption time of the child's first primary tooth. Those children whose mothers did not receive Stosstherapy had eruption times significantly later than children of supplemented mothers ($p<0.05$). This finding is consistent with other reports of episodes of malnutrition, prematurity, and neonatal nutrition on primary tooth eruption [18-21]. 25(OH)D deficiency may lead to delayed tooth eruption [21]. This association supports the hypothesis that Stosstherapy can influence the development of the primary dentition.

This investigation yielded several interesting findings with respect to maternal nutrition and prenatal health, oral hygiene practices of children, and dental caries. In general, mothers reported infrequent intakes of foods known to contain high levels of calcium and vitamin D such that they were unlikely to have obtained daily recommended amounts needed during pregnancy. For instance, daily milk consumption among mothers was low. Fish consumption was also relatively sparse. Therefore, we can assume that maternal intake of dietary calcium and vitamin D in general was minute.

Diets of First Nations people have changed significantly over recent decades. Unfortunately nutritional deficiencies have increased significantly over this time as well.

A majority of mothers stated that they bottle fed their child (87.9%) and generally ceased to do so at 29.5 ± 17.1 months. This is more than twice the recommended bottle weaning age of 12 to 14 months recommended by the AAPD [9]. Also, 86.1% of mothers admitted to putting their child to bed with the bottle, which is contrary to expert opinion [9]. A large segment of the children were brushing independently but 70.3% of mothers indicated that their children were brushing a few times a week or less and only 29.4% were doing so before bed, likely contributing to the extent of childhood decay in Garden Hill First Nation.

Forty-one percent of the children underwent dental treatment in Winnipeg. Children who underwent treatment in the provincial capital had a significantly higher deft score than children who did not undergo such treatment. This could be due to the more aggressive treatment approach of specialists who base clinical treatment decisions upon the child's future risk of decay, future access to dental care, and perhaps that children with severest forms of ECC are sent there for treatment. Analysis of variance also revealed that deft was associated with adding sugar to bottles. Higher deft scores were observed in children from households that did not have a fridge. Perhaps this can be interpreted that the presence of a fridge served as a proxy measure of SES, or more realistically indicated that those homes purchased milk since they had the capacity to store it between use. The association between dental caries rates and these two variables was confirmed through multiple regression statistical analysis.

A limitation of this study was the retrospective design of the interview. Many of the mothers indicated that they had difficulty in recalling specific information. Mothers also had difficulty with recalling the time interval from when they first noticed their child had caries and the time of the first dental visit. The use of retrospective interviews to recall past experiences continues to be a limitation of this type of research.

Another limitation of this study was that children should have been examined much sooner than what actually occurred. In order to assess the effect of vitamin D supplementation on the presence of enamel hypoplasia, subjects should have been identified and assessed as soon as their primary teeth erupted, starting between 6 to 9 months of age. Since many of the children enrolled into this study had caries and had already undergone dental treatment it was not possible to fully evaluate hypoplasia status. Several children also had missing primary maxillary incisors upon examination, which led the investigators to believe that they had been affected by caries. Had infants been examined as the primary maxillary incisors were erupting and then followed prospectively, hypothesized effects of modified Stosstherapy on the primary dentition may have been observed. One other limitation included variability in the frequency and timing of supplementation in the supplemented group while amelogenesis was occurring.

The AAPD has recently published its Oral Health Policy with respect to ECC [9]. Within this document, reference is made to the need for women to attain proper nutrition during the period when primary teeth are forming since it is known that hypoplasia of primary teeth is related to ECC [9]. In addition, it also recommends that children at high risk be screened at early ages within months of the eruption of the first tooth [9].

New front line prevention efforts need to be targeted to expectant mothers so that they may ensure that their nutritional intakes are sufficient during this stage when primary tooth enamel is forming [22]. Future epidemiological studies into prenatal nutrition, ECC and EH are needed and must be encouraged, especially those that are prospective in design. Evidence exists to encourage 400 IU of vitamin D daily during pregnancy (Cockburn 1980) but research is needed to determine effective dosage and schedules for intermittent supplementation for the prenatal population not receiving 400 IU daily.

6.6 Conclusions

Modified Stosstherapy was not significantly associated with a reduction in dental caries experience or the absence of enamel hypoplasia. Vitamin D supplementation was associated with eruption time of the first primary tooth with those not receiving it having later eruption times of the first primary tooth. The association between high dose vitamin D supplementation and eruption times supports the hypothesis that modified Stosstherapy can influence the development of the primary dentition.

Fifty percent of children experienced enamel hypoplasia. The mean deft score of 13.7 ± 3.2 was very high for this young cohort. High-risk behaviours for ECC, such as poor oral hygiene and bottle feeding practices were routine within this cohort, while maternal nutritional intakes of foods rich in vitamin D and calcium were infrequent. The high caries burden among children from this community reveals the need for effective prevention methods to lessen the disease burden.

Vitamin D may still play an integral role in the formation/calcification of healthy primary teeth. Should low concentrations be shown to be associated with increased risk for enamel hypoplasia, a correlation between vitamin D and caries rates is plausible. Prospective investigations of vitamin D and enamel hypoplasia are needed to substantiate the influence of vitamin D deficiency during pregnancy on the development of the primary dentition. Controlled trials of 25(OH)D supplementation may eventually prove to be beneficial in reducing enamel hypoplasia and ECC.

Appendix 6.1

CHILDREN'S TOOTH DECAY STUDY

VITAMIN D AND TEETH?: In Garden Hill, pregnant women and babies have been given high-dose vitamin D pills at the nursing station since about October 1995. This is done to protect babies from rickets. We think that vitamin D might also protect children from tooth decay. The rotten teeth of young children is called nursing caries or baby-bottle tooth decay. This tooth decay is very common in Garden Hill. Many of the children have had to go to Winnipeg to have dental surgery under general anesthetic.

WHY DO THIS STUDY?: We want to find out if the children who had high-dose vitamin D when they were babies, and whose mothers had high-dose vitamin D during pregnancy, have less tooth decay than children who did not get vitamin D.

WHAT ARE WE DOING?: We are asking questions of the mothers of all children who turn three during this study. We are also taking pictures of the teeth of the three year old children. We need to check the medical records of the mothers and children to see if, and when, they took high-dose vitamin D at the nursing station.

WHAT DO WE WANT FROM YOU?: Because you have a three year old child, we would like to ask you some questions about your diet, vitamin use, and the diet and dental health of your children. We would like to clean and photograph your child's teeth. We would like to look at your medical record and your child's medical record to find out when you or your baby had vitamin D, or if you did not have vitamin D.

RIGHT TO REFUSE: If you do not want to take part in this study you have the right to refuse. Or, you can change your mind or withdraw from the study at any time. You do not have to answer any questions you do not want to.

BENEFITS OF STUDY: The study will be of value to the community and to the health of future children. If vitamin D does lower the amount of tooth decay in children, this information could be used in other communities as well.

CONFIDENTIALITY: All information will remain confidential and no names of individuals will ever be released in reports or publications. If you have any questions please call Pam Smith (789-3473) or Dr. Michael Moffatt (789-3467) at the Department of Community Health Sciences, Faculty of Medicine, University of Manitoba.

INFORMED CONSENT TO PARTICIPATE IN CHILDREN'S TOOTH DECAY STUDY

I understand that I am being asked to take part in a study about vitamin D and children's tooth decay. The interviewer will ask me questions about my diet, vitamin use, and the diet and dental health of my children. My three year old child will have his/her teeth cleaned and photographed.

I have been given an oral and written explanation of the study and have had a chance to ask questions. I understand that I do not have to take part in this study. I can withdraw at any time and I do not have to answer any questions I do not want to.

I understand that this study will be of value to the health of future children. I have been assured that all information is confidential and individuals will not be identified in any reports or publications.

My signature below indicates that I understand and agree:

1) to be interviewed
2) to have my three year old child's teeth cleaned and photographed
3) to have our medical records checked for vitamin D exposure.

I, (print name) _____, agree to take part in the Children's Tooth Decay Study.

Signature of Participant:_____ Date:_____
Signature of Interviewer:_____Date:_____

INFORMED CONSENT TO HAVE MY CHILD'S TEETH EXAMINED BY A DENTIST AS PART OF THE CHILDREN'S TOOTH DECAY STUDY

I understand that my child has been invited to have his teeth examined by a dentist as part of the Children's Tooth Decay Study. The dental exams of selected children will be used to see whether the photographs can be used to correctly identify tooth decay.

I have been given an oral and written explanation of the study and have had a chance to ask questions. I understand that I do not have to have my child take part in this dental examination.

I understand that this study will be of value to the health of future children. I have been assured that all information is confidential and individuals will not be identified in any reports or publications.

My signature below indicates that I understand and agree to have my child examined by the dentist.

I, (print name)_____, agree to have my three-year-old child (child's name)_____ examined by a dentist as part of the Children's Tooth Decay Study.

Signature of Parent: _____ Date: _____

Signature of Interviewer: _____ Date: _____

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Chapter 7 – Protocol and Preliminary Data

A research study is proposed to investigate the association of vitamin D deficiency during pregnancy and enamel hypoplasia in the primary dentition. It will involve participant recruitment during the second trimester of pregnancy at which time serum testing and a questionnaire will be completed. Women and their infants will be followed prospectively until the infant is between 9 months and one year of age so that their child's newly erupted primary maxillary incisors can be examined.

7.1 Objectives

This proposed study will aim to:

1. To determine the vitamin D and calcium nutritional status of a group of urban Aboriginal women during pregnancy. This will be accomplished via serum analysis for circulating concentrations of 25-hydroxyvitamin D (25(OH)D), total calcium, inorganic phosphorus, and alkaline phosphatase. An interviewed questionnaire will also elicit information regarding dietary intakes, prenatal health, and socioeconomic factors.
2. Based on the 25(OH)D status and other serological concentrations of the mother, inferences regarding the vitamin D nutritional state of the developing fetus and subsequent newborn might be generated, as fetal and infant concentrations of 25(OH)D have been shown to be correlated with maternal levels [1-3].
3. To determine the incidence of enamel hypoplasia in the primary maxillary incisors of the infant as they erupt into the oral cavity.

4. To determine the association between maternal 25(OH)D status and the presence or absence of enamel hypoplasia in the primary maxillary incisors of the infant. This is of utmost interest as enamel hypoplasia has been identified as a risk factor for the development of early childhood caries (ECC) (baby bottle tooth decay) [4-8].

7.2 Methods

7.2.1 Study Design

A quantitative research strategy is proposed and will involve women being enrolled in a prospective cohort study during pregnancy. A serum sample from each participant will be collected during a regular prenatal visit. It is anticipated that the collection of these samples will coincide with routine prenatal blood screening. The desired period for serum sampling will be during the second trimester of pregnancy, as the maxillary primary incisors begin to calcify during weeks 13 and 17 in utero. Serum analysis will be conducted for 25(OH)D, as this is a reliable means of assessing overall vitamin D status [9-11, discussed in Chapter 2]. Serum analysis will also include 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 [12] 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 [13]. The relationship of these ions and metabolites has been reviewed in Chapter 2.

Each participant will also complete an interviewed questionnaire. A similar instrument was utilized by Smith to assess nutritional deficiencies in three northern Manitoba First Nations communities [14]. The questionnaire was also constructed with input from senior researchers and community health workers, including a registered dietician, all possessing good knowledge of this population.

The questionnaire is composed of specific sections including basic demographics, pregnancy and health, a nutrition and food security assessment, ECC, and mom's oral health profile. Other sections will pose questions regarding exposure to sunlight, and family composition and finances

The final component of the study, the infant dental examination, will occur following the eruption of the primary maxillary incisors. Clinical examination of each infant's, newly erupted maxillary incisors will be conducted by the principal investigator for the presence of enamel defects between 9 months and 1 year of age. Criteria used to score enamel hypoplasia in this clinical assessment will be based on established indices in the literature and criteria established by the World Health Organization for oral health surveys [15-17].

A prospective cohort study design has been selected. This will allow the natural progression of outcome to be observed, a useful approach when the exposure is not common. This may be beneficial, as serum 25(OH)D status will be considered as the exposure of interest. This same design also allows multiple outcomes of a single exposure to be examined and ensures that a temporal sequence can be established [18,19]. The researcher will be able to determine the incidence of enamel hypoplasia in the infants of those deficient in vitamin D and those with adequate vitamin D, which may

help uncover the potential role of vitamin D deficiency in enamel hypoplasia, and ECC development.

Although there are several benefits to this study design, the process of investigation will require considerable time and expense [18]. There will also be difficulties in maintaining the cohort over the period of the study and this design will not enable the impact of many other possible exposures to be evaluated [18,19]. A description of proposed methods to minimize participant withdrawals and dropouts appears later in this proposal.

7.2.2 Population

The target population for this investigation will be women of self-declared Aboriginal heritage residing in Winnipeg. Many Aboriginals live at or below the poverty level [20]. 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 [21]. Therefore, it is important to study this population group to determine the outcomes that nutritional deficiencies in utero have on the fetus and infant. In addition, many members from northern First Nations communities frequently migrate to southern regions of the province, especially Winnipeg, in search of new and better opportunities, often struggling to improve their quality of life.

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 [14]. One

purpose of this study is to determine whether analogous levels of vitamin D deficiency exist in an urban-based Aboriginal population of pregnant women, which has not been previously evaluated. This study is also targeted towards members of the Aboriginal community since the prevalence of ECC is higher in this population [22-24], even in urban centers. Enamel hypoplasia, a risk factor for ECC, is known to be more prevalent in lower SES populations [4,5,25].

7.2.3 Sample & Calculation of Sample Size

Determining appropriate sample size is important for the study results to have statistical significance upon analysis. Sample size has been reviewed by a senior biostatistician to ensure that this method is best suited for the nature of this research and has been 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 Aboriginals. As the known prevalence of vitamin D deficiency in some northern communities in Manitoba is over 80% [14], a reasonable estimate of prevalence in an urban population may be 50%.

Initial sample size calculation:

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

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

$n = [1.96^2 0.5 (1 - 0.5)] / 0.01 = \underline{\mathbf{96}}$

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 proposed sample size has been increased to 200 women. Enrolling this number of women would allow the project to suffer some loss of the cohort while still maintaining an adequate sample size of statistical significance necessary for analysis.

7.2.4 Instrumentation

Serum samples will be sent to Winnipeg's Health Sciences Centre (HSC), Department of Clinical Chemistry for laboratory analysis. The single serum sample will be analyzed for 25(OH)D, total calcium, inorganic phosphorus, and alkaline phosphatase. Reference ranges appear in Table 7.1.

Table 7.1 – Reference Ranges

	Range of Normal
25(OH)D (25(OH)D)	
	35 – 105 nnmol/L (winter) 37 – 200 nmol/L (summer)
Calcium	
	2.10 – 2.60mmol/L
Phosphorus	
<17 years	1.29 – 2.26 mmol/L
>16 years	0.81 – 1.45 mmol/L
Alkaline Phosphatase	
≤7 years	59 – 422 U/L
>17 years	30 – 120 U/L

Administration of the questionnaire will be by interview. The questionnaire has been developed with input from senior researchers and those health care professionals with knowledge of this population and has been pilot tested with the target population. The questionnaire has been divided into seven separate sections, each dealing with a specific theme.

The first section is the "participant profile" designed to collect basic demographic information from study volunteers including date, place of birth, address and contact information, and ascribed Aboriginal heritage.

The "pregnancy and health profile" will examine perceived prenatal health, and issues relating to prenatal care and well being. Inquiries regarding multivitamin use will be made, including whether their health care provider recommended multivitamins and whether there was compliance with the recommendation. Women will also be asked questions which may give insight as to their vitamin D and calcium status, including clinical symptoms of hypovitaminosis D. As a main objective of this study is to assess serum vitamin D status, participants will also be asked whether they have heard of

vitamin D and calcium, what they are important for, and what foods contain vitamin D and calcium. Responses to these questions may 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 delves into questions relating to food security, whether dietary practices have changed since learning of their pregnancy, and whether women are aware of the "Healthy Baby" Prenatal Benefit from the Province of Manitoba. Further questioning will focus on the consumption of calcium and vitamin D containing food products (i.e. fortified milk and dairy, fish, eggs), and whether participants would be open to various ways to enhance vitamin D and calcium intake. Recall that few foods naturally contain vitamin D, often leaving fortified milk as the main dietary source [26]. Intake of calcium and vitamin D containing foodstuffs will be assessed to determine if women and their developing fetuses may be at risk of having insufficient vitamin D concentrations.

Two other sections focus on oral health. The "ECC profile" will examine whether expectant mothers have heard of this disease, how they came to learn about it, whether they believe it is a normal part of childhood, and what they believe are its causes. It will also ask mothers whether they believe ECC is 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 will be assessed. "Mom's oral health profile" will assess dental attendance and whether mothers are experiencing dental problems.

The "exposure to sunlight" theme will evaluate participant exposure to sunlight during summer months for endogenous production, amount of sun exposure received, and duration of time. Use of sunscreens, clothing, and insect repellants will be reviewed.

The final theme, the "family & financial profile" will record their relational status, number of persons in the household, highest level of education, and issues related to annual income, to establish a socioeconomic (SES) profile. This is necessary so that the analysis can control for poverty and related issues as these may influence maternal health and nutrition, enamel hypoplasia, and ECC.

Lactose intolerance may be a considerable issue within this population [27,28], therefore assessing milk consumption alone may not prove to be the most beneficial method of determining risk of hypovitaminosis D. Other foods will also be considered to determine each participant's potential risk for vitamin D and calcium deficiencies. Vitamin D rich foods include fish, eggs, liver, and fortified dairy products including milk and cheese [29]. Calcium rich foods are usually dairy-based, although many other foods contain calcium [29], including some green vegetables. Daily recommendations (adequate intake) of vitamin D and calcium intake during pregnancy call for 5 ug/day (200 IU/day) and 1000 mg/day respectively [29]. For instance, 1 cup of milk contains approximately 2.3 ug of vitamin D and 300 mg of calcium [29]. However, controversy surrounding the recommended dietary allowances (RDA) for vitamin D exist and the real RDA remains obscure [30, discussed in Chapter 2].

The final component in the research protocol is the assessment of the integrity of the primary maxillary incisors. This examination will involve an assessment of the primary maxillary incisors (teeth predominantly affected by ECC) accompanied with

digital photographs of the teeth. Some lack of clarity regarding the precise diagnostic criteria for enamel hypoplasia exists. It may be useful to consider it as "chalky, gray-yellow, brown or black band, with or without pits and grooves" [31,32]. It may also help to view hypoplasia as "pitting, furrowing, or absence of enamel" [33]. The working definition of enamel hypoplasia that will be used for this study will be "a defect involving the surface of the enamel and associated with a reduced localized thickness of enamel" [16], either as pits or grooves. Indices for enamel hypoplasia exist, in addition to criteria established by the World Health Organization, and will serve as references for the assessment of enamel hypoplasia [15-17]. Specific locations of demarcated opacities, diffuse opacities and hypoplastic defects of the primary maxillary anterior incisors will be recorded. Both the presence and absence of enamel hypoplasia will be recorded for each erupted tooth along with simultaneous use of an enamel hypoplasia index.

7.2.5 Implementation

Aboriginal women seeking prenatal care at various core area community health clinics will be invited to participate. Sites that will serve as centers for the recruitment of study volunteers include the Outpatient Department of Women's Hospital, Health Action Centre, and Mount Carmel Clinic. Women's Hospital is situated at 735 Notre Dame Avenue and is part of Winnipeg's HSC. Health Action Centre is situated at 425 Elgin Avenue and is also affiliated with HSC. Mount Carmel Clinic 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 will be obtained through contacts made by primary care obstetricians and family physicians, prenatal nurses, and other health professionals providing prenatal care at these clinics.

Serum sampling will be utilized to assess whether clinical vitamin D deficiency exists. The primary care physician, clinic nurse, midwife or staff phlebotomist will collect the blood sample for this research. The proposed time frame for serum collection will be during the second trimester of pregnancy and it is planned that such serum draws coincide with routine prenatal serum screenings to minimize needless punctures.

The questionnaire is to be administered during this same visit to the clinic by the principal investigator, a research assistant, or existing staff at the various clinic sites. The questionnaire is designed to take 30 to 35 minutes to complete.

The principal investigator will conduct the dental examination, of each infant's primary maxillary incisors, at the health centers between 9 months and one year of age. Dental clinics at Mount Carmel Clinic and Health Action Centre will serve as sites for the dental examination. For participants enrolled at Women's Hospital, it is expected that many will bring their children to the children's clinic at Children's Hospital. Thus Children's Hospital will likely serve as a location for the dental examination in these instances. Should dental examinations not proceed at any of the sites, the Manitoba Institute of Child Health has granted permission to utilize an examination room for this process. Digital images of the primary maxillary incisors will be taken during the dental examination depending on consent from the mother. This may prove useful in identifying the differential patterns of enamel hypoplasia.

Cohort retention will likely pose a significant challenge. Follow-up to maintain this cohort will involve 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. It may be helpful to coordinate the dental examination visit

with infant vaccination appointments for convenience, and to improve the likelihood of maintaining the cohort.

An honorarium has been added for each participant to compensate them for out of pocket expenses incurred and to serve as an incentive for remaining in the study. This honorarium has been added on the recommendation of various senior researchers and community health professionals who have experience working with our target population. The amount proposed is $15.00 following the first visit and $15.00 following the infant dental exam. This amount is appropriate for the nature of each participant's role.

Subsequent to the collection and analysis of the data it is the intent of the research team to share all relevant findings with study volunteers and community leaders, and the academic and professional communities.

7.2.6 Ethics

This study protocol has been 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 participant information and consent form has been approved along with the questionnaire (Appendix 7.1). Recruitment posters have also been granted approval for use by the Health Research Ethics Board.

7.2.7 Analysis

Laboratory analysis will be conducted by the Department of Clinical Chemistry, HSC. Analysis will include 25(OH)D, total serum calcium, inorganic phosphorus, and alkaline

phosphatase. Preprinted laboratory requisitions have been created to facilitate this process. Specimens will be forwarded to the laboratory via current transportation and courier arrangements already established between the laboratory and participating health centers. Laboratory reports will be forwarded to Dr. Michael E.K. Moffatt to keep the principal investigator blinded of maternal 25(OH)D status while performing the dental examinations of the infant. Primary physicians will be contacted by a member of the research team should 25(OH)D concentrations be below 25 nmol/L.

Digital photos of primary maxillary anterior teeth and clinical examinations will be performed at the health centers. Results will be matched with the mother's questionnaire responses and the serum analysis data.

Statistical modeling and logistic regression will likely be employed in the analysis of the study data. NCSS will be used for the statistical analysis of data. Mean concentrations for serum metabolites will be reported along with the prevalence of vitamin D deficiency in this cohort, using the cutoff values used by the Department of Clinical Chemistry, and those in the scientific literature. Correlations between the serum concentrations of 25(OH)D, total calcium, inorganic phosphorus, and alkaline phosphatase will be explored. Logistic regression will be used to identify those variables that are significantly associated with 25(OH)D status, i.e. adequacy and insufficiency, and those strongly associated with the presence of enamel hypoplasia. Statistical modeling will also be performed to determine whether concentrations of 25(OH)D below a specific threshold can predict enamel hypoplasia.

7.2.8 Funding

Operating grants from the following agencies have been awarded to conduct this research:

- Manitoba Medical Service Foundation
- Children's Hospital Foundation of Manitoba Inc./Manitoba Institute of Child Health
- Dentistry Canada Fund
- Dean's Research Funds, Faculty of Dentistry, University of Manitoba

7.2.9 Significance of this Research

1. The nutritional status of expectant mothers belonging to the Aboriginal population in Winnipeg will be determined through serum assays and an interviewed questionnaire.
2. More information regarding the risk of nutritional insufficiencies for the developing fetus and infant will be known.
3. The incidence of enamel hypoplasia in children of urban Aboriginal mothers will be known.
4. The relationship between maternal 25(OH)D status during pregnancy and enamel hypoplasia infant will be evaluated.

The prevalence of ECC is still high for specific Native American populations [34,35] including the Aboriginal population within Manitoba (discussed in Chapters 5 and 6). Intraoral pain, dental infections, and malocclusions are common sequelae of ECC and many children require general anaesthesia in order to provide treatment. Over 1.7 million federal dollars annually are spent treating ECC under general anaesthesia for children solely from First Nations communities in Manitoba. Recognize that this figure

grossly underestimates the problem, as it does not give insight to the provincial expenditures required to treat this problem, which are considerable since the provincial program is the first insurer for those with dual benefits from First Nations and Inuit Health Branch (FNIHB) and provincial social assistance. It also does not reflect the large number of children who are currently on waiting lists for treatment and those who have limited access to care. There is also a tremendous burden placed upon pediatric health care facilities forced to deal with this pediatric disease. For instance, approximately $1/6^{th}$ of the current operating room time at Children's Hospital in Winnipeg is devoted to treating ECC (Dr. Moffatt – personal communication 2002). By focusing research efforts towards a preventable pediatric disease, there is the potential to reduce child suffering along with relieving the burden on pediatric health facilities. This could ultimately lead to a reallocation of scarce resources.

Enamel hypoplasia has been identified as a risk factor for ECC development as it affects the enamel's resistance to bacterial invasion. This prospectively designed cohort study is unique since few, if any, studies in the area of ECC have undertaken such a design.

While determining the association between vitamin D deficiency in utero and the presence of enamel hypoplasia is a major objective of this planned research, results from the serum analysis and questionnaire responses will also have tremendous importance in determining the vitamin D status and the prevalence of deficiency in this urban population. Data from this longitudinal study will provide the first documented prevalence of 25(OH)D concentrations among a cohort of pregnant Aboriginals residing in southern Manitoba. This in itself will be of extreme importance to future expectant

mothers and those health care professionals providing prenatal care as more attention may need to be devoted to enhancing diets and educating women during pregnancy if vitamin D insufficiencies are found to be significant in this cohort. It may also reveal the need for more intervention and support of improved maternal nutrition during prenatal periods.

More may be learned including the effects of season, SES, diet, and other factors on maternal 25(OH)D concentrations. Factors known to be associated with maternal 25(OH)D (reviewed in Chapter 3) can also be assessed at the end of this investigation and may assist in validating and clarifying associations. For instance, it will be possible to assess the relationship between maternal SES, age, gravid history, and antenatal care with 25(OH)D as insufficient information currently exists to support their relationships. This may aid health care professionals profile and identify women who may be at risk of having low 25(OH)D concentrations during pregnancy, so that interventions can be implemented to reduce the risk of development disturbances to the developing fetus and infant.

Preliminary Data

7.3 Results

Enrollment commenced in June, 2002 at Health Action Centre while recruitment at Women's Hospital, HSC began during July 2002, serving as the prime site for enrollment to date. Participant enrollment at Mount Carmel Clinic began during September 2002. Participant consent was obtained from all study volunteers. As of November 13, 2002, 70 participants were enrolled, the majority completing the questionnaire and serum draws between the months of July and October (Table 7.2). All subjects enrolled between June 19, 2002 and November 13, 2002 were included in this analysis.

Table 7.2 – Frequency of Participants

Health Facility	*n*	*Percent*
Women's Hospital (HSC)	53	75.7
Health Action Centre	12	17.1
Mount Carmel Clinic	5	7.1

7.3.1 Serum Results

Serum analysis constituted an essential component of this study. Enrolled participants had serum samples drawn to assess circulating levels of 25(OH)D (nmol/L), total calcium (mmol/L), inorganic phosphorus (mmol/L), and alkaline phosphatase (U/L). Completed laboratory results for 61 of the 70 subjects were available at the time of the analyses. All laboratory reports from the serum analyses had participant identifying information removed to keep the primary investigator blinded to vitamin D status for the second stage of this prospective study. This prevented any linkage of serum and questionnaire data at this present time. Preliminary serum results for participants can be seen in Appendix 7.2.

Figure 7.1 presents the distribution of 25(OH)D results for the study population as of mid November 2002. Mean results along with their respective standard deviations, ranges and median values for the various metabolites were calculated (Table 7.3).

Figure 7.1 – Distribution of Participant 25(OH)D Concentrations

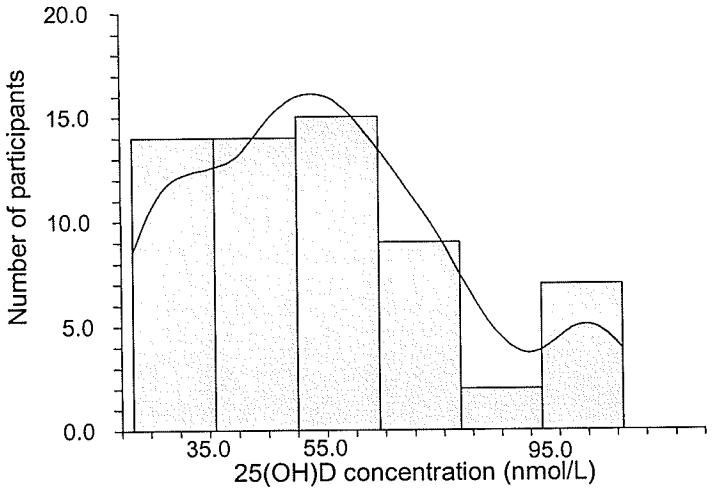


Table 7.3 – Mean, Standard Deviation (S.D.), Range, and Median

Serological Test	*Mean*	*± S.D.*	*Range*	*Median*
25(OH)D (nmol/L)	55.69	22.61	25 – 106	54
Total Calcium (mmol/L)	2.25	0.10	2.05- 2.43	2.23
Inorganic Phosphorus (mmol/L)	1.17	0.21	0.69 – 2.28	1.16
Alkaline Phosphatase (U/L)	116.41	62.17	46 – 372	98

The calculated mean value for 25(OH)D was found to be in the normal range when 35 nmol/L was used to determine deficiency, but is also indicative of hypovitaminosis D [36]. Serum values of 25(OH)D were grouped according to various

defined ranges of normal. 35 nmol/L is the current lower threshold limit of normal, with the normal range for vitamin D being 35 to 200 nmol/L (Clinical Chemistry, HSC). Values below this level are indicative of vitamin D deficiency. Others recommend that values of ≤40 nmol/l are indicative of vitamin D insufficiency [37] and deficiency [38]. Table 7.4 presents the frequency of participants based on the various threshold cut-off values.

Table 7.4 – Frequency by 25(OH)D Thresholds

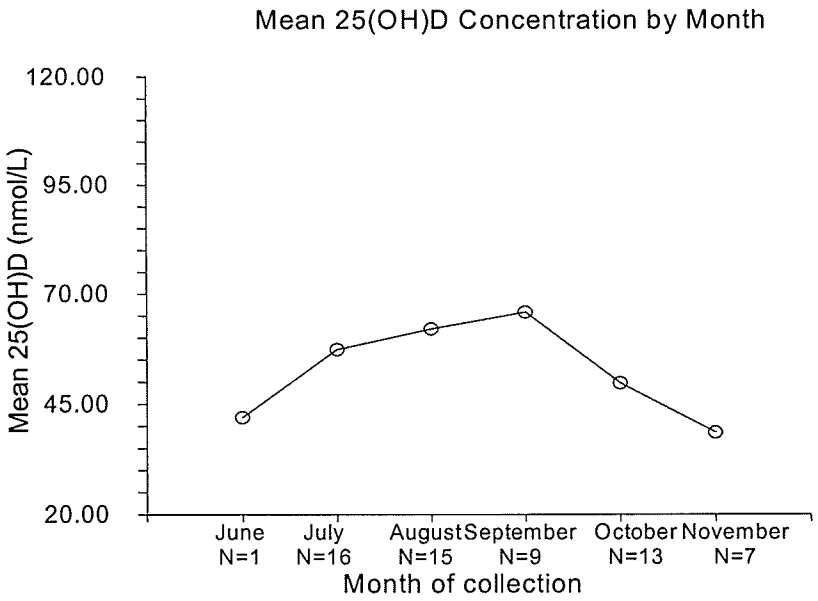
25(OH)D Threshold	*n*	*Percent*
< 35 nmol/L	12	19.7
< 40 nmol/L [39,40]	17	21.9
< 50 nmol/L [36,41]	27	44.3
<80 nmol/L [36,39]	52	85.2
≥100 nmol/L [36]	4	6.6

The calculated mean value for total calcium was within the range of normal (2.10 – 2.60 mmol/L) and only two participant had a total serum calcium concentration below 2.10 mmol/L. The mean inorganic phosphorus value was also in the normal range for those 17 years of age and older, while the mean alkaline phosphatase value was also in the normal range but at the higher cut-off value for those 17 years and over. Serum concentrations for some participants fell outside normal ranges. For instance, twenty-one participants had low inorganic phosphorus concentrations, and one had an elevated inorganic phosphorus level. Individual concentrations of alkaline phosphatase also fell outside normal ranges with 14 having concentrations above the upper threshold and one below the lower threshold.

As all identifying information was removed from the laboratory reports, with the exception of age, inquiries of interactions between participant characteristics or questionnaire responses with serum results could not be examined at this stage of the investigation. Analysis of variance revealed that maternal age was not associated with 25(OH)D status ($p=0.82$). Likewise, there was no difference in the 25(OH)D concentrations of adolescent participants (<18 years) and adult participants ($n=31$, 58.65 ± 24.04 nmol/L vs. $n=23$, 57.30 ± 20.58 nmol/L, $p=0.83$). Analysis of variance demonstrated that there were no significant associations between mean maternal age and whether participants were vitamin D deficient (<35 nmol/L) ($p=0.19$), or insufficient (< 40 nmol/L) ($p=0.21$).

Monthly and seasonal variation of 25(OH)D concentrations were found (Figure 7.2). Maternal 25(OH)D values differed significantly when the mean concentration from participants sampled between June to September ($n=41$) were compared with those from October to November ($n=20$) (60.51 ± 21.29 nmol/L vs. 45.8 ± 22.53 nmol/L, $p<0.05$). Mean concentrations during the peak summer season (July through September) were higher than those concentrations sampled during the autumn months of October and November. Mean 25(OH)D concentrations between the months of July (57.31 ± 19.67 nmol/L), August (62.00 ± 23.12 nmol/L), and September (65.78 ± 22.49 nmol/L) did not vary greatly ($p>0.05$). Significant differences in mean maternal 25(OH)D existed between July and November ($p<0.05$), August and November ($p<0.05$), and September and November ($p<0.05$). No significant differences existed when other monthly mean values were compared.

Figure 7.2



Finally, multiple regression was performed with the maternal 25(OH)D being the continuous outcome variable of choice. Variables in this analysis included the remaining serum metabolites, participant age, and month of sample collection. None of these variables were significant predictors of serum vitamin D concentrations in this cohort. Correlations between 25(OH)D, calcium, phosphorus, and alkaline phosphatase were also investigated. Multiple regression analysis revealed that total calcium and inorganic phosphorus concentrations were correlated ($p<0.05$) but no other serum metabolites were correlated.

Logistic regression also did not find any significant associations between deficient (< 35 nmol/L) and sufficient (≥ 35 nmol/L) concentrations and total calcium, inorganic phosphorus, or alkaline phosphatase.

7.3.2 Questionnaire Results

Participant responses to a selected number of questions from the comprehensive questionnaire were examined. Of specific interest were responses pertaining to demographics, knowledge of vitamin D, prenatal health and nutrition intakes, and the use of multivitamins. 70 completed questionnaires were available at the time of analysis.

The distribution of participants according to their self-declared Aboriginal heritage was determined (Table 7.5). The mean age was 18.7 ± 4.2 years, while the average level of education completed by subjects at the time of enrollment was grade 9.6 ± 1.4. Forty-four participants (62.9%) indicated that this was their first pregnancy (primigravid).

Table 7.5 – Self-declared Heritage

Aboriginal Heritage	*N*	*Percent*
Status Indian	48	68.6
Non Status Indian	7	10.0
Metis	13	18.6
Other	2	2.9

Specific interest pertained to whether participants had heard of vitamin D, whether they knew what vitamin D was important for, and if they were able name at least one correct food item known to contain vitamin D. The same knowledge of calcium was assessed. In addition, women were questioned about their knowledge of the Healthy Baby Prenatal Benefit from the Province of Manitoba and whether they were receiving

this bonus. The number, percent, and respective confidence intervals of their responses appear in Table 7.6.

Table 7.6 – Participant Knowledge of Vitamin D, Calcium, and Provincial Benefit

Question	*n*	*Percent*	*95% C.I.*
Heard of vitamin D	45	64.3	11.2
Knew what vitamin D was important for	22	31.4	11.0
Identified at least one food item containing vitamin D	10	14.3	8.2
Heard of calcium	67	95.7	4.8
Knew what calcium was important for	48	68.6	10.9
Identified at least one food item containing calcium	47	67.1	11.0
Heard of Healthy Baby Provincial Benefit	61	87.1	7.9
Receiving Healthy Baby Provincial Benefit	38	55.1	11.7

Multivitamin supplementation is frequently recommended during pregnancy. When asked, 63 (90.0%) subjects reported that their doctor recommended that they take vitamins during their pregnancy. Further questioning revealed that 65.2% of participants took vitamins daily, 13.0% took them a few times a week, 1.5% rarely did so, while 20.3% indicated that they had not yet taken vitamins during their pregnancy.

Further analysis revealed that women were more likely to take prenatal vitamins if recommended to do so by their doctor (chi square = 16.0, df 1, $p<0.001$). Likewise, bivariate analysis revealed that subjects were also more likely to take vitamins more frequently if doctors recommended vitamin supplement use to them (chi square = 21.6, df 3, $p<0.001$).

A majority of women indicated that they drank milk on a daily basis (57.1%, CI 11.6), 30.0% (CI 10.7) did so several times a week, while 9 rarely or never drank milk (12.9%, CI 7.9). Only 12 women (17.1%, CI 8.8) indicated that intake of dairy products upset their stomach, and 82.9% of women (n=58) stated they had increased their intake of milk and dairy products since becoming pregnant. Bivariate analysis revealed that women who reported some dairy intolerance did increase their intake during pregnancy compared to before pregnancy (chi square = 12.93, df = 2, $p<0.05$).

Responses to specific questions in the infant oral health section of the questionnaire were also of interest at this time. For instance, had participants ever heard of ECC, did they believe it was a normal part of childhood, and did they believe it could be prevented? Responses to these questions along with their confidence intervals appear in Table 7.7. The most common explanations by participants for the etiology of ECC were bottle use, sweet liquids in the bottle, too much sugar or candy in the diet, and lack of proper infant oral hygiene.

Table 7.7 – Knowledge of ECC

Question	*n*	*Percent*	*95% C.I.*
Heard of ECC	55	78.6	9.6
ECC was a normal part of childhood	21	30.0	10.7
ECC could be prevented	64	91.4	6.6

Further demographic information for the existing cohort was gleaned from the interview. The majority of study participants (n=58) reported a yearly income below $18,000 (82.9%), while 11.4% of women stated that they were unsure of their annual

income. The relational status of participants was also collected and the majority of women reported they were single (58.6%) while another large contingent reported being in existing common law relationships (35.7%). Only 3 participants said they were married while another was separated from her spouse.

Analysis of Variance was performed to determine whether any interactions existed between those variables assessed from the questionnaire. Mean age was associated with the site of recruitment. Participants attending the outpatient department of Women's Hospital, HSC were younger (17.4 years) than participants recruited at Health Action Centre (23.1 years) and Mount Carmel Clinic (20.8 years), $p<0.001$. When mean age was compared again, this time combining the community health centers, this too revealed that mean age was associated with clinic location ($p<0.001$) with those attending the outpatient department of Women's Hospital being significantly younger (17.4 years vs. 22.4 years).

Bivariate analysis revealed that first pregnancies were associated with clinic location, with those recruited at Women's Hospital being more likely to be primigravid, Chi Square = 11.0, df = 2, $p<0.01$. This is likely due to increased number of adolescents attending the Outpatient Department of Women's Hospital.

7.4 Discussion

These findings give insight into the serum 25(OH)D status of a group of urban Aboriginal women during the summer and early autumn season in Winnipeg. The cross sectional look at the vitamin D status of Aboriginal women during pregnancy is helpful. In addition responses to the interviewed questionnaire give further insight into the demographic composition of this cohort, their nutritional intake, and their knowledge of ECC.

A total of 70 women were already enrolled into this prospective cohort at the time of this report which equaled 35.0% of the overall desired sample size. Participant recruitment began in June 2002 with subjects from each of the 3 clinic sites taking part. 75.7% of subjects were recruited from Women's Hospital while the remainder came from the two inner-city community health centres. On average, 3 to 4 participants were recruited weekly.

7.4.1 Serum Results Discussion

Laboratory reports for 61 women were available at the time of analysis, or 87.1% of the cohort as of November 13, 2002. The mean 25(OH)D (55.69 ± 22.61 nmol/L) was in the range of normal yet nearly $1/5^{th}$ of women demonstrated vitamin D deficiencies, defined by concentrations below 35 nmol/L. The debate over a universally acceptable threshold defining serum 25(OH)D deficiency (reviewed in Chapter 4) has even lead for calls to raise this threshold to at least 40 nmol/L and beyond [39]. While there is ample evidence of the difficulty residents of northern Canada face in achieving adequate vitamin D, from both diet and endogenous sources [14,27,28], these preliminary findings indicate that residents in southern regions of Manitoba may suffer from

insufficiencies. In fact the prevalence of vitamin D insufficiencies during this summer period (June to end of September) was a little higher (20.0%) than those of young non-white Canadian women, including Aboriginals, residing in Toronto (17.1%) during the same season [42].

Monthly and seasonal variations in maternal circulating concentrations were observed, adding to the limited research among the seasonal effects on vitamin D status of Canadian women, especially expectant mothers from the Aboriginal community. Mean 25(OH)D concentrations for expectant mothers sampled during the fall were significantly lower than the mean level for women enrolled during the summer season, which is consistent with other findings [43]. Significant monthly variation existed between July and November, August and November, and September and November.

While there has been documented evidence of the association between age and vitamin D status in healthy adults [39], there is little evidence to suggest that age influences the 25(OH)D levels during pregnancy [44, reviewed in Chapter 3]. These preliminary data too did not demonstrate a significant relationship between age and measured serum concentration nor was there a difference in serum concentrations between adolescents and adult expectant mothers.

The mean value of circulating calcium was also found to be in the normal range, with only one participant having a value below the lower limit of 2.10 mmol/L. A number of study subjects were found to have low serum levels of inorganic phosphorus, which may require additional investigation, as phosphorus is also necessary for fetal development.

Multiple regression analysis indicated that total calcium (mmol/L) and inorganic phosphorus (mmol/L) were found to be correlated, and is consistent with the findings of a recent study of vitamin D insufficieny among western Canadians [39]. No correlation existed between 25(OH)D, phosphatase, inorganic phosphorus, or total calcium.

7.4.2 Questionnaire Results Discussion

While a thorough questionnaire was constructed to evaluate a multitude of issues, only select questions were assessed for this preliminary analysis. The selection of these three clinics proved to be good places to recruit Aboriginal women seeking prenatal care. Self declared Aboriginal heritage revealed that 73.6% of the women enrolled at Women's Hospital were Status Indians, 11.3% were Non Status Indian, while another 11.3% were Metis. Heritage of those recruited at Health Action Centre revealed that 60.0% were Status Indian, while the remainder, were Metis. The majority (60.0%) from Mount Carmel also identified themselves as Status Indians with the remainder being equally distributed as Metis and Non Status. Of the entire cohort (n=70), 68.6% had identified themselves as being Status Indian. Winnipeg is a good urban centre for those investigating the health of urban Aboriginal populations as this group has a higher birth rate than the general population, and many migrate to Winnipeg from rural First Nations communities.

While these sites indicate that they are good locations to capture the sample of prenatal Aboriginal women, the difficulty following enrollment will be the maintenance of this highly mobile urban population. Low SES, housing, and other social factors often result in these women moving frequently [21]. In order to minimize the effects of significant withdrawals and losses to follow-up, an honorarium has been implemented.

Contacts by mail and by phone will also be necessary. Fortunately, we have the ability to over recruit study participants to deal with any expected attrition in the study population.

A majority of participants (64.3%) stated they had heard of vitamin D, yet only 22 (31.4%) knew what vitamin D was important for, and only 10 correctly identified at least one food item containing vitamin D (14.3%), such as milk, fish, liver, or eggs [29]. Further, the age of those indicating they had heard of vitamin D was significantly less than those unfamiliar with vitamin D. There is a possibility that younger women were demonstrating performance bias exists and the introduction of vitamin D during the recruitment and completion of the informed consent document may have altered or biased their responses. The limited knowledge regarding the role of vitamin D and dietary sources may not be unique to this population, as many in the general public are unfamiliar with the actions and origins of this vitamin and hormone. As vitamin D adequacy has tremendous implications for health and wellness, public awareness may need to be heightened if more people are to possess this basic knowledge.

The same questions were posed with respect to calcium. In this case the majority of participants had heard of calcium (95.7%), however, a lesser number could answer what calcium was important for (68.6%) while 67.1% correctly named at least one food item known to contain calcium. Participants had a better understanding of calcium's role in health than vitamin D which is likely comparable to the masses.

A provincial benefit was introduced to ensure that expectant mothers of lower SES are able secure better nutrition during pregnancy. Considering that the prenatal period is a period of critical development for the fetus, this benefit has the potential to improve the health status of expectant mothers and their infants. Considering that many

reported an annual income below $18,000 the need for government support exists in order to improve and enhance their diets. While 87.1% had heard of this benefit just over half of those enrolled were receiving this government grant at the time of the proctored interview. As participants were enrolled during their second trimesters and at the midpoint of pregnancy, a disturbing fact is that many were likely not in receipt of the beneficial effects to the prenatal diet that this benefit was intended to bring about. Many also do not seek prenatal care prior to this period and in order to qualify for the program, applications must be received by a certain stage of pregnancy, which may also disqualify some most in need of improved nutrition.

As limited income often translates into difficulties in purchasing a well balanced diet, and knowing that the endogenous synthesis of vitamin D is halted during significant portions of the year in this geographic location [45], the only other source for vitamin D can come from vitamin supplements. However, the difficulty with relying on dietary supplements to obtain essential amounts of vitamin D is compliance. This is exhibited in this cohort as the frequency of prenatal vitamin intake varied considerably with only 2/3rds of participants admitting to daily use.

Lactose intolerance has been raised as an issue among some Aboriginals yet only 12 participants reported that intake of dairy products upset their stomach, and nearly half admitted to increasing their use of dairy products during pregnancy. Of similar interest is that 57.1% reported they drank milk on a daily basis, with only 9 rarely or never drinking milk. Perhaps milk may be a reasonable method to improve the 25(OH)D status of this population.

ECC is common in many Aboriginal populations in North America [22-24,34] including urban Aboriginals; therefore it was not surprising that more than 75% of participants had heard of this dental syndrome afflicting very young children. However, the shocking statistic was that 21 women (30.0%) believed that ECC was a normal part of childhood, possibly indicating the commonality of this disease in their community. Changing community awareness about ECC is important to reduce the perception that ECC is a normal occurrence so that people can realize that caries need not exist in children. What is promising is that nearly all still believed that this disease was preventable.

As this data represents preliminary data from this prospective investigation, the full statistical analyses cannot yet be performed. In addition, serum results have had all identifying information removed to eliminate assessor bias for the dental assessment stage of this study. This precludes any investigation of those factors whose association with maternal circulating 25(OH)D during pregnancy were reviewed in Chapter 3 (i.e. vitamin supplementation, dietary restrictions, SES, amount of sun exposure, etc.). At the end of this investigation, all data will be combined so that closer examinations can be made to see whether these associations hold true for this urban Aboriginal population. What will prove interesting will be whether achieving recommended dietary intakes of fortified foods and use of multivitamins actually serves to prevent insufficiencies [42].

While prospective research methods offer tremendous benefit, they do require significant resources and maintaining the cohort becomes a significant challenge. In order to investigate the relationship between vitamin D status in utero and the presence of

enamel hypoplasia, a risk factor for ECC, prospective studies offer the best opportunity to establish a link between exposure and outcome.

7.5 Conclusions

While the mean 25(OH)D concentration was within the range of normal, 25(OH)D deficiencies and insufficiencies were found among urban Aboriginal expectant mothers in Winnipeg during the summer and early autumn seasons. 25(OH)D concentrations did demonstrate seasonal variation with summer values being significantly higher than early autumn.

Questionnaire responses indicated that the majority of participants were Status Indians of low income. The majority possessed little knowledge of vitamin D and prenatal multivitamin use was not universal. Dietary intakes of foods containing vitamin D and calcium varied, although nearly half stated they had increased their intake of dairy products.

While ECC was a recognized childhood disease, many believed it was a normal part of childhood, indicating that ECC awareness and prevention is needed for this population.

UNIVERSITY OF MANITOBA | Faculty of Medicine

Appendix 7.1

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
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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: _____

Appendix 7.2 – Blinded Serum Results of Study Participants (H = high, L = low)

Report Number	*25(OH)D (nmol/L)*	*Calcium (mmol/L)*	*Inorganic Phosphorus (mmol/L)*	*Alkaline Phosphatase (U/L)*
1	49	2.37	1.16 L	94
2	78	2.22	1.13	58
3	106	2.20	0.76 L	372 H
4	54	2.24	0.69 L	60
5	35	2.19	1.11 L	262
6	42	2.31	1.00	51
7	46	2.33	1.23	50
8	56	2.31	1.41	67
9	50	2.35	1.17 L	164
10	60	2.27	1.27 L	138
11	67	2.26	1.27	77
12	93	2.21	1.22 L	109
13	93	2.21	1.02	188 H
14	69	2.22	1.22	125 H
15	56	2.16	1.12	61
16	25 L	2.37	1.28 L	53 L
17	46	2.30	0.99	67
18	73	2.20	1.16 L	157
19	74	2.43	1.28 L	96
20	31 L	2.21	0.94 L	78
21	69	2.41	1.31	73
22	57	2.18	1.08 L	155
23	61	2.32	1.20	152 H
24	55	2.32	1.39	79
25	62	2.15	1.25	60
26	54	2.33	2.28 H	121 H
27	59	2.12	1.13 L	165
28	28 L	2.11	1.29	172
29	80	2.33	1.24	118
30	100	2.05 L	1.16 L	68
31	31 L	2.13	1.15	156 H
32	36	2.20	1.09	212 H
33	68	2.31	1.20	108
34	64	2.37	1.44	96
35	105	2.10	1.17 L	110
36	39	2.19	1.15	64
37	78	2.16	1.23	129 H
38	46	2.20	1.05	274 H
39	75	2.36	1.31	85
40	80	2.13	1.14	148 H
41	103	2.20	1.15	132 H
42	53	2.42	1.30	70

Report Number	*25(OH)D (nmol/L)*	*Calcium (mmol/L)*	*Inorganic Phosphorus (mmol/L)*	*Alkaline Phosphatase (U/L)*
43	25 L	-	1.08	107
44	34 L	2.24	1.10	211 H
45	63	2.28	1.01	98
46	39	2.18	1.08	86
47	43	2.18	1.04 L	123
48	43	2.40	1.48 L	46
49	97	2.16	0.95	53
50	46	2.40	1.14	155 H
51	26 L	2.05 L	0.82	86
52	38	2.19	1.08 L	219
53	25 L	2.38	1.28	96
54	48	2.17	1.10	74
55	56	2.23	1.20	117
56	24 L	2.31	1.18	61
57	27 L	2.13	1.03 L	83
58	64	2.43	1.30	159 H
59	30 L	2.32	1.21 L	71
60	22 L	2.19	1.01 L	103
61	47	2.42	1.29	79

Health Sciences Centre

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Telephone # (204) 787-1534

Appendix 7.3

RESEARCH PROJECTS
LABORATORY TEST REQUISITION
FOR CLINICAL CHEMISTRY AND
HEMATOLOGY
Lab Contact: Sandy Crowson
Telephone # (204) 787-4395

ACCESSIONING INFORMATION:	PATIENT INFORMATION
Project Name: Prenatal nutritional deficiency and early childhood caries in an urban aboriginal population. **Physician**: Dr. Michael Moffatt Co-ordinator: Kirsten Ryan Mailing Address: CE208 – 840 Sherbrook Telephone #: 2441 HSC Account #: U of M 385-2339-03 Project #: RI01:158 **RCOD/Loc: MOFP1371** **Patient Type: X**	HSC #: **Name**: **DOB:** Dr.: Male ☐ Female ☐ **COLLECTION INFORMATION:** **Date**: **Time**:

TESTS REQUIRED	SPECIMEN REQUIRED
(phlebotomy) R8 ☐ yes ☐ no D25, Ca, Phos, ALP	One red-top tube (if transport to lab will be delayed store D25 frozen)

LAB ACCESSIONING INSTRUCTIONS:

1. Accession tests and/or profiles as listed above.

R:\Office\Research\Forms\Requ-New.DOC

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Chapter 8 – Significant Findings, Implications, and Future Research Directions

8.1 Significant Findings

An essential regulator of tissue calcification, vitamin D is both synthesized endogenously and obtained through exogenous sources. A systematic review determined that maternal serum concentrations of 25(OH) are linked to several factors, although the weight of evidence varies, with the most strongly associated factors being vitamin D supplementation, sun exposure and season, and ethnicity. This review also determined that concentrations of 25(OH) do not appear to change significantly during pregnancy. There was also some evidence that maternal levels are associated with religious practice, diet, and geography. Little or no evidence existed to support associations with socioeconomic status (SES), maternal age, antenatal care, gravid history, and number of fetuses.

Another systematic review found that deficient concentrations of 25(OH)D (<35nmol/L) during pregnancy have been documented for many different populations around the globe, including mothers from northern Manitoba First Nations communities [1], while insufficiencies (< 40 nmol/L) may also occur in the general adult population [2].

Prenatal 25(OH)D deficiencies may predispose rickets, hypocalcemia, and delayed fetal growth. Dental tissues are also at risk, resulting in disrupted amelogenesis (i.e. enamel defects), and delayed tooth eruption. Enamel hypoplasia in primary teeth has particular significance, as it is a risk factor for development of early childhood caries (ECC) [3]. This is a most serious health problem in northern communities, where vitamin D deficiencies are also prevalent. To effectively deal with the problem of rickets

and its sequelae, modified Stosstherapy has been implemented for a few northern communities, whereby high dose vitamin D supplementation is prescribed, during pregnancy and early infancy.

It was hypothesized that this modified Stosstherapy might reduce the incidence of enamel hypoplasia and ECC, although a study in a northern Manitoba community was unable to support this hypothesis (discussed in Chapter 6), although no supplementation was associated with later eruption times. 50% of the children had enamel hypoplasia, the mean deft was 13.7 ± 3.2, and many had recognized risk factors for ECC including the addition of sugar to bottles and infrequent brushing.

A prospective study has been designed to investigate the relationship between prenatal 25(OH)D levels and the incidence of enamel hypoplasia of the primary maxillary incisors, teeth commonly affected by ECC. Preliminary data from this longitudinal study has revealed that nearly 25% of participants were 25(OH)D insufficient during pregnancy (< 40 nmol/L), and 20% were 25(OH)D deficient (< 35 nmol/L). However, the mean 25(OH)D concentration was 55.69 ± 22.61 nmol/L, close to the low normal value [4], indicative of hypovitaminosis D. Seasonal variation in 25(OH)D concentrations was also observed, with autumn values being significantly lower than those in the summer.

Participants displayed limited knowledge of vitamin D and only 65.2% were taking daily prenatal vitamin supplements. Awareness of ECC was also common, with 30% believing this pediatric disease was a normal childhood phenomenon. The majority of participants were single, earning less than $18,000 per annum, and had a grade 9 education. This stimulated conclusions that poverty and a lack of education may be significant influences in nutritional deficiencies, enamel hypoplasia, and ECC.

8.2 Implications

Many variables associated with maternal concentrations of 25(OH)D have serious implications for both mothers and developing fetuses in states of deficiency. An increased awareness of associations could help health professionals identify those at greatest risk for deficiencies. Influencing factors could then be highlighted and reviewed with expectant mothers to establish their vitamin D profiles, since 25(OH)D assays are prohibitive and not routine in remote Aboriginal communities.

Maternal 25(OH)D is affected by season, yet some urban Aboriginal women's 25(OH)D concentrations are deficient during summer months, indicating both a lack of endogenous synthesis and exogenous intake. As mean concentrations decline during fall and winter periods, dietary intakes may not be sufficient to maintain normal serum concentrations. Finances, dietary habits, availability of nutritious foods, and education are known to influence dietary intake, and may be responsible for these deficiencies [5]. In addition, it is likely that more women will become vitamin D deficient as seasons change, since their low SES and dietary habits will likely not enhance 25(OH)D levels. Considering that the majority of women indicated an annual income of below $18,000, the inability to purchase nutritious foods is a reality.

In fact, vitamin D insufficiencies may not be just unique to urban Aboriginal women during pregnancy, as they may be prevalent in other low-income populations [6]. It is likely that others of low SES, living in this geographic region of limited endogenous synthesis may also have vitamin D insufficiencies. This may also be true for immigrants whose cultural practices limit outdoor activity, ethnic dress reduces sun exposure, or diet is low in vitamin D. Along with nutritional deficiencies, both enamel hypoplasia and

ECC are more prevalent in such SES groups [7]. The quality of life and lifestyles of the growing urban Aboriginal population must therefore change if gains are to be made in improving the vitamin D status of mothers and their children to reduce the prevalence of ECC.

Vitamin D deficient mothers place both themselves and their fetuses at risk, since the impact on the intrauterine environment has tremendous implications for fetal development. Enhancing prenatal concentrations of 25(OH)D can ensure the optimal calcification of fetal tissues and child health. As this is the most crucial period of development affecting the health of children, it must be the prime focus of prevention interventions.

Obtaining sufficient serum concentrations of this metabolite may yield the most benefit in preventing a host of diseases and associated morbidities. The possibility of improving 25(OH)D should be explored as an overall preventive practice for improving health. Considering that associations have been proposed with hypertension, schizophrenia, certain cancers, autoimmune disorders such as multiple sclerosis (MS) and Type 1 diabetes, the need to improve our understanding of 25(OH)D, much of this mounting evidence is currently unnoticed by health care providers [8].

Vitamin D deficiency among urban residents may be common [2] and often neglected as an aspect of overall health. Limited public awareness and understanding by health professionals, and urban lifestyle may contribute to an increasing health burden of this population. Inadequate vitamin D also impacts on immunity, decreasing host resistance to combat infections [9]. This may conceivably decrease host resistance to the cariogenic microorganisms associated with ECC.

Improving serum concentrations through increased sun exposure may be a challenge considering the lobby to prevent skin cancer, and the need to modify cultural practices. Therefore, it is not the most appropriate preventive strategy. Dietary supplementation may be the simplest and most tolerable means to improving 25(OH)D, although compliance may reduce the effectiveness of daily supplementation. The use of high dose supplements at key intervals during pregnancy and adult life may therefore be warranted.

Vitamin D may yet become a preventive strategy for ECC, since vitamin D deficiencies can predispose enamel hypoplasia and reduce immunity to infections of cariogenic bacteria [9-11], both reducing host resistance. The American Academy of Pediatric Dentistry (AAPD) (2002) recommends that an enhanced prenatal diet is one strategy to prevent ECC, as enamel hypoplasia contributes to the progression of this disease. This in addition to early screening of children during the first year of age may help to minimize the severity and development of ECC [3]. Improving the maternal nutritional status may have the greatest potential to improve the intrauterine environment, and is an appropriate time for parental education on ECC prevention. Supplementation during pregnancy may eventually become the preferred method of ensuring vitamin D sufficiency, in addition to serving as a preventive strategy for ECC.

8.3 Future Research Directions

Studies of expectant mothers of different ethnic backgrounds residing in Manitoba may be necessary, considering the multitude of health conditions ascribed to deficiencies of 25(OH)D. More epidemiological research is imperative to clarify such relationships. This may not only help propel vitamin D sufficiency as a future preventive strategy, but

will also assist in understanding the relationships between prenatal 25(OH)D status and such variables as SES, diet, maternal age, and antenatal care.

Such investigations will also assess the relationship between vitamin D status of mothers and enamel hypoplasia in children, and help explore the exact relationship between 25(OH)D and the development of ECC. Interventions to alter feeding modalities and curb purportedly high-risk behaviours have done little to reduce ECC. More longitudinal studies assessing such risk factors as prenatal environment factors are also required, since changes in the multifactorial etiology of ECC have had limited success in reducing the prevalence of this disease.

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