

**THE EFFECT OF VITAMIN D SUPPLEMENTATION DURING PREGNANCY AND
LACTATION ON MATERNAL & INFANT 25-HYDROXYVITAMIN D
CONCENTRATION**

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Abstract

BACKGROUND: Adequate vitamin D during pregnancy and lactation is important for optimal health of mother and infant. Due to low levels of vitamin D occurring in breast milk, exclusively breastfed infants are recommended by Health Canada to be supplemented with 400 IU/day of vitamin D. A potential solution to this is maternal vitamin D supplementation during pregnancy and lactation to raise maternal, infant serum 25-hydroxyvitamin D (25OHD) and breast milk vitamin D content.

OBJECTIVE: To determine the effect of three dose regimens of supplemental vitamin D (400 IU/d, 1000 IU/d and 2000 IU/d) during pregnancy and lactation on maternal and infant 25-hydroxyvitamin D concentrations at 8 weeks post-partum.

METHODS: In a double-blind, randomized controlled trial healthy pregnant women ($n=226$) between 13-24 weeks of gestation were recruited from Vancouver, Canada and randomized to take one of three doses of supplemental vitamin D₃ (400 IU/d, 1000 IU/d or 2000 IU/d) until 8 weeks postpartum. Maternal blood was collected at baseline, 36 weeks gestation, and maternal and infant blood were collected 8 weeks postpartum.

RESULTS: Mean 25OHD was 66 nmol/L at baseline and 21% of participants had a 25OHD < 50 nmol/L. At 8 weeks postpartum, maternal serum 25OHD concentrations [mean (95% CI)] were highest in the 2000 IU/d [87 (83, 90) nmol/L] followed by the 1000 IU/d [78 (74,81) nmol/L] and the 400 IU/d [69 (66, 73) nmol/L] group using intent to treat analysis. Likewise, at 8 weeks serum 25OHD concentrations were highest in infants whose mothers received 2000 IU/d [75 (67, 83 nmol/L)] followed by the 1000 IU/d [52 (45, 58) nmol/L] and the 400 IU/d [45 (38,52) nmol/L].

CONCLUSION: Maternal vitamin D supplementation of 2000 IU/d during pregnancy and lactation was found to be protective against vitamin D deficiency in infants for the first two months after birth. Generally, vitamin D supplementation increased maternal and infant 25OHD concentrations in a dose response manner.

Preface

This thesis is based on work of a clinical research study conducted by myself, the candidate, Nancy Chen, under the supervision of Dr. Tim Green with guidance from Dr. Peter von Dadelszen, Dr. Sheila Innis, Dr. Hope Weiler and Dr. Susan Whiting. This study was designed by Dr. Tim Green, Dr. Sheila Innis, Dr. Antonia Shand and Dr. Peter von Dadelszen. The research presented in this thesis is part of a larger research project investigating the effects of maternal vitamin D supplementation throughout pregnancy and lactation on maternal and infant responses. Our research team included graduate students, research assistants, volunteers and myself.

Under the supervision of Dr. Green, I was responsible for and have participated in all aspects of recruitment, data collection, administration roles, coordination, sample collection and analysis, data analysis and the completion of both maternal and infant clinic visits. The data analysis and writing of this thesis were primarily my work. This study is a large randomized controlled trial with 226 participants each seen over a 9 month time period. At the time of publication of this thesis, all clinic visits for the study have been completed. Kaitlin March, a previous graduate student, focused her thesis on the data up to parturition. My thesis focuses on the baseline and final clinic visits for all 226 participants, with concentration on maternal and infant 25OHD outcomes at 8 weeks postpartum. Additional sample analysis from this study including breast milk and other sample analysis and data analysis is still ongoing. Sections of this thesis will be submitted for publication as a manuscript in peer reviewed journals. Ethical approval for this research study was provided by the UBC Clinical Research Ethics Board (H09-01261). This trial is also registered with the Clinical Trials Registry (NCT011128).

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List of Abbreviations

1,25OH ₂ D	1,25-dihydroxyvitamin D
25OHD	25-hydroxyvitamin D
AI	Adequate Intake
AT	As-Treated
ANOVA	Analysis of Variance
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BSAP	Bone Specific Alkaline Phosphatase
CBC	Complete Blood Cell Count
CCHS	Canadian Community Health Survey
CHMS	Canadian Health Measures Survey
CI	Confidence Interval
DBP	Vitamin D Binding Protein
DBRCT	Double-blind Randomized Controlled Trial
DRI	Dietary Reference Intakes
FFQ	Food Frequency Questionnaire
GDM	Gestational Diabetes Mellitus
IOM	Institute of Medicine
ITT	Intention to Treat
IU	International Units
NR	Not Reported
PTH	Parathyroid Hormone
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
SD	Standard Deviation
SES	Socioeconomic Status
T1DM	Type 1 Diabetes Mellitus
UL	Tolerable Upper Level of Intake
UV	Ultraviolet
VDR	Vitamin D Receptor

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Chapter 1: Literature Review

1.1 Introduction

Vitamin D, first recognized as a vitamin in the 20th century, is a fat-soluble vitamin this is best known for its role in regulating calcium and phosphorus homeostasis and bone health[2]. In recent years, more attention has been brought to vitamin D due to the discovery that most cells have vitamin D receptors and several tissues possess the enzymatic capability to convert vitamin D to its active form, calcitriol [3]. In addition to the functions of vitamin D in bone health, the potential of vitamin D in the prevention of chronic diseases and its role in autoimmune diseases, cardiovascular diseases, and infectious diseases have been of particular interest [3].

Vitamin D is unique in that it can be obtained from the diet as well as synthesized endogenously through exposure to ultraviolet B (sunlight) light. With adequate sun exposure, the human body is capable of synthesizing sufficient levels of vitamin D to achieve satisfactory vitamin D status. Factors that influence UV exposure include latitude, season, clothing, skin colour, time of day, and lifestyle factors. As a result, vitamin D deficiency can be common especially during winter months [3, 4]. When there is insufficient exposure to sunlight, one can improve their vitamin D status by taking supplements or through the consumption of foods rich in vitamin D. Natural food sources of vitamin D are uncommon, with major sources limited to fatty fish and organ meats [5]. Currently, Health Canada and the Institute of Medicine recommend a vitamin D intake of 600 IU/d for all adults from 19-70 years of age, including pregnant and lactating women [2]. In contrast, the Canadian Paediatric Society recommends 2000 IU/d for pregnant and lactating women [6]. Experts have called for

further studies to examine the vitamin D intake needed to achieve optimal vitamin D concentration in women during pregnancy and lactation [7].

To assess vitamin D status, the circulating concentration of serum 25-hydroxyvitamin D (25OHD) is measured. Serum 25OHD is the best indicator of vitamin D status due to its longer half-life compared to the active form, 1, 25 dihydroxyvitamin D and it also represents both dietary intake and endogenous synthesis [8]. To date, concentrations for vitamin D sufficiency are still under debate. The Institute of Medicine recommends a serum 25OHD concentration of 50 nmol/L as adequate for all adults and infants based on evidence on bone health. In contrast, the Canadian Paediatric Society and the Endocrine Society have suggested that a concentration of 75 nmol/L is required for sufficiency [2, 6, 9]. Adequate vitamin D status is important for all age groups, in particular during pregnancy and lactation to ensure maternal and infant health. Inadequate vitamin D concentrations in mothers and infants can lead to poor infant bone mineralization that may persist into later life [10]. In infants, vitamin D deficiency results in skeletal deformities and growth retardation, and in its most severe form, rickets [3]. In adults, low vitamin D can lead to osteomalacia or softening of the bones and muscle weakness [3].

In Canada, women receive limited sun exposure during winter months, and it has been reported that women of childbearing age may receive less than 400 IU/d of vitamin from dietary sources and supplement intake [3, 11]. Consequently, vitamin D insufficiency is prevalent in pregnant women and women of childbearing age [12]. As maternal vitamin D status during pregnancy and lactation is related to infant vitamin D concentration, inadequate maternal vitamin D concentrations can contribute to low breast milk vitamin D content and infant vitamin D deficiency [13]. It is uncertain whether mothers and their infants may benefit

from higher than the recommended 600 IU/d of vitamin D and cut-offs for blood biomarkers in vitamin D sufficiency (i.e. 25OHD) are under debate [2, 14]. Human milk is considered the best nutrition source for infants although it is a poor source of vitamin D. As a result, vitamin D supplementation of 400 IU/d is recommended for breast-fed infants [15]. In addition, there have been concerns with infant vitamin D supplementation including the potential for infant overdose [13, 16]. Fortunately, over 80% of pregnant women already take a prenatal supplement, and maternal vitamin D supplementation has been shown to improve both mother and infant vitamin D status [12, 13]. Currently, there are gaps in evidence regarding adequate maternal vitamin D supplementation and serum 25OHD concentrations and its relation to infant 25OHD concentrations. Given the limited experimental evidence that has examined vitamin D supplementation throughout pregnancy and lactation, the objectives of my research are to:

1. To determine the effect of 3 dosages of supplemental vitamin D starting at 13-24 weeks of pregnancy on maternal serum 25-hydroxyvitamin D at 8 weeks postpartum.
2. To determine the effect of 3 dosages of supplemental vitamin D starting at 13-24 weeks of pregnancy on infant serum 25-hydroxyvitamin D at 8 weeks postpartum.

The first sections of this literature review summarize vitamin D metabolism and physiological functions, assessment of vitamin D status, and vitamin D deficiency and sufficiency. These are followed by a discussion on the consequences of vitamin D deficiency for both mother and infant as well as current recommendations for vitamin D supplementation for pregnant Canadian women and their infants. Possible problems associated with infant supplementation are addressed and potential solutions are examined.

Furthermore, maternal supplementation and its impact on maternal serum 25OHD concentrations and infant outcomes are summarized and reviewed.

1.2 Vitamin D Metabolism and Physiological functions

Vitamin D is a prohormone that can be obtained through the diet, dietary supplements, or produced endogenously in the skin as a result of ultraviolet blue (UVB) radiation [3]. UVB wavelengths between 290-315nm penetrate the skin and convert 7-dehydrocholesterol to previtamin D, which is then slowly isomerized to cholecalciferol (vitamin D₃) [1, 3]. Vitamin D also exists in a second form called ergocalciferol (vitamin D₂), derived from exposing plant sources such as fungi and yeast to UVB radiation [17]. Vitamin D₂ cannot be produced endogenously but is found in fortified foods and supplements. It differs from vitamin D₃ in its molecular structure with an extra double bond and methyl group and is less bioavailable compared to vitamin D₃ [17].

Vitamin D from both endogenous and exogenous sources is converted to the major circulating form of vitamin D, 25-hydroxyvitamin D (25OHD), in the liver by vitamin D-25-hydroxylase (cytochrome P-450 enzymes) [1, 3]. 25OHD must go through one final hydroxylation by the enzyme 25-hydroxyvitamin D-1 α -hydroxylase (Cyp27b1) to become the metabolically active form 1,25-dihydroxyvitamin D (1,25(OH)₂D), also known as calcitriol [3, 18]. This occurs mainly in the kidneys, but other tissues also possess 25-hydroxyvitamin D-1 α -hydroxylase activity [19]. As a fat-soluble molecule, vitamin D metabolites are transported in the circulation by binding to carrier proteins called vitamin D binding proteins (DBP), which have the highest affinity for 25OHD followed by 1,25(OH)₂D and then vitamin D [20].

The majority of biological activities exerted by $1,25(\text{OH})_2\text{D}$ require binding to a high-affinity receptor known as the vitamin D receptor (VDR) [19]. VDRs can regulate the transcription of a number of target genes involved in calcium homeostasis and cell differentiation [21]. The main physiological functions of vitamin D involve calcium homeostasis. Vitamin D functions to increase serum calcium concentrations by stimulating active intestinal absorption of calcium and phosphate, and osteoblasts leading to bone resorption [1]. This allows calcium to be mobilized from the bone when it is absent or low from the diet [1]. Calcium-sensing proteins in the parathyroid gland stimulate secretion of parathyroid hormones (PTH), which in turn elevate 1α -hydroxylase leading to synthesis of $1,25(\text{OH})_2\text{D}$ which stimulates intestinal absorption of calcium, renal reabsorption of calcium, as well as mobilization of calcium from bone [1].

The presence of $1,25(\text{OH})_2\text{D}$ stimulates osteoclast activity and increases the efficiency in which intestinal calcium and phosphorus is absorbed [3, 19]. 1α -hydroxylase is also inhibited by $1,25(\text{OH})_2\text{D}$, fibroblast growth factor-23 (FGF23) and high calcium or phosphorus [22]. PTH levels are the main determinant of $1,25(\text{OH})_2\text{D}$ levels and along with serum calcium and phosphorus concentrations help to tightly regulate the renal production of $1,25(\text{OH})_2\text{D}$ [3].

Roles of vitamin D beyond calcium homeostasis have been suggested due to the presence of 1α -hydroxylase in breast, prostate, colon, lung, pancreatic- β cells, parathyroid and monocytes cells and tissues [19, 23]. These non-calcitropic actions of vitamin D have been primarily attributed to the extra-renal expression of 1α -hydroxylase, which acts to increase $1,25(\text{OH})_2\text{D}$ concentration [23]. Autocrine and paracrine actions of $1,25(\text{OH})_2\text{D}$ in

cell proliferation, cell differentiation and immune regulation have also been described [23]. In addition, the presence of VDRs in parathyroid gland cells, skin keratinocytes, promyelocytes, lymphocytes, colon cells, pituitary gland cells and ovarian cells suggest that vitamin D may have additional roles in these cells [24].

1.2.1 Vitamin D Metabolism and Physiological Changes in Pregnancy and Lactation

During pregnancy and lactation, calcium metabolism changes significantly to accommodate the calcium demands for fetal bone accretion, breast milk synthesis, and restoration of maternal bone stores [25]. During pregnancy, approximately 25-30 g of calcium is transferred to the fetus with the majority of the transfer taking place in the third trimester at an estimated rate of 250 mg/d [25]. Maternal 1,25(OH)₂D circulating concentrations increase from 50 to over 100% during pregnancy along with increases in DBP while PTH levels remains relatively constant [25]. Synthesis of 1,25(OH)₂D increases due to the acceleration of 1 α -hydroxylase in the kidneys of the mother and placenta [26]. Moreover, markers of bone turnover such as bone alkaline phosphatase (BAP) have been shown to increase in expression during pregnancy. Maternal intestinal calcium absorption is also increased significantly, serving as a physiological adaptation to ensure that the fetus receives adequate vitamin D [25]. According to a recent review by Kovacs, although 25OHD crosses the placenta freely during pregnancy, the fetus does not deplete maternal 25OHD stores [18]. Furthermore, concentrations of maternal 25OHD are higher than fetal concentrations, allowing for 25OHD to cross the placenta as the major D source for the fetus. This transfer explains why cord blood 25OHD levels are approximately 75% (range 50-100%) of maternal levels [22]. 1,25(OH)₂D does not cross the placenta; however, the placenta and fetal tissues

are able to synthesize 1,25(OH)₂D directly to contribute towards infant circulating concentrations of 1,25(OH)₂D [18, 27].

During lactation, vitamin D can pass readily into breast milk while only limited amounts of 25OHD and no 1,25(OH)₂D can pass through to breast milk [28]. There are also no increases in circulating 1,24(OH)₂D concentrations or changes in intestinal calcium absorption compared to non-lactating women. During this period, 200-240 mg of calcium is secreted into the breast milk per day [29]. Bone turnover increases with a possible net loss of 5-10% of bone mineral content during the 3-6 months of postpartum [22]. Despite the loss of mineral content, the maternal skeleton restores the depleted mineral content within 3-12 months post-weaning. Therefore, the skeleton is not weakened in the long term by the temporary skeletal resorption of calcium during lactation [18]. Some studies have found decreased renal calcium excretion in women during lactation although there have been mixed results [25]. Reproductive hormones such as prolactin, placental lactogen, and estrogen also play a role in calcium homeostasis and bone metabolism during this time to ensure that calcium needs are met for both mother and infant [25, 30].

1.3 Assessment of Vitamin D Status

Vitamin D status can be assessed using several biomarkers including PTH, 1,25(OH)₂D and 25OHD concentrations. Serum PTH concentrations are increased when 25OHD concentrations are decreased. However, it is estimated that PTH concentrations plateau when 25OHD reaches a threshold of approximately 61 nmol/L [31]. Furthermore, PTH concentrations are also affected by calcium absorption, which can be influenced by multiple factors. Additional variables such as ethnicity, life stage, calcium and phosphorus intake, and

physical activity can also influence PTH concentrations making it a poor indicator of vitamin D status [32].

An alternate biomarker of vitamin D status, serum 1,25(OH)₂D concentrations is under tight regulation by PTH, serum calcium and phosphorus, which allows for it to remain normal in mild vitamin D deficiency. The half-life of 1,25(OH)₂D is 15 hours compared to the half-life of 25OHD which is 15 days [21]. Both the insensitivity of 1,25(OH)₂D to mild vitamin D deficiency and its short half-life, make 1,25(OH)₂D a poor indicator of vitamin D status.

In contrast, the hydroxylation of vitamin D to 25OHD is poorly regulated, thus allowing for its concentration to fluctuate with vitamin D intake and endogenous vitamin D production, making it a better predictor of vitamin D status [33]. Consequently, serum 25OHD concentration is considered the best indicator of vitamin D status.

1.3.1 Defining Vitamin D Deficiency and Insufficiency

There remains debate over what concentration of serum 25OHD is optimal for all health outcomes. The Institute Of Medicine, which sets dietary recommendations for Canadians and Americans, indicated that 25OHD concentrations ≥ 50 nmol/L is sufficient for all people based on bone outcomes, and concentrations between 30 – 50 nmol/L are considered inadequate. The IOM committee thought that there was insufficient evidence to base recommendations on non-bone outcomes. Some organizations and experts have called for much higher cutoffs for 25OHD, up to 100 nmol/L, for non-bone health outcomes [3]. According to the Endocrine Society, a circulating concentration of 25OHD ≥ 75 nmol/L is recommended for satisfactory development of pregnancy and fetus [34]. In addition, although 25OHD concentrations increase with vitamin D intake, this relationship is not

entirely linear and the extent to which 25OHD concentrations reflect health outcomes is not well known. It is suspected that that depending on the physiological measures used, the optimal serum concentration of 25OHD will vary at each life stage [2]. For the purposes of our study we will be examining two cutoffs, 50 and 75 nmol/L.

1.3.2 Vitamin D Deficiency

Vitamin D deficiency can result from dietary inadequacy, limited exposure to sunlight, or absorption and renal metabolic issues. Severe vitamin D deficiency can result in rickets in children, a condition characterized by soft bones and skeletal deformities and osteomalacia in adults, a condition resulting in soft and weak bones. Insufficient vitamin D may also contribute to the development of osteoporosis by limiting calcium absorption [2].

In vitamin D deficient conditions, only 10-15% of dietary calcium, and 60% of phosphorus is absorbed, compared to an intestinal calcium absorption from of 30 to 40% and phosphorus absorption of approximately 80% with adequate 1,25 dihydroxyvitamin D and vitamin D receptor interactions [1, 3, 35] Dietary sources of calcium are favored to balance calcium concentrations under normal physiological conditions but when this system fails, calcium mobilization from bones is stimulated to meet systematic demands and can ultimately result in the loss of calcium from bone and increase the risk of osteoporosis [1]. The IOM has set 25OHD concentration <30 nmol/L as indicative of deficiency [2]. Individuals with rickets and osteomalacia almost always have a 25OHD concentration less than 30 nmol/ [2].

In pregnancy, associations between low 25OHD and increased risk for gestational diabetes, bacterial vaginosis, preterm birth and preterm delivery have been observed in some

but not all studies [36]. More research is needed to establish whether there is a causal association.

1.3.3 Factors Affecting 25-hydroxyvitamin D

25-hydroxyvitamin D concentrations in circulation generally reflect dietary intake, supplement use and sun exposure. UVB exposure is a major contributor to 25OHD and thus anything affecting the number of UVB photons penetrating the epidermis will influence vitamin D status [37]. These include season, latitude, climate, use of sunscreen, clothing, tanning, as well as ethnicity, and skin color. For example, the pigment melanin in darker skin individuals can decrease the skin's ability to synthesize vitamin D from UVB light [2]. In countries such as Canada with higher latitudes, there is little to no UVB light during winter months to allow for any vitamin D synthesis and the population is reliant on exogenous sources of vitamin D. Unfortunately, dietary sources of vitamin D are limited, and include organ meats, egg yolks and fatty fish. Contributors to 25OHD concentrations in Canadians include fortified milk and margarine, with 100 IU of vitamin D per 250mL and 530 IU per 100g, respectively [5]. Whiting and her colleagues examined the vitamin D status of Canadians relative to the 2011 DRIs using plasma 25OHD from a representative sample of subjects from the Canadian Community Health Survey [38]. It was found that 1 in 4 Canadians aged 6-79 years of age did not meet the IOM and Health Canada cutoffs of adequacy for vitamin D status (50 nmol/L) and this percentage increased to one third in winter months [38]. Due to the seasonal variability of sun exposure and lack of abundant dietary sources, it is especially important to obtain a consistent source of vitamin D such as vitamin D supplementation to prevent any consequences of deficiency.

1.4 Consequences of Inadequate Vitamin D during Infancy

Inadequate vitamin D status during pregnancy and lactation can lead to inadequate vitamin D status of neonates as they depend solely on their mothers for vitamin D [7]. Inadequate vitamin D concentration in neonates results from low maternal vitamin D concentration during pregnancy, exclusive breastfeeding without infant vitamin D supplementation, and/or a lack of vitamin D intake after birth from breast milk and/or formula [39, 40]. During gestation, approximately 30 g of calcium is transferred to the fetus via the placenta [41]. This process requires adequate 1,25(OH)₂D to be synthesized from 25OHD [42]. Maintaining adequate 25OHD concentration during pregnancy is necessary for providing calcium for fetal bone mineral accretion, most of which occurs mostly during the third trimester [43]. Vitamin D deficiency in infants, synthesis of 1,25(OH)₂D is limited, and consequently only 10-15% of dietary calcium is absorbed, compared to 60-80% when the infant has adequate vitamin D [3, 40, 44]. Severe vitamin D deficiency can cause impaired bone mineralization and may result in cardiomyopathy associated with hypocalcemia [45]. In rare cases of severe maternal vitamin D deficiency, the fetus may develop rickets in utero and can manifest this at birth [46].

In particular, inadequate infant vitamin D can lead to rickets and has been associated with other negative health outcomes such as type 1 diabetes mellitus, asthma and poor dentition.

1.4.1 Rickets

Adequate vitamin D intake is necessary for healthy skeletal development in infancy. Rickets, a condition characterized by growth retardation, weak and toneless muscles, skeletal deformities that result from poor bone mineralization, is the result of severe vitamin D

deficiency [47]. Clinical manifestations are most obvious in areas of rapid bone growth, such as the costochondral junctions and long bone epiphyses leading to classic symptoms such as rachitic rosary and bowed legs, respectively [47]. Rickets is caused by the failure to mineralize newly formed unmineralized bone tissue and cartilage [48]. The condition can be divided into three stages: the first stage is characterized by decreased 25OHD, increased parathyroid hormones (PTH), osteopenia and hypocalcemia. This is followed by further rises in PTH and swelling of the collagen matrix in the second stage and severe bone changes and evident hypocalcemia in the final stage [44]. Before rickets is physically obvious, vitamin D deficiency usually results in hypocalcemic seizures, irritability, lethargy, and a higher susceptibility to respiratory infections during infancy [40]. There is also an increased demand for calcium during infancy due to increased growth velocity, further emphasizing the importance of adequate vitamin D [44].

The peak incidence of rickets typically present between 3-12 months of age in infants, with most cases observed before 18 months of age [47]. However, vitamin D deficiency occurs months before rickets is physically obvious [49]. Inadequate vitamin D status in mothers can also result in lower bone mineral content and rickets in infants [50]. Since body calcium accretion is highest from 28 weeks in utero to birth when fetal growth is the most rapid, deficiency at birth and early infancy is typically attributable to inadequate maternal vitamin D status [48]. This is supported by evidence demonstrating the strong association between maternal and fetal circulating 25OHD [3].

In the 1900s, autopsy studies conducted in Boston and The Netherlands found that 80-90% of children had rickets [47]. The epidemic of rickets was eradicated with the introduction of foods and oils with antirachitic activity and encouragement of adequate sun

exposure [47]. In recent years, rickets has begun to resurface and has been recognized as a continuing global health concern through a series of cross sectional and retrospective studies [51-53]. A study looking at cases of rickets in medical centers of North Carolina found that African American infants who were breastfed and not given a vitamin D supplement had a higher incidence of rickets [54]. Data from the Canadian Pediatric Surveillance Program suggests that vitamin D deficiency rickets is still a continuing problem among infants and children in Canada [39]. A survey from 2325 pediatricians over the span of two years (2002-2004) examined the incidence of vitamin D-deficiency rickets and found the annual incidence rate to be 2.9 in 100,000 (95% CI: 2.2-3.7) children [39]. This is probably an underestimate because rickets is not a reportable condition and milder forms of rickets may go undetected. Children with rickets in Canada are almost all exclusively breastfed infants born to mothers deficient in vitamin D and were not supplemented after birth [39]. This rise in vitamin D deficiency and rickets is associated with the promotion of exclusive breastfeeding without vitamin D supplementation, especially in infants whose mothers have inadequate vitamin D levels, and decreased sun exposure in infants and immigrant groups in more temperate regions [40, 48]. Breast milk is generally a poor source of vitamin D and children under 1 year are recommended be kept out of the sun. Therefore, Health Canada recommends that exclusively breastfed infants be supplemented with 400 IU/d vitamin D [15].

1.4.2 Other

Low vitamin in infancy has been associated with a number of conditions later on in life including type 1 diabetes mellitus, poor dentition, and asthma.

1.4.2.1 Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus (T1DM) is a disease that results from the autoimmune destruction of insulin producing β -cells in the pancreatic islets [55]. Vitamin D has been linked to the prevention of T1DM as it is suggested to have a protective effect against autoimmune diseases by enhancing immunity [56]. Early in the first trimester, circulating maternal concentrations of $1,25(\text{OH})_2\text{D}$ rises, and doubles by the end of the third trimester [57]. The early rise in $1,25(\text{OH})_2\text{D}$ is thought to be necessary for immunological adaption by the mother, which is needed for the maintenance of a normal pregnancy and thus increasing the likelihood of a healthy infant [57]. In addition, factors with a positive influence on vitamin D status, such as warmer seasons and positive latitude gradient, have also been associated with a lower incidence of T1DM [58, 59].

Several studies have looked into the effects of vitamin D supplementation on Type 1 Diabetes prevention. A birth-cohort study followed 10821 Finnish infants born in 1966 until December 1997 and reported their diagnosis of T1DM [60]. Vitamin D intake and incidence of rickets was recorded at one year of age and subjects were followed until diagnoses of diabetes (n=81), emigration (n=565), death (n=215), or until the end of the study [60]. Children who were given 2000 IU of vitamin D per day was found to have a rate ratio of 0.12 (95% CI: 0.05-0.89) compared to those who did not take a supplement; in other words, intake of a vitamin D supplement was associated with a 88% decreased risk of type 1 diabetes [60]. However, very few women did not supplement their infants with vitamin D (n=32) and two cases of T1DM were found among these infants. It is reasonable to expect that this group may differ from other women in the study in ways other than the fact they supplemented their infants.

Another study in Norway examined the link between low maternal 25OHD levels and increased incidence of T1DM in the offspring in 29072 women [56]. Of these, 109 serum samples were available from subjects whose children developed T1DM before 15 years of age versus 219 serum samples from control subjects. Compared to children who did not have T1DM, those who were diagnosed with T1DM during childhood were born to mothers with lower 25OHD concentrations during pregnancy (66 vs.73 nmol/L, $p=0.021$) [56]. Although the results from both studies show an association between 25OHD levels and T1DM, the studies were observational in design and do not show cause and effect. Additional longitudinal studies conducted in the US and Finland have shown conflicting results [61, 62].

According to a meta-analysis conducted by Zipitis and Akobeng on vitamin D supplementation in infancy and incidence of T1DM later in life, infants who were supplemented with vitamin D were found to have a reduced risk of diabetes compared to those who were not (pooled odds ratio 0.71, 95% CI: 0.60 to 0.84) [63]. It was suggested that factors such as timing and dose of supplementation may play a role as well. The results were drawn from observational studies, and further randomized control trials involving supplementation are needed to determine whether there is a causal effect in addition to the optimal dosage and duration of supplementation.

1.4.2.2 Poor Dentition

Inadequate vitamin D levels during pregnancy and infancy has been linked to poor dentition in infants. Pregnant women (n=1139) in Edinburgh were divided into two groups and were given a 400 IU/d vitamin D supplement or a placebo [64]. At three years of age, 61 infants (31 control group, 30 treatment group) had their teeth examined by a pediatric dentist;

of those with an enamel defect, 15 were from the control group, and only 2 were from the treatment group ($p < 0.001$) [64].

More recently, a prospective study conducted at the University of Manitoba assessed 25OHD levels in 207 pregnant women (89.8% of Aboriginal heritage) and risk factors for Early Childhood Caries in their toddlers (16.1 ± 7.4 mos.). Mothers of infants with dental caries were found to have significantly lower 25OHD concentrations during their 2nd trimester than those with infants who did not have dental caries (41 ± 20 vs. 52 ± 27 nmol/L, $p = 0.045$) [65]. Overall, inadequate vitamin D appears to have some association with dental caries and poor dentition later in life, but again there is a lack of high quality evidence such as randomized control trials [65].

1.4.2.3 Asthma

Vitamin D is thought to be important in the development of the fetal immune system and functions of the lungs [66]. Several studies have explored the link between vitamin D deficiency and asthma. Vitamin D signaling pathways may affect smooth muscle contraction, airway inflammation, prostaglandin regulation, and airway remodeling [30, 67, 68]. In a cross-sectional study, the associations between maternal asthma, infant respiratory infection severity, and maternal 25OHD were examined in 340 mother-infant pairs [69]. Overall, the study population consisted of 70% Caucasian mothers, 19% African American mothers the incidence of asthma among this group was 21% [69]. In the Caucasian women group, a 35 nmol/L increase in 25OHD concentration was associated with a decreased risk of bronchiolitis in their infants (adjusted odds ratio, 0.54; 95% CI, 0.33-0.86). Maternal vitamin D concentrations were not associated with bronchitis in their infants [69].

Similar results were observed from the New Zealand Asthma and Allergy Cohort Study. This prospective birth cohort study examined cord blood 25OHD levels and early life risk of respiratory infection, wheezing, and asthma in 1105 infants [70]. Out of the 922 newborn cord blood samples tested for 25OHD, the median 25OHD concentration was 44 nmol/L (interquartile range: 29-78) [70]. Follow-up with the infants found that 25OHD levels were inversely associated with risk of respiratory infection at 3 months of age and risk of wheezing at 15 mos, 3 years and 5 years of age ($p < 0.05$) [70]. No association was found between 25OHD levels and asthma by 5 years of age [70].

More recently, Bener et al compared serum vitamin D concentrations in asthmatic (n=483) and non-asthmatic (n=483) children in Qatar (n=966) [71]. Asthmatic children were found to have significantly lower serum vitamin D concentrations compared to non-asthmatic

children ($p < 0.001$). In this population 68.1% of all children diagnosed with asthma were classified as vitamin D deficient (serum 25OHD < 50 nmol/L) compared with what in the children who did not have asthma [71]. Results of the study should be regarded with caution as children entering the study already presented the disease condition and other predictors of vitamin D status such as sun exposure may be a result of the disease condition and not a predictor of asthma.

In a recent review by Gupta et al of vitamin D and asthma in children, evidence has shown associations between vitamin D and immunity and lung health, specifically the anti-inflammatory role in asthma [72]. Intervention trials are suggested to examine the effect of vitamin D supplementation on asthma control and exacerbations [72]. To date, most studies characterizing asthma and vitamin D are observational and further clinical human trials are needed before any recommendations can be made.

1.5 Current Recommendations

Due to the risk of rickets and other adverse outcomes associated with vitamin D deficiency, it is important to maintain adequate vitamin D concentrations during infancy. To achieve this, Health Canada recommends a vitamin D intake as Dietary Reference Intakes (DRIs). The DRIs are a nutrient reference intake based on the intakes of healthy individuals in the population established by a group of experts in Canada and US, a process that is undertaken by the Institute of Medicine (IOM) of the National Academies, a non-governmental body in the US [14]. Due to conflicting messages regarding vitamin D recommendations, in 2010 the IOM released an updated report for DRIs on vitamin D and calcium based on recent data on health outcomes associated with calcium and vitamin D [2]. The review of over a thousand studies examined the association of vitamin D with outcomes

including cardiovascular disease, diabetes, metabolic syndrome, cancer, immune response, preeclampsia, and neuropsychological functions with mixed and inconclusive results. The DRIs were established based on the strong body of evidence supporting the role of vitamin D in promoting bone health. Currently, the IOM and Health Canada sets the Recommended Dietary Allowance (RDA) at 600 IU/day for all ages and genders up to 70 years of age [2, 14]. This includes all pregnant and lactating women. It should be noted that prior to 2010, there was insufficient evidence to establish the Estimated Average Requirement (EAR) to set the RDA for vitamin D recommendations. An Adequate Intake (AI) based on approximations of the nutrient intake of a group of healthy individuals was used instead [73]. The AI for vitamin D was set at 200 IU/day for everyone under 70 years of age before the update [73].

1.5.1 Recommendations and Vitamin D Status in Infants and Mothers

The current recommendations for vitamin D intake differ between various organizations and government agencies. Based on the vitamin D DRIs published by the IOM, infants from 0-12 months of age are recommended to take 400 IU of vitamin D per day while women during pregnancy have a RDA of 600 IU per day [2]. These recommendations are used by Health Canada and the American Academy of Pediatrics (AAP) [40, 74]. For higher-risk infants in winter months, the Canadian Paediatric Society recommends an oral vitamin D supplement of 800 IU daily [75].

Furthermore, the American Academy of Pediatrics advises infants under 6 months of age to avoid direct sunlight [76]. At this life stage, infants have low levels of melanin in their skin, putting them at increased risk for sunburns [76]. Due to the risk of skin cancer, Health Canada and the Canadian Dermatology Association recommends that infants under one year be kept out of direct sunlight [77]. For infants over six months who are exposed to sunlight,

Health Canada recommends the use of sunscreen [77]. With a lack of sun exposure, vitamin D status of infants is thus dependent on in utero accumulation and dietary intake.

Breast milk generally has low vitamin D content (1-10 IU/250 mL), thus breastfed infants who are not exposed to sunlight are not likely to acquire adequate amounts of vitamin D from breast milk alone [78, 79]. Consequently, it is recommended that both breastfed and partially breastfed infants be supplemented with 400 IU/day of vitamin D beginning at birth until one year of age or when the infant's diet supplies at least 400 IU of vitamin D [15, 40]. However, it is debatable how well these guidelines are followed. According to the Canadian Health Measures Survey of data collected in 2002, 53% of breastfed infants were supplemented with vitamin D drops, though the quantity and frequency was not specified [80]. Moreover, the tolerable upper intake level (UL) for infants of 1000 IU/d for infants 0-6 months was based on a no-observed-adverse-effect level of 1800 IU/d [81].

1.5.2 Current Vitamin D Status of Infants

With the potential for inadequate vitamin D intake and decreased sun exposure, many infants are at risk for rickets [40]. This is supported by results from the surveillance program launched by Canadian Pediatric Society in 2002 to study the incidence of vitamin D deficiency rickets in Canadian children [15]. Of the 69 cases reported in the first 18 months, 85% were from breastfed babies, and 86% had not received vitamin D supplementation [15]. Furthermore, the authors in the 2001-2006 National Health and Nutrition Examination Survey (NHANES) estimated that approximately 320 000 US children between 1-11 years of age have 25OHD levels less than 25 nmol/L (95% CI: 220 000-430 000) [82]. A recent study by Crocker et al examined the rate of supplementation in 2 months old infants in Vancouver

and Richmond, British Columbia [83]. Of the 577 mothers who participated in the survey, 90% breastfed their infants at 2 months, 57% exclusively. 61% of infants had a vitamin D intake between 300-500 IU/day [83]. However, this population has a unique ethnic mix and the data does not represent the rest of Canada.

Although giving infants a vitamin D supplement of 400 IU/day has been shown to achieve a target 25OHD concentration of over 50 nmol/L, a cross-sectional survey among 365 healthy infants and toddlers (age 8-24 months) in Boston found that 12% still had 25OHD below 50 nmol/L [36, 84]. Green et al measured serum 25OHD in Asian and Caucasian mother-infant (2-4 months of age) pairs in the Vancouver area [85]. They found that of the 58% of infants who were given a vitamin D supplement after birth, mean 25OHD was 23.5 nmol/L higher in infants who were supplemented with ≥ 400 IU of vitamin D compared to those who received less or no supplementation ($p=0.003$) [85]. However, this study was limited in its sample size ($n=65$) [85].

More recently, Gallo et al conducted a double-blind RCT in 132 one month old healthy breastfed infants from Montreal. They found plasma 25OHD concentrations of 75 nmol/L or greater in 97.5% of infants at 3 months [83]. In this study, infants were randomized to one of four doses of vitamin D supplement (400 IU/d, 800 IU/d, 1200 IU/d, or 1600 IU/d) for 11 months [83]. At 3 months, 55% (95% CI: 38, 72) of infants in the 400 IU/d group, 81% (95% CI: 65, 91) in the 800 IU/d group, 92% (95% CI: 77, 98) in the 1200 IU/d group, and 100% in the 1600 IU/d group achieved a plasma 25OHD of greater than 75 nmol/L [83]. However, this concentration was not sustained at 12 months in any of the treatment groups [83]. More prospective studies examining current supplementation practices of Canadian infants may be needed to gain a better perspective for future recommendations. **Table 1.2** and **Table 1.3**

show evidence from earlier studies as well as the more recent Gallo et al study on infant supplementation during lactation on infant vitamin D status.

1.5.3 Current Vitamin D Status of Canadian Women

As infant vitamin D status is influenced by maternal vitamin D status, I will review recent studies investigating the vitamin D status of pregnant women in Canada. The most recent Canadian population wide data on 25OHD values of women of reproductive age is from the Canadian Health Measures Survey 2007-2009 [36]. In women aged 20-39 years of age, the mean 25OHD levels were found to be 69.5 nmol/L with a 5th-95th percentile range of 31.0-121.1 nmol/L [36]. Whiting and her colleagues reviewed the vitamin D status of Canadians and found that in winter months, significant differences occurred between plasma 25OHD in women between 20-39 years of age who did take a supplement vs. those who did not (mean 25OHD 70.3 nmol/L vs. 55.7 nmol/L) respectively [38].

In a study examining the vitamin D status of pregnant women in Vancouver, Li et al reported that despite taking prenatal supplements containing vitamin D, a quarter of the women (24%) had 25OHD levels below 50 nmol/L [12]. Vitamin D deficiency is even more common in more vulnerable populations. A cross-sectional study in Boston assessed plasma 25-hydroxyvitamin D in 40 healthy mother-infant pairs from a high risk population (majority black, winter season, northern latitude) and found that 50% of mothers and 65% of infants were vitamin D deficient (defined as 25OHD less than 30 nmol/L) immediately postpartum [86]. Furthermore, Weiler et al examined the vitamin D status in 50 healthy Canadian mothers and their newborn infants and found that 46% of healthy mothers and 35% of their

infants were deficient in vitamin D [87]. Vitamin D deficient infants were found to have lower whole-body bone mineral content relative to body weight [87].

The IOM has stated in their report that pregnant women do not need more vitamin D than their non-pregnant counterparts [2]. However, given the existing evidence, some debate still remains over the amount of vitamin D levels that are adequate for pregnant women to achieve sufficient levels for mothers and their infant.

1.6 Vitamin D Content of Breast Milk

Due to the many benefits of breast milk to both mother and infant, Health Canada recommends that infants be exclusively breastfed for the first six months of life. However, breast milk is typically low in vitamin D and 95% of children with vitamin-deficiency rickets have been breastfed [36]. Early studies have determined breast milk to contain approximately 50-60 IU/L of vitamin D, which would explain the high occurrence of rickets in breast fed infants [88]. Exclusively breastfed infants are fed between 6-18 times in a 24hr period, with milk consumption varying from 0 – 240 g per feed [89]. Standard infant formulas in the United States and Canada typically contain approximately 400 IU/L of vitamin D and infants usually drink up to 1 L of formula/day in the first month of life [40].

In human milk, vitamin D is present mainly as vitamin D and 25OHD since other metabolites like $1,25(\text{OH})_2\text{D}_3$ are at insufficient concentrations to measure their activity [75]. Due to the high association vitamin D binding protein has for 25OHD compared to other vitamin D metabolites, there is a fairly consistent circulating concentration of 25OHD, with minimal variation with regards to UVB exposure and dietary vitamin D intake [90]. This allows for 25OHD concentrations in breast milk to provide a consistent supply of vitamin D

for the infant. As compared to 25OHD, vitamin D₃, the parent compound, shows greater variability depending on sun exposure and dietary intake [90]. In breast milk, vitamin D₃ is at 20-30% of maternal circulating concentrations, and thus vitamin D in milk reflects maternal intake and UV exposure [90-92]. To achieve sufficient vitamin D concentrations in breast milk will require a constant source of vitamin D to be available to the lactating mother [75].

Breast milk vitamin D concentration depends on the vitamin D status of the lactating mother, and significant correlations have been found between plasma and breast milk vitamin levels [75, 90]. In lactating women, vitamin D₃ was found to be the predominant form of vitamin D in breast milk [90]. Because vitamin D₃ is rapidly converted to 25OHD in the mother, a constant source would be required to maintain vitamin D levels in breast milk [75]. UVB exposure may not be a realistic option due to its variability and availability in winter months and higher latitudes [75]. A practical, safe alternative to achieve adequate levels of vitamin D in breast milk is maternal vitamin D supplementation. **Table 1.1** shows a summary of studies that have examined the average content of vitamin D in breast milk.

1.6.1 Quantifying Vitamin D in Breast Milk

Vitamin D content in breast milk was first measured successfully in the 1980s using ligand-binding analysis. Metabolites of vitamin D were analyzed in human and bovine milk using extraction and chromatographic purification procedures coupled with ligand binding assays [93]. Radioactive internal standards were added for each metabolite to trace recovery. Following extraction and chromatographic procedures, purification for vitamin D and its metabolites was conducted using high performance liquid chromatography (HPLC) before quantification by ligand binding analysis [93]. Due to the large amount of lipids present in breast milk, the difficulty of identifying vitamin D, a fat-soluble compound, is greatly

increased. Lipids can interfere with ligand binding assays and result in falsely elevated results [75]. Several methods involve the use of functional assays in the alkaline removal of lipids and lengthy chromatographic steps to separate and purify the anti-rachitic sterols using HPLC and finally ligand binding assays to determine the amount of vitamin D and 25OHD [93, 94]. However, it is difficult to determine vitamin D compounds using HPLC due to problems with sensitivity and specificity [95].

More recently, Kamao et al explored the use of liquid chromatography–tandem mass spectrometry in quantifying fat-soluble vitamins [95]. Analysis was completed using human breast milk samples from 82 lactating mothers. 10 mL of breast milk was first saponified and extracted for vitamin D [95]. Stable isotope-labeled compounds (d_7 -D₃ and d_6 -25(OH)D₃) were used as internal standards. Cookson-type reagent was used for derivatization and determination of vitamin D compounds. Vitamin D content was determined using liquid chromatography-tandem mass spectrometry in positive ion mode. The mean concentration of vitamin D and 25 hydroxyvitamin D₃ in breast milk was found to be 0.088 ng/mL and 0.081 ng/mL respectively [95]. The authors claim that this assay would be useful for large-scale studies since it allows for more specific and sensitive detection of fat-soluble vitamins [95].

1.7 Problems with Infant Supplementation

Despite the recommendations for infant vitamin D supplementation, a number of problems exist. In 2010, the Food and Drug Administration issued warnings for the potential risk of overdose with liquid vitamin D drops in infants based on reported overdoses of over the counter vitamin D supplements, and varying concentrations in liquid vitamin D supplements for infants [16, 96, 97]. For infants age 6 months and younger, the tolerable

upper intake level (UL) is set for 1000 IU of vitamin D per day [98]. Excessive amounts of vitamin D can lead to loss of appetite, excessive thirst, frequent urination, nausea and vomiting, constipation, abdominal pain, muscle weakness, muscle and joint aches, confusion, fatigue, and in rare cases, kidney damage [16].

In addition, adherence to infant vitamin D supplementation has been a concern. Data from 7266 women from the Canadian Community Health Survey indicate that amongst mothers who had breastfed for more than 1 week (~4700 women), approximately 50% had given their infants a vitamin D supplement, although the frequency or dosage was not reported [99]. According to data from the Infant Feeding Practices II, only 5.3% of breastfed infants in the US were supplemented with oral vitamin D at 1 month of age, and 9.4% and 10.3% were supplemented at 2 and 3 months, respectively [100]. Of all infants studied, including those who were both breastfed and formula fed, only 11.4% met the recommendations for 400 IU/day of vitamin D at 1 month, and 16.4% and 20.1% at 2 and 3 months, respectively [100]. The overall findings suggest that most US infants are not meeting the recommendations for vitamin D supplementation or consuming adequate amounts of vitamin D [100]. In Vancouver, Canada, rates of supplementation have been shown to be ~80% for infants at 2 months of age [83]. However, this study comprised of a unique population and the results cannot be generalized to the rest of Canada.

Breastfeeding has been described as “an unequalled way of providing ideal food for the healthy growth and development of infants” by the World Health Organization [101]. In addition to child health benefits, important health benefits for breastfeeding such as decreased risk of breast and ovarian cancer and reduced postpartum bleeding are described

for mothers [102]. However, recommendations for vitamin D supplementation may suggest breast milk to be inadequate. Some breastfeeding proponents indicate that recommending vitamin D supplementation gives the message that breast milk is not a complete source of nutrition for baby [103]. Furthermore, guidelines for supplementation may decrease maternal confidence in breastfeeding and some pediatricians are concerned that recommending vitamin D supplements would cause parents to switch to formula [103]. Furthermore, Thiele and her colleagues have stressed the importance of reducing any barriers for exclusive breastfeeding [13].

1.7.1 Adverse Outcomes of Hypervitaminosis D

Although vitamin D toxicity from supplements is not common, and usually only occurs as a result of consuming pharmacologic doses of vitamin D for a prolonged period of time [104]. Dietary sources and sun exposure are unlikely to lead to excessive vitamin D since dietary sources do not typically contain high amounts of vitamin D and physiological feedback mechanisms are available for degradation of excess vitamin D produced via UVB exposure [14]. The tolerable upper intake level (UL) is set by Health Canada at 4000 IU/day for adults and 1000 IU/day for infants under 12 months based on studies investigating the highest intake with no adverse effects [14, 15]. Vitamin D intoxication can lead to hypercalciuria, a urinary calcium excretion that exceeds 6.2 mmol/L over a 24 hr period and in rare cases can cause hypercalcemia, a serum calcium concentration > 2.5 mmol/L [2]. Hypercalciuria and hypercalcemia results in weakness, muscle twitches, dementia, renal consequences and may be fatal if untreated [2]. Intoxication of vitamin D can be treated with a diet low in calcium and phosphorus, discontinuation of intake, intravenous hydration with

saline, loop diuretics, calcitonin, glucocorticoids and biophosphates [105]. Several studies have shown higher levels to be safe for intake although the dose and duration that causes toxicity is highly debated [14, 46].

1.8 Alternatives to Infant Supplementation

Due to the potential complications of vitamin D supplementation for infants and possible interference with breastfeeding promotion, we will explore a safe and cost-effective alternative to infant vitamin D supplementation: maternal supplementation.

1.8.1 Maternal Vitamin D Supplementation and Breast Milk and Infant Outcomes

Since exclusively breastfed infants who do not receive vitamin D supplements are at increased risk for developing vitamin D deficiency and/or rickets, and infant supplementation has been shown to have its complications, determining the amount of vitamin D in supplements needed to provide sufficient vitamin D in mothers and promote adequate vitamin D activity in breast milk for the infant will be the most optimal solution [75]. Early studies in the 1980s by Greer et al. revealed that a mother's ultraviolet light exposure, and dietary supplementation of vitamin D were able to increase the vitamin D content in her breast milk [92, 106].

During the first 6 months of life, the vitamin D status of the infant depends initially on the vitamin D status of the mother during pregnancy and later on the diet of the infant and exposure to sunlight [107]. The antirachitic activity of the mother's milk varies due to season, from vitamin D intake and amount of sun exposure the mother attains [103]. Approximately 20% of maternal circulating vitamin D and 25OHD is transferred to the infant through the mother's milk [103]. During lactation, the main form of vitamin D transferred to

breast milk is as vitamin D₃ as opposed to circulating 25OHD, which is transferred in a lesser amount. Due to the short half-life of vitamin D₃, it is suspected that a dietary supplement would need to be taken on a daily basis to maintain balanced circulating levels to the mother to sustain an adequate supply in the breast milk [108].

I will explore the existing evidence on studies that have examined the relationship between vitamin D status of mothers and the vitamin D status of breastfed infants (**Table 1.4**). Hollis and Wagner investigated the effects of maternal supplementation during lactation to prevent low vitamin D for mother and infant [91]. 18 fully lactating mothers were enrolled 1 month after delivery. In addition to a multivitamin with 400 IU of vitamin D₃, each participant was given either 1600 IU vitamin D₂ or 3600 IU vitamin D₂ for a 3 month period. Results from the study showed that circulating 25OHD₂ concentrations in infants were reflective of maternal intake and breast milk vitamin D [91]. Breast milk antirachitic activity (including both vitamin D₂ and 25OHD₂) in the 1600 IU group increased from baseline of 35.5 ± 3.5 IU/L to 69.9 ± 3.0 IU/L ($p < 0.001$) at the end of the 3-month period [91]. More significant increases were seen in the 3600 IU group, from 40.4 ± 3.7 IU/L to 134.6 ± 48.3 IU/L ($p < 0.001$) [91]. Infants whose mothers were in the 3600 IU group also had higher 25OHD₂ concentrations compared to those in the 1600 IU group ($p < 0.003$) [91]. This was the first prospective study to evaluate vitamin D supplementation in lactating mothers and examine how vitamin D in breast milk affects 25OHD concentrations in the infant. Instigators used vitamin D₂ as the supplement of choice to account for the transfer from mother to infant since the amount of vitamin D₂ from other sources would be unlikely. However previous research has questioned the potency of vitamin D₂ in comparison to

vitamin D₃ [17]. This study also had a fairly small sample size, and it is uncertain whether this data can be applied to other populations.

To further investigate the effects of high dose vitamin D supplementation in breastfeeding mothers and their infants, a RCT conducted by Wagner et al enrolled 19 lactating mothers 1 month postpartum and provided them with one of two treatments (6400 IU vitamin D₃ vs. 400 IU vitamin D₃) for six months [109]. Infants whose mothers were on the 400 IU/day treatment also received a vitamin D supplement of 300 IU/day [109]. In mothers on the 6400 IU/d treatment, breast milk vitamin D content increased from 82 IU/L to 873 IU/L ($p < 0.003$) during the study period [109]. High dose supplementation in breastfeeding mothers indicates that exclusive maternal supplementation of 6400 IU/day was able to achieve equivalent levels of vitamin D in infants as compared to infants who received an oral vitamin D supplement [109]. No further benefit was found for infants with higher 25OHD levels than achieved with 300 IU per day. This was a pilot study exploring the effects of maternal supplementation as an alternative to provide sufficient vitamin D levels through breast milk to the infant.

More recently, a middle-eastern study measured the vitamin D status of exclusively breastfed infants as a result of combined maternal and infant vitamin D supplementation [110]. 90 healthy lactating mothers were divided into two treatment groups of 2000 IU/day or 60 000 IU/month vitamin D₂, with all of their infants given 400 IU/day vitamin D₂, for a 3 month period [110]. Infant serum 25OHD levels increased significantly from baseline of 13.9 ± 8.6 to 49.6 ± 18.5 nmol/L ($p < 0.0001$) in mothers given 2000 IU/d from 13.7 ± 12.1 to 44.6 ± 15.0 nmol/L ($p < 0.0001$) in mothers given 60 000 IU/month [110]. Overall, the combined

supplementation of mother and infant was able to increase infant serum 25OHD threefold from a state of deficiency.

A review conducted by Kovacs in 2012 explored the role of vitamin D in pregnancy and lactation in clinical studies and animal models [36]. The author concludes that pregnant and lactating women should require the same amount of vitamin D as non-pregnant women since the body adapts to meet the increased demands of vitamin D [36]. However, there are many issues associated with infants born with vitamin D deficiency. As seen in a previous study, healthy infants who were supplemented with 400 IU/d of vitamin D still presented with 25OHD levels below 50 nmol/L – of these participants, one-third exhibited evidence of demineralization [84]. Further research in the United Kingdom suggested that maternal vitamin D supplementation was significantly associated with increased bone mineral mass in children [10]. Higher 25OHD levels at birth also increases the likelihood that the breastfed infant will have sufficient vitamin D stores until supplemental foods are introduced [10]. A reasonable approach to ensure that neonates are born with adequate 25OHD levels would be to ensure that mothers have adequate vitamin D throughout pregnancy and lactation. Several studies have already demonstrated the link between maternal supplementation and breast milk and infant 25OHD [58-60, 62, 63]. A recent review by Principi et al that examined clinical studies in the last 15 years investigating the implications of vitamin D deficiency during pregnancy and the optimal vitamin D supplementation for maternal and infant health concluded that further studies are needed to determine the amount of vitamin D required for health of mother and infant. [7]. To date there are no randomized controlled trials that have investigated the effects of maternal supplementation throughout pregnancy and lactation on maternal and infant 25OHD.

Figure 1.1 - Hydroxylation of Vitamin D₃ [1]

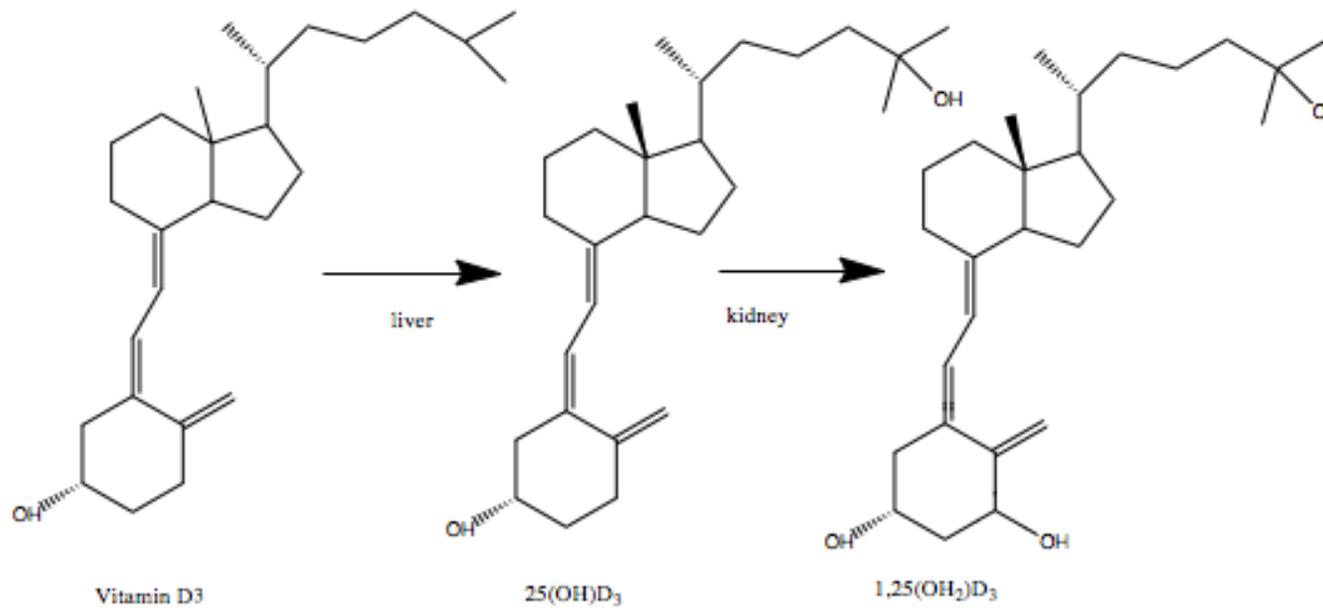


Table 1.1 – Vitamin D Content in Breast Milk

Reference	Year	Reported concentration of Vitamin D /L	Additional Comments
Wagner et al	2009	33-68 IU/L	Human milk's total vitamin D or its antirachitic activity obtained from mothers receiving 400 IU/day typically contains 33–68 IU/L
Wagner et al	2006	45.6-78.6 IU/L	Mean milk antirachitic activity of mothers receiving 400 IU of vitamin D per day
Makin et al	1983	40 IU/L	Human breast milk collected from 6 women 1-8 days postpartum Reported 45-75 IU/L of antirachitic activity
Reeve et al	1982	33 IU/L	Human milk contains 163 ng/mL of 25-hydroxyvitamin D3, which gives about 33 IU/L of vitamin D activity
Hollis et al	1981	20-70 IU/L	Antirachitic activity of human milk was defined with sensitive assay technology

Table 1.2 - Vitamin D Supplementation in Infants During Lactation

Reference	Year	Population (n)	Duration	Infant Vitamin D Supplement Dose and Form	Total circulating 25OHD in Infant (nmol/L)		Overall outcomes
					Baseline	End of Study	
Greer and Marshall	1989	Breastfed white infants (n=46)	6 months	400IU/d D ₂ (n=24)	NR	92.4 ± 29.7	25OHD ₃ significantly higher in the unsupplemented human milk-fed group compared with the supplemented group (<i>p</i> < 0.01) Unsupplemented breastfed infants did not show vitamin D deficiency in first 6 mos
				Placebo (n=22)	NR	58.8 ± 24.9	
Greer and Searcy	1981	Healthy term infants (n=18)	12 weeks	Placebo (n=9)	NR	NR	Infants receiving placebo had significant decrease in bone mineralization and in serum 25-hydroxyvitamin D concentrations compared to supplemented group
				400 IU/d (n=9)	NR	NR	

Table 1.3 - Effect of Different Dosages of Oral Vitamin D Supplementation on Vitamin D Status in Healthy, Breastfed Infants

Reference	Year	Population (n)	Duration	Infant Vitamin D Supplement Dose and Form	<u>Plasma 25OHD Concentrations of 75 nmol/L or greater (% [95% CI])</u>	<u>Total circulating 25OHD in Infant (nmol/L [95% CI])</u>	<u>Overall outcomes</u>
					3 months	3 months	
Gallo et al	2013	132	11 months	400 IU/d (n=39)	55 [38, 72]	78 [71, 84]	25OHD concentration was not sustained in 97.5% of infants at 12 mos in any of the groups Only 1600 IU/d of vitamin D supplementation was able to achieve 75 nmol/L in 97.5% of infants at 3 mos; however this dose may be associated with hypercalcemia
				800 IU/d (n=39)	81 [65, 91]	102 [90, 114]	
				1200 IU/d (n=38)	92 [77, 98]	134 [118, 150]	
				1600 IU/d (n=16)	100	180 [154, 207]	

Table 1.4 – Randomized control trials of Vitamin D supplementation during lactation on maternal, and infant vitamin D concentration

Reference	Year	Population (n)	Duration	<u>Maternal Vitamin D Supplement</u>	<u>Infant Vitamin D Supplement</u>	<u>Total circulating 25OHD in Infant (nmol/L)</u>		<u>Overall outcomes</u>
				Dose and Form	Dose and Form	Baseline	End of Study	
Hollis et al	2004	Healthy lactating mothers (n=18)	3 months	1600 IU vitamin D ₂ and 400 IU vitamin D ₃ as multivitamin	N/A	20 ± 3 (<i>p</i> <0.02)	70 ± 10 (<i>p</i> <0.02)	DRI of 400 IU/d insufficient for status of mothers and infants ; maternal intake of 4000 IU/d is safe and provide sufficient vitamin D for mothers and Infants
				3600 IU vitamin D ₂ and 400 IU vitamin D ₃ as multivitamin	N/A	34 ± 8 (<i>p</i> <0.01)	77 ± 13 (<i>p</i> <0.01)	
Wagner et al	2006	Lactating women (n=19)	6 months	400 IU/d D ₃	300IU/d D ₃		Same between groups	400 IU/d did not sustain 25OHD levels
				6400 IU/d D ₃	None		Same	Infant levels achieved exclusively through maternal supplementation were equivalent to levels in infants who received oral vitamin D supplementation

Reference	Year	Population (n)	Duration	<u>Maternal Vitamin D Supplement</u>	<u>Infant Vitamin D Supplement</u>	<u>Total circulating 25OHD in Infant (nmol/L)</u>		<u>Overall outcomes</u>
				Dose and Form	Dose and Form	Baseline	End of Study	
Saadi et al	2009	Healthy breastfeeding mothers and their infants (n=90 mothers, n=92 infants)	3 months	2000 IU/d D ₂ 60 000 IU/mo D ₂	400 IU/d vitamin D ₂ 400 IU/d vitamin D ₂	13.9 ± 8.6 (<i>p</i> <0.0001)	49.6 ± 18.5 (<i>p</i> <0.0001)	Combined supplementation of mother and infant was able to increase infant serum 25OHD threefold from a state of deficiency

Chapter 2: Research Study

2.1 Purpose

The primary purpose of this study is to examine the effect of maternal vitamin D supplementation on maternal and infant serum 25-hydroxyvitamin D concentrations at 8 weeks postpartum. This study will offer critical data for health care workers, policy makers and parents for future vitamin D recommendations and information on infant supplementation to promote optimal health for mothers and infants.

2.2 Hypothesis and Objectives

Hypothesis:

1. In a dose response manner, mothers who are supplemented with the highest dosage of vitamin D (2000 IU/day) will have higher breast milk 25-hydroxyvitamin D, and serum 25-hydroxyvitamin D at 8 weeks postpartum than mothers on lower dosages (400 IU/day and 1000 IU/day).
2. Infants born to mothers who are supplemented with the highest dosage of vitamin D (2000 IU/day) will have higher serum 25-hydroxyvitamin D compared to infants born to mothers who are supplemented with lower dosages (400 IU/day and 1000 IU/day).

Objectives: The overall goals of the study are to determine the effect of vitamin D supplementation during pregnancy and lactation on maternal and infant vitamin D concentration. My research and analysis will focus specifically on maternal and infant vitamin D concentration at 8 weeks postpartum.

1. To determine the effect of 3 dosages of supplemental vitamin D starting at 13-24 weeks of pregnancy on maternal serum 25-hydroxyvitamin D at 8 weeks postpartum.

2. To determine the effect of 3 dosages of supplemental vitamin D starting at 13-24 weeks of pregnancy on infant serum 25-hydroxyvitamin D at 8 weeks postpartum.

2.3 Methods

2.3.1 Overview

This is a double-blind randomized control trial consisting of 226 healthy pregnant women in Greater Vancouver. Participants were randomized to one of three dosages of vitamin D (400 IU/d, 1000 IU/d, or 2000 IU/d) for the duration of the study. The study consisted of three clinic visits at baseline, 36 weeks gestation and 8 weeks postpartum. Participants were asked to complete a demographic and lifestyle questionnaire, a food frequency questionnaire (FFQ) to account for vitamin D and calcium intake through food, and a sun exposure questionnaire to approximate for sun exposure and use of sunscreen. A non-fasting blood and urine sample was taken at each of the visits. A breast milk sample and infant blood sample were also collected at 8 weeks postpartum. Additional measures such as anthropometrics (height and weight), blood pressure, and skin colour were also collected.

2.3.2 Sample Size

A sample size of 52 participants per group were estimated to allow for estimation of the dose required for majority of women (97.5%) to achieve a serum 25OHD > 50 nmol/L, within 10% of the true dose with 95% confidence. The primary outcome measure is maternal and infant 25OHD concentrations at 8 weeks postpartum. For the mothers, a minimum mean difference of 10 nmol/L 25OHD between any two dosages would require a sample of 55 women per dose, assuming a standard deviation of 25 nmol/L [111]. We assumed a 20%

attrition rate for a total recruitment of 226 women – approximately 70 subjects for each dosage of vitamin D.

2.3.3 Participant Recruitment and Selection

This research was approved by The University of British Columbia and Children’s and Women’s Clinical Research Ethics Board H09-01261 and informed written consent was given by each participant (**Appendix A**). A convenience sample of 226 healthy pregnant women was recruited from Vancouver and the Lower Mainland (49°N) between June 2010 to August 2012. Active recruitment (researchers approached potential participants in person) of participants took place at BC Women’s Hospital Ultrasound and Crossroads Obstetrics and Gynecology Clinic. Participants were passively recruited through brochures and flyers in clinics, flyers at hospitals and coffee shops, word of mouth, blogs and newspaper advertisements (**Appendix B, C & D**). Recruitment also took place in the lower mainland through various Midwifery clinics including: South Community Birthing Program, Ravensong, The Midwifery Group, Pomegranate Community, Open Door Midwifery, and various other local community programs such as prenatal classes.

Pregnant women aged 18-42 years between 13-24 weeks of gestation with singleton pregnancies and identified as “low-risk” were identified as eligible for the study (Inclusion criteria). Women were not eligible for the study if they had any co-morbidities such as diabetes, cardiac or renal disease, communicable diseases (HIV/AIDS), chronic hypertension/pre-eclampsia, autoimmune diseases or conditions associated with vitamin D malabsorption such as celiac disease. Clinic visits took place on-site at BC Women and Children’s Hospital and a standard study protocol was used for each participant.

2.3.4 Procedures

Participants who consented to the study attended three clinic visits at BC Women and Children's hospital, with each visit varying from approximately 30-60 minutes in length. An identification number from 001 to 226 was assigned to each participant. At baseline participants were assigned to a supplement dose for the duration of the study.

Randomizations were blocked by ethnicity (Caucasian vs. non-Caucasian) then participants were randomized without bias to one of three dosages of vitamin D within their ethnicity block. An inclusion/exclusion criteria form was filled out by participants to make sure they met all the inclusion criteria for enrollment (**Appendix E**). After participants consented and met all inclusion criteria, they were asked to complete a socio-demographic and pregnancy history questionnaire (country of birth, ethnicity, infant's ethnicity, education, occupation, total family income, self-reported pre-pregnancy weight, due date, past pregnancy history, smoking status, alcohol consumption, diet and supplement and medication use) (**Appendix F**), and a sun exposure questionnaire (sunscreen use, tanning practice, time spent outdoors) (**Appendix G**). In instances where a participant reported that they belonged to more than one ethnic group, a single ethnic group was assigned based on the ethnic group the participant felt they most identified with. If two or more non-European ethnicities were reported the participant was matched to the non-European ethnic category that matched with the cultural origin of her ancestors. Researchers completed anthropometric measurements using a stadiometer to the nearest 0.1 cm for height, a standing floor scale to the nearest 0.1 kg for weight, and an electronic blood pressure monitor to assess for blood pressure and heart rate. Participants completed a food frequency questionnaire (FFQ) with focus on vitamin D and

calcium intake over the previous month. Laboratory staff at BC Women and Children's Hospital collected a blood sample and spot urine sample from participants at each visit.

2.3.5 Supplements

A vitamin D₃ supplement of 400 IU/d, 1000 IU/d or 2000 IU/d was provided to each participant along with a standard prenatal multivitamin with no vitamin D upon entrance of the study (Table 2.1). The study did not use any placebo as it would be unethical to randomize women to a supplement that contained no vitamin D since most prenatal multivitamins contain 200-400 IU/d of vitamin D. Two sets of supplements were provided for participants throughout the study – at the baseline clinic visit (~22 weeks gestation) and clinic visit 2 (~36 weeks gestation). Participants were given instructions to finish the first set of supplements before starting the next set. Compliance was measured at clinic visit 2 and the final visit with a questionnaire assessing how often the vitamin D supplement was consumed over the last week, and over the last two months (**Appendix I**). The investigators also counted the remaining vitamin D pills at the final visit. Participants who consumed over 80% of their supplements in the last two months prior to the clinic visit were defined as compliant.

The vitamin D tablets were identical in size and colour and contained one of three dosages of vitamin D₃ with vegetable grade magnesium stearate as a lubricant and microcrystalline cellulose and dicalcium phosphate dehydrate as fillers. The investigators and participants were both blinded to the supplement dose by coding through Natural Factors Inc., the manufacturer of all supplements utilized in the study. Proper dosage for the vitamin D supplements were ensured through analysis of vitamin D tablets at multiple time points throughout the study by Natural Factors Inc. External evaluation of samples were conducted by Dr. Ronald Horst at Heartland Assays LLC (Table 2.2).

2.3.6 Dietary Assessment

Maternal vitamin D and calcium intake from food sources including fortified foods and supplements over the previous month was estimated using a semi-quantitative FFQ validated for use in a variety of ethnic groups (**Appendix H**). The FFQ was developed and validated in a group of healthy Canadian young adults of diverse ancestry for rapid assessment of vitamin D intake during late winter of 2007 (n=107). Results of the FFQ was highly correlated with 7-day food records and serum 25OHD concentrations ($r=0.529$, $p < 0.001$; $r=0.481$, $p < 0.001$, respectively) [112]. Participants are allowed to choose the frequency of consumption for each food item (never or less than once per month up to 2+ times per day) listed in the FFQ as well as quantify a serving size (small, medium, large based on the reference serving size listed). Information on nutritional supplements taken in the previous month leading up to the visit was also collected from participants. Participants also provided additional information on any supplements taken throughout the study (such as omega 3s or probiotics). The FFQ's were analyzed by Dr. Susan Whiting, from the University of Saskatchewan College of Pharmacy and Nutrition, who developed the questionnaire. EHSA Food Processor (Version 8.0, EHSA Research, Ore), which incorporated the 1997 Canadian Nutrient File from Health Canada, was used to determine the calcium and vitamin D content for each food item in the FFQ [112]. Updates were made on the amount of fortification for foods that were recently approved in Canada (soy beverage, orange juice) [112].

2.3.7 Blood and Urine Samples

At each clinic visit, phlebotomists took blood samples by venipuncture at the outpatient blood laboratory at BC Children's Hospital. Each participant provided three tubes

of blood into vacutainers at each clinic visit – two samples for the preparation of plasma in a 2 mL and 6 mL plastic tube with potassium ethylene diamine acetic acid (K₂ EDTA) as an anticoagulant and one sample for the preparation of serum in a 10 mL plastic serum tube with Increased Silica Act Clot Activator. The 2 mL plasma tube was used for measuring complete blood cell count (CBC) by the Hematopathology lab at BC Children’s Hospital. At delivery, nurses from BC Women’s Hospital collected a 6 mL blood sample from the umbilical cord.

For the preparation of plasma, blood samples were centrifuged within 30 minutes (3700 g, 15 minutes). For the preparation of serum, blood samples were centrifuged within 1 hour (3000 g, 10 minutes). Plasma and serum were aliquoted into 2 mL microtubes and stored in -80°C at the Child and Family Research Institute, Vancouver for subsequent analysis. Prior to preparation for 25OHD analysis in November 2011, April 2012 and September 2012, samples did not undergo any freeze-thaw cycles and were stored up to 20 months. Serum samples were shipped on dry ice to Dr. Hope Weiler at McGill, Montreal, Quebec for analysis. Serum 25OHD was determined by the DiaSorin LIAISON[®] 25-OH Vitamin D Total Assay (DiaSorin Inc, Stillwater, MN, USA), a competitive chemiluminescence immunoassay (CLIA) for the quantitative determination of 25OHD and other hydroxylated vitamin D metabolites (25OHD₂ and 25OHD₃) in human serum. The assay has high specificity (25OHD₂ 104%; and 25OHD₃ 100%) with a measurement range of 10-374 nmol/L. The intra-assay coefficient of variation (CV)% was 8% for the low 25OHD control (37 nmol/L); and 8% for the high 25OHD control (123 nmol/L), with an accuracy of mid-range of manufacturer specifications at 88% for low control and 90% for high control. Previous supplementation trials have demonstrated that hypercalciuria, the first indicator of hypervitaminosis D, is not caused by serum levels of 25OHD > 300 nmol/L. For our study,

we defined hypervitaminosis D as 25OHD > 225 nmol/L, the same cut-off used by the United States Food and Drug Administration (FDA) [113, 114].

DiaSorin LIAISON[®] BAP Ostase (DiaSorin Inc, Stillwater, MN, USA) was used for determining serum bone-specific alkaline phosphatase (BSAP), a serum marker for osteoblastic bone formation. The DiaSorin LIAISON[®] BAP Ostase uses CLIA to quantitatively determine BSAP concentrations in serum. The intra-assay CV% was 17% for the low BAP control (14 ug/L) and 13% for the high BAP control (63 ug/L) with an accuracy for the mid-range of manufacturer specifications of 95% for low control and 98% for high control. The assay allows for a measurement range of 1.5 µg/L to 120 µg/L with an analytical sensitivity is ≤ 0.1 µg/L and functional sensitivity is 1.5 µg/L.

Spot urine samples were collected during each clinic visit in a 200 mL urine cup. The urine was then aliquoted and stored in 2 mL microtubes at -80°C for subsequent analysis. Maternal serum and urine creatinine, calcium and phosphate were measured on laboratory equipment from Johnson & Johnson, Ortho-Clinical Diagnostics VITROS[®] 5600 System according to standardized methodology and laboratory normative data at BC Children's Hospital. The cut-offs used for serum calcium were 2.22 – 2.67 mmol/L, serum phosphate 0.87 – 1.52 mmol/L, serum creatinine 39 -106 µmol/L and urine calcium to creatinine ratio < 0.7 mg/dL as indicated by BC Women's and Children's Hospital [115].

2.3.8 Breast Milk Samples

Participants were instructed to provide a breast milk sample two hours post-feed at the last clinic visit. A full expression of breast milk (including foremilk and hindmilk) from one breast was requested whenever possible. Participants were allowed to bring in a breast milk sample collected the morning before the clinic visit and kept in the refrigerator, or pump

during the clinic visit using an electric breast pump (Medela Swing™ Breastpump). Samples of breast milk ranging from approximately 10-100 mL were collected and mixed evenly before freezing in 50 mL falcon tubes at -80°C for subsequent analysis.

2.3.9 Data Analysis

Statistical analysis was performed using SPSS Statistics 20.00 for Macintosh (SPSS Inc., Chicago, IL 2012). Normality of data was checked using a histogram and found to be normally distributed. The deficiency cut-off was set as 25OHD < 25 nmol/L [30]. Vitamin D sufficiency was examined using two cutoffs, 50 and 75 nmol/L [2]. Both intent-to-treat (ITT) analysis and as-treated (AT) analysis was conducted for data analysis. ITT analysis was primarily used to reduce any bias by carrying forward the last data value for a participant if she withdraws from the study or is lost to follow-up. Since these deviations from protocol are not always random, carrying forward data from participants allows for reduction of bias. Many clinical trials use ITT model to compare outcomes between assigned randomizations without assuming compliance with dosing regimen [114]. This approach allows for an unbiased assessment of treatment efficacy and can be applied in a community or public-health context as the level of adherence could be similar to that observed in the community [116]. As-treated analysis was also completed on data from participants who adhered to protocols and completed the study. This approach allows for investigators to examine study outcomes under ideal conditions since it only includes participants with complete data sets. However, AT analysis can be prone to potentially important biases as it excludes data of participants who withdrew or were lost to follow-up.

One-way analysis of variance (ANOVA) and General Linear Model was used to compare differences between treatment groups at 8 weeks postpartum. Estimated Marginal

Means was used to take out the effect of baseline 25OHD concentrations and Bonferroni adjustment for multiple comparisons was used. Estimates were considered statistically significant if $p < 0.05$. Chi-square test was used to compare categorical variables.

2.3.10 Other

Participants received a \$25 gift voucher to a grocery chain for every clinic visit attended as well as bus fare or parking pass to compensate for transportation costs to BC Women and Children's Hospital. At the last clinic visit at 8 weeks postpartum, all mothers are given vitamin D drops and all exclusively breastfeeding mothers are recommended to supplement their infants once a day with 400 IU of vitamin D₃. After the study was completed, a letter addressing study summary, participant's 25OHD concentration, infant 25OHD concentration (if available), and breast milk vitamin D content (if available), and recommendations on vitamin D intake was mailed to participants.

Table 2.1 – Natural Factors Prenatal Multivitamin Composition

<i>Vitamins</i>	
Beta carotene (provitamin A)	1500 IU
Vitamin A	1500 IU
Vitamin B1 (thiamine mononitrate)	3 mg
Vitamin B2 (riboflavin)	3.4 mg
Niacinamide	20 mg
Vitamin B6 (pyridoxine hydrochloride)	10 mg
Vitamin B12 (cyanocobalamin)	12 mcg
D'Pantothenic acid (calcium pantothenate)	10 mg
Folic Acid	1 mg
Biotin	30 mcg
Vitamin C (sodium ascorbate/ascorbic acid)	100 mg
Vitamin E (d-alpha tocopheryl acid succinate)	30 IU
<i>Minerals</i>	
Calcium (citrate)	250 mg
Magnesium (oxide)	50 mg
Iron (ferrous fumarate)	27 mg
Potassium (citrate)	5 mg
Zinc (citrate)	25 mg
Manganese (gluconate)	1 mg
Iodine (potassium iodide)	0.15 mg
Copper (copper gluconate)	2 mg
Chromium (chromium HVP chelate)	25 mcg
Manganese (manganese citrate)	5 mg
Molybdenum (molybdenum)	25 mcg
Selenium (selenium HVP chelate)	25 mcg
HVP hydrolyzed vegetable protein	
Non-medicinal ingredients: Microcrystalline cellulose, croscarmellose sodium, coating (carbohydrate gum, polyethylene glycol), vegetable grade magnesium stearate (lubricant).	

Table 2.2 Vitamin D supplement dosage quality control (IU)

	400 IU Tablet	1000 IU Tablet	2000 IU Tablet
Natural Factors Inc.			
January 17, 2011	473	1266	2523
August 3, 2011	463	1211	2209
Heartland Assays LLC			
March 27, 2012	484	1144	2379

Chapter 3: Results

3.1 Recruitment and Randomization

Figure 3.1 shows the participant enrollment, randomization and follow-up through the course of the study. From July 2010 to August 2012, healthy women with singleton pregnancies recruited from BC Women's and Children's Hospital and lower mainland were assessed for eligibility. Participants who met the inclusion criteria were enrolled. 226 women attended the baseline clinic visit at BC Women's Hospital and were randomized to one of three treatment groups: 76 women in the 400 IU/d group, 76 women in the 1000 IU/d group and 74 women in the 2000 IU/d group. At 8 weeks postpartum, the retention rate was 77 %. 30 participants were lost to follow-up, and 22 participants withdrew from the study due to various reasons including personal, illness, or busyness. A small number of women (n=6) failed to attend their second clinic visit at 36 weeks gestation were mailed their vitamins and continued on in the study and attended their postpartum clinic visit. Reasons stated for missing the clinic visit at 36 weeks gestation include early delivery, pre-term delivery, miscalculated estimated delivery dates, bed-rest or the women were out-of-town. At 8 weeks postpartum, 59 in the women in the 400 IU/d group, 57 women in the 1000 IU/d group and 56 women in the 2000 IU/d group attended the clinic visit and completed the study. Infant blood samples were collected from 51 infants whose mothers were in the 400 IU/d group, 44 infants in the 1000 IU/d group and 45 infants in the 2000 IU/d group.

3.2 Baseline Participant Characteristics

Baseline participant characteristics are shown in **Table 3.1**. Participants were randomized to three treatment groups and no significant differences were found between groups for any of the measures examined. For example, week of gestation, vitamin D and

calcium supplement intake before study entry and gravidity were not found to be different between treatment groups. Baseline mean serum 25OHD concentrations of all participants was 66 (95% CI: 63, 69) nmol/L. There were 5 (2.2%) women in total with 25OHD concentration below 30 nmol/L; classifying them as vitamin D deficient under Institute of Medicine guidelines. Overall 15 (7 %) of women at baseline were below the Estimated Average Requirement for 25OHD of 40 nmol/L and a total of 48 women (21.2%) had 25OHD <50 nmol/L, the cutoff set as the RDA for 25OHD by the IOM.

Of the women, 64% had a pre-pregnancy BMI between 18.5-24.9 at baseline. The majority (72%) of women were of Caucasian ethnicity (White/European). The remaining non-Caucasian participants included Chinese (n=21), Hispanic (n=10) East Indian (n=4), Aboriginal (n=5), South-East Asian (n=8), Japanese (n=4), Korean (n=1), South Asian (n=6) and Black (n=1). Most of the participants had at least college or undergraduate education and had a household income greater than \$50,000. For the majority of participants it was not their first pregnancy (maternal gravidity > 0). More women enrolled in the study during spring and summer (April-September) (n= 147) than fall and winter (October-March) (n=78) months. Average dietary vitamin D intake at baseline was approximately 200 IU/day for all participants. None of these characteristics were found to be significantly different between groups.

3.3 Compliance to Treatment

Study protocol compliance was measured as participants taking more than 80% of their vitamin D tablets in the two months leading up to the clinic visit. At 36 weeks gestation, overall compliance was 86%. In the 400 IU/d, 1000 IU/d, 2000 IU/d treatment groups, compliance over the last two months were reported to be 81%, 83% and 95% respectively

(**Table 3.2**), with no significant differences between treatment groups ($p=0.063$). At the final clinic visit at 8 weeks postpartum overall compliance was 72%. Participant compliance in groups taking 400 IU/d, 1000 IU/d and 2000 IU/d were 68%, 68% and 81% ($p=0.243$) respectively.

3.4 Maternal Serum 25OHD at 8 Weeks Postpartum

Mean (95% CI) maternal serum 25OHD at 8 weeks postpartum are shown in **Table 3.3**, **Table 3.4** and **Table 3.5**. Using intent to treat (ITT) analysis and after adjusting for baseline 25OHD concentrations, the maternal 25OHD concentration at 8 weeks postpartum was 69 (66, 73) nmol/L, 78 (74,81) nmol/L, 87 (83, 90) nmol/L in the 400 IU/d, 1000 IU/d, and 2000 IU/d treatment groups respectively. Serum 25OHD was significantly different between groups, with the mothers taking the highest dose of vitamin D supplement (2000 IU/d) having the highest serum 25OHD concentration. The 400 IU/d treatment group was significantly different from the 1000 IU/d treatment group ($p=0.004$), as were 1000 IU/d group from the 2000 IU/d group ($p=0.002$) and the 400 IU/d group from the 2000 IU/d group ($p=0.000$). Compared to the 400 IU/d treatment group, the mothers in the 1000 IU/d group had a mean 25OHD concentration that was 8 nmol/L higher, and the 2000 IU/d treatment group had a mean 25OHD concentration that was 17 nmol/L higher.

Differences between treatment groups were even more pronounced when examining the data using the as-treated (AT) analysis (including only values for those participants who attended all clinic visits and adhered to study protocol). At 8 weeks postpartum, after adjusting for baseline 25OHD concentrations, 25OHD concentration was 71 (67, 75) nmol/L, 79 (75,83) nmol/L, 91 (87, 95) nmol/L in the 400 IU/d, 1000 IU/d, and 2000 IU/d treatment groups respectively. Serum 25OHD concentrations were significantly different between

treatment groups with the 400 IU/d treatment group was significantly different from the 1000IU treatment group ($p=0.022$), as were 1000 IU/d group from the 2000 IU/d group ($p=0.000$) and the 400 IU/d group from the 2000 IU/d group ($p=0.000$). Overall, the 1000 IU/d group had a mean 25OHD concentration that was 8 nmol/L higher than the 400 IU/d group, and the 2000 IU/d group had a mean 25OHD concentration that was 20 nmol/L higher than the 400 IU/d group.

A similar trend is also observed in those participants who were over 80% compliant, with an overall mean 25OHD concentration of 83 (95% CI: 80, 86) nmol/L. Serum 25OHD concentrations in 400 IU/d, 1000 IU/d and 2000 IU/d treatment groups were 75 (95% CI: 69,80), 82 (95% CI: 77, 87) and 93 (95% CI: 88, 98) respectively. Significant differences were observed between 1000 IU/d group and the 2000 IU/d group ($p=0.006$) and the 400 IU/d group from the 2000 IU/d group ($p=0.000$) but not between the 400 IU/d and 1000 IU/d group ($p=0.180$). Compared to the 400 IU/d treatment group, 1000 IU/d treatment group had a mean 25OHD concentration that was 7 nmol/L higher and the 2000 IU/d treatment group had a mean 25OHD concentration that was 19 nmol/L higher.

3.5 Infant Serum 25OHD at 8 Weeks Postpartum

The second objective of my research is to determine the effect of maternal vitamin D supplementation during pregnancy and lactation on infant vitamin D concentration at 8 weeks postpartum. Mean infant 25OHD concentrations are displayed in **Table 3.6**, **Table 3.7** and **Table 3.8**. Overall, infants had a mean 25OHD concentration of 57 (52, 61) nmol/L at 8 weeks postpartum. Infants whose mothers were in the 2000 IU/d treatment group had significantly higher 25OHD concentration than the 400 IU/d and 1000 IU/d treatment groups. The 400 IU/d and 1000 IU/d treatment groups were not significantly different from each

other ($p=0.499$). 25OHD concentrations of infants whose mothers were in the 2000 IU/d treatment group significantly differed from the 400 IU/d ($p=0.000$) and 1000IU ($p=0.000$) treatment groups, although it was not significantly different between the 400IU and 1000IU group ($p=0.499$). Compared to the 400 IU/d treatment group, the 1000 IU/d treatment group had a mean 25OHD concentration that was 7 nmol/L higher, and the 2000 IU/d treatment group had a mean 25OHD concentration that was 30nmol/L higher. Mean 25OHD concentration in the 400 IU/d, 1000 IU/d, 2000 IU/d treatment groups were 45 (38, 52) nmol/L, 52 (45, 58) nmol/L, 75 (67, 83) nmol/L respectively.

There was a similar trend found in 25OHD concentration of infants whose mothers were over 80% compliant to study protocol. The overall mean 25OHD concentration of these infants were slightly higher at 59 nmol/L compared to the mean 25OHD of all infants at 57 nmol/L. Mean 25OHD concentration in the 400 IU/d, 1000 IU/d, 2000 IU/d treatment groups were 44 (35, 53) nmol/L, 52 (44, 59) nmol/L, 78 (69, 87) nmol/L respectively. Although there were no significant differences observed between the 400 IU/d and 1000 IU/d groups ($p=0.491$), there were significant differences between the 1000 IU/d and 2000 IU/d group ($p=0.000$) and 400 IU/d and 2000 IU/d group ($p=0.000$).

All mothers were asked not to supplement their infant with vitamin D; however, some mothers did not follow this advice. Thus, additional analysis was completed on infants who had not been supplemented with vitamin D drops. The infants who were supplemented with vitamin D drops for more than one week after birth were excluded from the analysis to avoid potential falsely elevated serum 25OHD concentrations. The serum concentrations we found were essentially identical to the analysis completed with all infant serum samples. Mean 25OHD concentration in the 400 IU/d, 1000 IU/d, 2000 IU/d treatment groups were 40 (32,

47) nmol/L, 50 (43, 58) nmol/L, 73 (64, 81) nmol/L respectively. The infants whose mothers were on the 400 IU/d and 1000 IU/d groups did not differ significantly from each other ($p=0.163$), however, significant differences were seen between the 1000 IU/d and 2000 IU/d groups ($p=0.001$) and the 400 IU/d and 2000 IU/d groups ($p=0.000$).

3.6 Serum Vitamin D Not Achieving 50nmol/L and 75nmol/L Cutoffs

Based on 25OHD concentrations observed at 8 weeks postpartum, I examined the proportion of mothers and their infants from each treatment group who did not meet the 50, and 75 nmol/L cutoffs. Other commonly used cutoffs were also evaluated (**Table 3.8, Table 3.9, Table 3.10, Table 3.11**). At 8 weeks postpartum, in the ITT analysis, 10% (22/226) women had a serum 25OHD below 50 nmol/L, compared to 8% (14/172) in the AT analysis, and only 3% (4/118) in those who were over 80% Compliant. In both ITT and AT analysis, there were significantly more women diagnosed with vitamin D insufficiency in the 400 IU/d and 1000 IU/d treatment groups compared to the 2000 IU/d treatment group (ITT: $p = 0.045$, AT: $p = 0.025$). Using ITT analysis only 2.7% (2/74) women in the 2000 IU/d treatment group were below the 50 nmol/L cutoff but none of the women were below 50 nmol/L in the AT analysis and over 80% compliance, however this difference was only statistically significant between treatment groups in the AT analysis (AT: $p = 0.025$).

There were significantly less mothers whose serum 25OHD concentrations were below 75 nmol/L in the 2000 IU/d treatment group compared to the 400 IU/d, and 1000 IU/d treatment groups in ITT, AT, and >80% Compliant analysis (ITT: $p = 0.000$, AT: $p = 0.000$, >80% Compliant: $p = 0.021$). However, the number of mothers with serum 25OHD below 75 nmol/L did not differ significantly between the 400 IU/d and 1000 IU/d treatment groups across the ITT, AT and >80% analysis.

In infants, significant differences between treatment groups were observed in those who were below 50 nmol/L and 75 nmol/L cutoffs. Other commonly used cutoffs were also examined (**Table 3.12, Table 3.13, Table 3.14**). Significantly less infants had an insufficient vitamin D concentration at 8 weeks postpartum when their mothers were in the 2000 IU/d treatment group compared to the 400 IU/d and 1000 IU/d treatment groups (2000 IU/d: 11.1% (5/45) vs. 1000 IU/d: 45.5% (20/44) and 400 IU/d: 58.8% (30/51) [$p=0.000$]). Similar trends were seen in infants whose mothers were compliant to study protocol (2000 IU/d: 5.9% (2/34) vs. 1000 IU/d: 43.3% (13/30) and 400 IU/d: 59.4% (30/32) [$p=0.000$]). Less infants whose mothers were in the 2000 IU/d treatment allocation had a serum 25OHD below 75 nmol/L compared to the 1000 IU/d and 400 IU/d treatment groups in overall analysis, >80% Compliant analysis and Non-Supplement analysis (Overall: $p = 0.004$, >80% Compliant: $p = 0.022$, Non-Supplement: $p = 0.006$).

3.7 Dietary Vitamin D and Calcium Intake

Dietary vitamin D and calcium intake at baseline and 8 weeks postpartum were examined using ITT analysis (**Table 3.15**) and AT analysis (**Table 3.16**). At baseline, the mean \pm SD dietary vitamin D intake was 193 ± 106 in the 400 IU/d group, 191 ± 111 in the 1000 IU/d group and 207 ± 127 in the 2000 IU/d group. Using ITT analysis at 8 weeks postpartum, the mean \pm SD dietary vitamin D intake was in the 400 IU/d treatment group was 180 ± 104 , 197 ± 103 in the 1000 IU/d treatment group and 190 ± 126 in the 2000 IU/d treatment group. There were no significant differences between groups at baseline ($p=0.641$) or 8 weeks postpartum using ITT analysis ($p=0.650$). Similar trends were observed in AT analysis with no significant differences between groups. There were also no trends observed

in changes in dietary vitamin D intake (IU) from baseline to 8 weeks postpartum between treatment groups in both ITT and AT analysis.

3.8 Bone Specific Alkaline Phosphatase

Total maternal and infant circulating bone-specific alkaline phosphatase (BSAP) are shown in **Table 3.17** (ITT) and **Table 3.18** (AT). The mean \pm SD maternal BSAP ($\mu\text{g/L}$) concentration was 8.34 ± 2.49 at baseline in the 400IU group, 8.10 ± 2.35 1000 IU/d group, and 8.00 ± 2.46 in the 2000 IU/d group. After adjusting for baseline BSAP values, at 8 weeks postpartum, the mean (95% CI) BSAP was 14.7 (13.1, 16.3) in the 400 IU/d group, 14.5 (12.8, 16.1) in the 1000 IU/d group, and 16.0 (14.4, 17.7) in the 2000 IU/d group. There were no significant differences found at baseline ($p = 0.687$) or 8 weeks postpartum using ITT analysis ($p = 0.532$). Compared to the 400 IU/d treatment group, the 1000 IU/d group was on average 0.223 (-2.615, 3.060) $\mu\text{g/L}$ higher in BSAP concentration and the 2000 IU/d group was on average -1.3 (-4.2, 1.5) $\mu\text{g/L}$ lower in BSAP concentration. In the AT analysis, analogous trends were observed at 8 weeks postpartum after adjusting for baseline BSAP and there were no significant differences found between treatment groups ($p = 0.290$).

BSAP concentration ($\mu\text{g/L}$) in infants at 8 weeks postpartum was found to be higher than BSAP concentrations observed in their mothers. In infants whose mothers were in the 400 IU/d group at BSAP concentrations (mean \pm SD) was 116.2 ± 67.9 , and 116.7 ± 57.3 in the 1000 IU/d group and 128.2 ± 75.0 in the 2000 IU/d group. BSAP concentrations were slightly higher in infants in the 2000 IU/d group compared to the 400 IU/d and 1000 IU/d groups however this difference was not statistically significant ($p = 0.653$).

3.9 Safety Measures

Serum and urine creatinine, calcium and phosphate across treatment groups at baseline and 8 weeks postpartum are reported as means \pm SD in ITT analysis (**Table 3.19**) and in AT analysis (**Table 3.20**). Urine calcium to creatinine ratios and changes in urine calcium to creatinine ratio from baseline to 8 weeks are also reported. There were no differences in any measures at baseline or 8 weeks postpartum except for urine phosphate ($p = 0.001$). Subjects were randomly allocated to the three treatment groups it is likely that the difference observed is purely due to chance.

At 8 weeks postpartum, 2 participants exceeded the normal laboratory cut-offs for serum creatinine ($> 104 \mu\text{mol/L}$) and 14 participants exceeded the normal cut-off for serum calcium (2.7 mmol/L). No participants had low serum creatinine ($< 39 \mu\text{mol/L}$) at 8 weeks postpartum, and 1 participant had low serum calcium at 8 weeks ($< 2.2 \text{ mmol/L}$). There was one participant with low serum phosphate ($< 0.87 \text{ mmol/L}$) and 46 participants whose serum phosphate values exceeded the recommended range ($> 1.52 \text{ mmol/L}$). Using ITT analysis, 44 participants were identified with elevated urinary calcium to creatinine ratio ($> 0.20 \text{ mg/dL}$) at 8 weeks postpartum. Data on participants with elevated serum calcium and urine calcium creatinine ratio are displayed by treatment group in **Table 3.21**, **Table 3.22**.

Figure 3.1 - Participant Flow Diagram in Pregnancy and Lactation Study

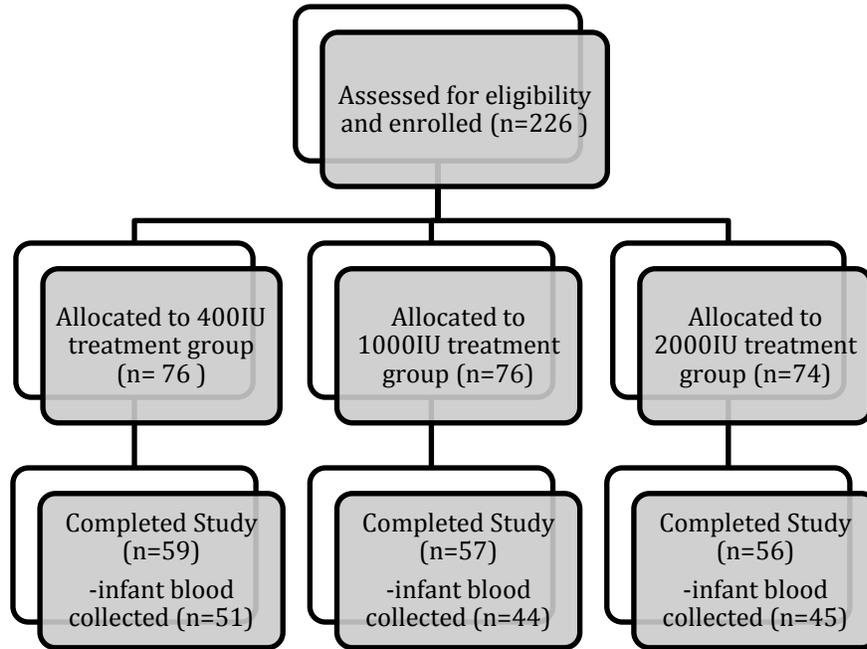


Table 3.1 - Baseline Participant Characteristics

Characteristic	400 IU Group (n=76)	1000 IU Group (n=76)	2000 IU Group (n=74)	<i>p</i> value
Age (yrs), mean \pm SD	33.2 \pm 4.2	33.4 \pm 4.4	34.5 \pm 4.6	0.170
Week of Gestation at baseline, mean \pm SD	20.9 \pm 2.5	19.8 \pm 2.8	19.9 \pm 3.3	0.038
Pre-pregnancy BMI, mean \pm SD	23.7 \pm 7.8	23.3 \pm 6	22.6 \pm 5	0.565
18.5-24.9, n (%)	48 (68)	44 (62)	53 (78)	
24.9-29.9, n (%)	10 (14)	20 (28)	12 (18)	
\geq 30, n (%)	13 (18)	7 (10)	3 (4.4)	
Ethnicity, n (%)	76 (33.6)	76 (33.6)	74 (32.7)	0.895
White	56 (73.7)	55 (72.4)	52 (70.3)	
Non-white	20 (26.3)	21 (27.6)	22 (29.7)	
Education, n (%)	76 (34.5)	73 (33.2)	71 (32.3)	0.249
High School	3 (3.9)	3 (4.1)	6 (8.5)	
College	16 (21.1)	19 (26.0)	9 (12.7)	
Undergraduate or more	57 (75)	51 (69.9)	56 (78.9)	
Household income per year, n (%)	75 (34.6)	74 (34.1)	68 (31.3)	0.592
< \$ 50,000	12 (16.0)	16 (21.7)	15 (22.0)	
\$50,000 - \$100,000	23 (30.7)	21 (28.4)	22 (32.4)	
\geq \$100,000	40 (53.3)	37 (50.0)	31 (45.6)	
Season at study entry, n (%)	76 (33.6)	76 (33.6)	74 (32.7)	0.767
April - September	51 (67.1)	47 (61.8)	49 (66.2)	
October - March	25 (32.9)	29 (38.2)	25 (33.8)	
Dietary vitamin D intake (IU), mean \pm SD	193 \pm 106	191 \pm 111	207 \pm 126	0.641
Supplement vitamin D intake (IU), mean \pm SD	456 \pm 335	419 \pm 228	449 \pm 277	0.705
Dietary calcium intake (IU), mean \pm SD	815 \pm 339	836 \pm 459	877 \pm 431	0.647
Supplement calcium intake (IU), mean \pm SD	249 \pm 94	279 \pm 151	262 \pm 136	0.363
25OHD (nmol/L) at baseline, mean \pm SD	68 (63, 73)	64 (59, 68)	67 (63, 69)	0.369
Baseline serum 25OHD (nmol/L), n (%)				
< 40 nmol/L, n (%)	5 (6.6)	6 (7.9)	4 (5.4)	0.831
< 50 nmol/L, n (%)	18 (23.7)	21 (27.6)	9 (12.2)	0.056
< 75 nmol/L, n (%)	47 (61.8)	58 (76.3)	52 (70.3)	0.152
> 100 nmol/L, n (%)	4(5.3)	3 (4.0)	4 (5.4)	0.909

Table 3.2 – Participant Compliance by Supplement Dosage

Compliance	400 IU	1000 IU	2000 IU	<i>p</i> Value
Compliance at 36 weeks gestation ¹ , <i>n</i> (%)	46 (80.7)	50 (83.3)	55 (94.8)	0.063
Compliance at 8 weeks postpartum, <i>n</i> (%)	39 (68.4)	38 (67.9)	30 (80.8)	0.243

¹Compliance calculated as taking 80% or more of 60 vitamin D tablets two months prior to clinic visit

* Results are significantly different if $p < 0.05$, Bonferroni post-hoc analysis

Table 3.3 - Maternal Serum 25OHD at 8 Weeks Postpartum (ITT)

Variable	400 IU (n=76)	1000 IU (n=76)	2000 IU (n=74)
25OHD (nmol/L) at baseline, mean (95% CI)	68 (63, 73)	64 (59, 68)	67 (63, 69)
25OHD (nmol/L) at 8 weeks postpartum, adjusted for baseline (95% CI)	69 (66, 73) ^a	78 (74, 81) ^b	87 (83, 90) ^c
Difference from 400 IU adjusted for baseline 25OHD (95% CI)		8 (2, 15)	17 (11, 24)

*ITT, Intent to Treat model used

^{abc}Rows without common superscript are significantly different ($p < 0.05$)

Table 3.4 – Maternal Serum 25OHD at 8 Weeks Postpartum (AT)

Variable	400 IU (n=59)	1000 IU (n=57)	2000 IU (n=56)
25OHD (nmol/L) at baseline, mean (95% CI)	68 (63, 73)	64 (59, 68)	67 (63, 69)
25OHD (nmol/L) at 8 weeks postpartum, adjusted for baseline (95% CI)	71 (67, 75) ^a	79 (75, 83) ^b	91 (87, 95) ^c
Difference from 400 IU adjusted for baseline 25OHD (95% CI)		8 (1, 15)	20 (13, 27)

*AT, As-Treated model used

^{abc}Rows without common superscript are significantly different ($p < 0.05$)

Table 3.5 – Maternal Serum 25OHD at 8 Weeks Postpartum (>80% Compliant)

Variable	400 IU (n=38)	1000 IU (n=38)	2000 IU (n=42)
25OHD (nmol/L) at baseline, mean (95% CI)	68 (63, 73)	64 (59, 68)	67 (63, 69)
25OHD (nmol/L) at 8 weeks postpartum, adjusted for baseline (95% CI)	75 (69, 80) ^a	82 (77, 87) ^a	93 (88, 98) ^b
Difference from 400 IU adjusted for baseline 25OHD (95% CI)		7 (2, 16)	19 (10, 27)

^{ab}Rows without common superscript are significantly different ($p < 0.05$)

Table 3.6 – Mean Infant Serum 25OHD at 8 Weeks Postpartum

Variable	400 IU (n=51)	1000 IU (n=44)	2000 IU (n=45)
25OHD (nmol/L) at 8 weeks (95% CI)	45 (38, 52) ^a	52 (45, 58) ^a	75 (67, 83) ^b
Overall Mean [nmol/L (95% CI)]	57 (52, 61)		

^{ab}Rows without common superscripts are significantly different ($p < 0.05$)

Table 3.7 – Mean Infant Serum 25OHD at 8 Weeks Postpartum (>80% Compliant)

Variable	400 IU (n=32)	1000 IU (n=30)	2000 IU (n=34)
25OHD (nmol/L) at 8 weeks (95% CI)	44 (35, 54) ^a	52 (44, 59) ^a	78 (69, 87) ^b
Overall Mean [nmol/L (95% CI)]	59 (53, 64)		

*Infants whose mothers who were over 80% compliant to study protocol

^{ab}Rows without common superscripts are significantly different ($p < 0.05$)

Table 3.8 – Mean Infant Serum 25OHD at 8 Weeks Postpartum (Non-Supplement*)

Variable	400 IU (n=40)	1000 IU (n=32)	2000 IU (n=36)
25OHD (nmol/L) at 8 weeks (95% CI)	40 (32, 47) ^a	50 (43, 58) ^a	73 (64, 81) ^b
Overall Mean [nmol/L (95% CI)]	54 (49, 59)		

*Infants who were given vitamin D drops for 0 days to < 1 week after birth

^{ab}Rows without common superscripts are significantly different ($p < 0.05$)

Table 3.9 – Mothers Not Achieving 40, 50, 75 nmol/L at 8 Weeks Postpartum by Treatment Group (ITT)

Supplement Dose	Serum 25OHD (nmol/L), n(%)			
	<30	<40	<50	<75
400 IU (<i>n</i> = 76)	1 (1.3)	3 (4.0)	10 (13.2)	45 (60)
1000 IU (<i>n</i> = 76)	0 (0)	3 (3.9)	10 (13.2)	42 (55.3)
2000 IU (<i>n</i> =74)	0 (0)	1 (1.4)	2 (2.7)	22 (29.7)
<i>p</i> value	--	--	0.045	0.000

ITT, Intent to Treat model used

*Chi-Square test was used to evaluate difference between groups

Table 3.10 – Mothers Not Achieving 40, 50, 75 nmol/L at 8 Weeks Postpartum by Treatment Group (AT)

Supplement Dose	Serum 25OHD (nmol/L), n(%)			
	<30	<40	<50	<75
400 IU (<i>n</i> = 59)	1 (1.7)	2 (3.4)	7 (11.9)	31 (53.4)
1000 IU (<i>n</i> = 57)	0 (0)	2 (3.5)	7 (12.3)	31 (54.4)
2000 IU (<i>n</i> =56)	0 (0)	0 (0)	0 (0)	11 (19.6)
<i>p</i> value	--	--	0.025	0.000

AT, As-Treated model used

Table 3.11 – Mothers Not Achieving 40, 50, 75 nmol/L at 8 Weeks Postpartum by Treatment Group (>80% Compliant)

Supplement Dose	Serum 25OHD (nmol/L), n(%)		
	<40	<50	<75
400 IU (<i>n</i> = 38)	0	2 (5.1)	18 (47.4)
1000 IU (<i>n</i> =38)	0	2 (7.9)	18 (47.4)
2000 IU (<i>n</i> =42)	0	0 (0)	9 (21.4)
<i>p</i> value	--	--	0.021

Supplement Dose	Serum 25OHD (nmol/L), n(%)			
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*Chi-Square test was used to evaluate difference between groups

Table 3.12 – Infants Not Achieving 40, 50, 75 nmol/L at 8 Weeks Postpartum by Treatment Group

Supplement Dose	Serum 25OHD (nmol/L), n(%)			
	<30	<40	<50	<75
400 IU (n= 51)	22 (43)	24 (48)	30 (58.8)	51 (100)
1000 IU (n=44)	7 (16)	14 (33.3)	20 (45.5)	44 (100)
2000 IU (n=45)	1 (2.2)	2 (4.4)	5 (11.1)	40 (88.9)
<i>p</i> value	0.000	0.000	0.000	0.004

*Chi-Square test was used to evaluate difference between groups

Table 3.13 – Infants Not Achieving 40, 50, 75 nmol/L at 8 Weeks Postpartum by Treatment Group (>80% Compliant)

Supplement Dose	Serum 25OHD (nmol/L), n (%)			
	<30	<40	<50	<75
400 IU (n=32)	15 (46.9)	16 (51.6)	19 (59.4)	32 (100)
1000 IU (n=30)	4 (13.3)	8 (28.6)	13 (43.3)	30 (100)
2000 IU (n= 34)	0 (0)	1 (2.9)	2 (5.9)	30 (88.2)
<i>p</i> value	0.000	0.000	0.000	0.022

*Infants born to mothers who were >80% Compliant

*Chi-Square test was used to evaluate difference between groups

Table 3.14 – Infants Not Achieving 40, 50, 75 nmol/L at 8 Weeks Postpartum by Treatment Group (Non-Supplement*)

Supplement Dose	Serum 25OHD (nmol/L), n(%)			
	<30	<40	<50	<75
400 IU (n=40)	21 (52.5)	23 (57.5)	28 (70.0)	40 (100)
1000 IU (n=32)	5 (15.6)	12 (40.0)	16 (50.0)	32 (100)
2000 IU (n=36)	1(2.8)	2 (5.6)	4 (11.1)	33 (91.7)
<i>p</i> value	0.000	0.000	0.000	0.046

*Chi-Square test was used to evaluate difference between groups

*Infants who were given vitamin D drops for 0 days to < 1 week after birth

Table 3.15 – Dietary Vitamin D and Calcium Intake During Pregnancy and Lactation (ITT)

Measure	400 IU (n=69)	1000 IU (n=65)	2000 IU (n=67)	<i>p</i> Value
Daily dietary vitamin D intake (IU)				
Baseline (13-24 wks gestation), mean ± SD	193 ± 106	191 ± 111	207 ± 126	0.641
8 weeks postpartum (IU), mean ± SD	180 ± 104	197 ± 103	190 ± 126	0.650
Change in dietary vitamin D intake (IU), mean (95% CI)	12.51 (-10.59, 35.60)	-6.27 (-27.76, 15.24)	17.67 (-11.77, 47.11)	
Daily dietary calcium intake (mg)				
Baseline (13-24 wks gestation), mean ± SD	814 ± 339	836 ± 459	877 ± 431	0.647
8 weeks postpartum (IU), mean ± SD	757 ± 359	840 ± 419	762 ± 487	0.453
Change in dietary calcium intake (IU), mean (95% CI)	54.27 (-31.65, 140.20)	3.72 (-99.69, 107.12)	117.96 (-10.23, 246.15)	

ITT, Intent to Treat Model used

Table 3.16 – Dietary Vitamin D and Calcium Intake During Pregnancy and Lactation (AT)

Measure	400 IU (n=58)	1000 IU (n=55)	2000 IU (n=57)	<i>p</i> Value
Daily dietary vitamin D intake (IU)				
Baseline (13-24 wks gestation), mean ± SD	193 ± 106	191 ± 111	207 ± 126	0.641
8 weeks postpartum (IU), mean ± SD	174 ± 106	190 ± 100	181 ± 126	0.760
Change in dietary vitamin D intake (IU), mean (95% CI)	23.94 (-6.37, 52.23)	2.98 (-24.46, 30.41)	22.63 (-15.74, 61.00)	
Daily dietary calcium intake (mg)				
Baseline (13-24 wks gestation), mean ± SD	814 ± 339	836 ± 459	877 ± 431	0.647
8 weeks postpartum (IU), mean ± SD	737 ± 353	812 ± 381	739 ± 504	0.559
Change in dietary calcium intake (IU), mean (95% CI)	88.36 (-9.90, 186.62)	22,12 (-93.94, 138.20)	132.00 (-17.36, 281.36)	

AT, As-Treated Model used

Table 3.17 – Total Maternal and Infant Circulating Bone-Specific Alkaline Phosphatase (BSAP) Concentrations (µg/L) During Pregnancy and Lactation (ITT)

Measure	400 IU (n=76)	1000 IU (n=76)	2000 IU (n=73)	<i>p</i> Value
BSAP (ug/L) at baseline, mean ± SD	8.3 ± 2.5	8.1 ± 2.4	8.0 ± 2.5	0.687
Range	(2.2, 13.9)	(3.9, 16.0)	(2.9, 17.0)	
BSAP (ug/L) at 8 wks postpartum adjusted for baseline, mean (95% CI)	14.7 (13.1, 16.3)	14.5 (12.8, 16.1)	16.0 (14.4, 17.7)	0.372
Difference from 400IU adjusted for baseline 25OHD (95% CI)		0.22 (-2.6, 3.1)	1.33 (-4.2, 1.5)	
Infant BSAP (ug/L) at 8 wks postpartum, mean ± SD	116.2 ± 67.9	116.7 ± 57.3	128.2 ± 75.0	0.653
Range	(42, 453)	(40, 366)	(55, 414)	

ITT, Intent to Treat Model used

Table 3.18 – Total Maternal and Infant Circulating Bone-Specific Alkaline Phosphatase (BSAP) Concentrations ($\mu\text{g/L}$) During Pregnancy and Lactation (AT)

Measure	400 IU (n=59)	1000 IU (n=57)	2000 IU (n=56)	<i>p</i> Value
BSAP ($\mu\text{g/L}$) at baseline, mean \pm SD	8.34 \pm 2.49	8.10 \pm 2.35	8.00 \pm 2.46	0.687
Range	(2.2, 13.9)	(3.9, 16.0)	(2.9, 17.0)	
BSAP ($\mu\text{g/L}$) at 8 wks postpartum adjusted for baseline, mean (95% CI)	16.31 (14.4, 18.2)	16.1 (14.1, 18.0)	18.1 (16.1, 20.1)	0.290
Difference from 400IU adjusted for baseline 25OHD (95% CI)		0.24 (-3.1, 3.6)	1.8 (-1.6, 5.2)	
Infant BSAP ($\mu\text{g/L}$) at 8 wks postpartum, mean \pm SD	116.2 \pm 67.9	116.7 \pm 57.3	128.2 \pm 75.0	0.653
Range	(42, 453)	(40, 366)	(55, 414)	

AT, As-Treated Model used

Table 3.19 - Serum and Urine Calcium, Creatinine and Phosphate at Baseline and 8 Weeks Postpartum (ITT)

Measure	400 IU (n=73)	1000 IU (n=74)	2000 IU (n=71)	<i>p</i> Value
Serum Creatinine				
Baseline (µmol/L), mean ± SD	49.7 ± 7.8	49.2 ± 7.4	48.9 ± 8.0	0.842
8 weeks postpartum (µmol/L), mean ± SD	67.7 ± 13.8	70.3 ± 47.3	68.4 ± 15.6	0.857
Urine Creatinine				
Baseline (µmol/L), mean ± SD	8531 ± 5846	6706 ± 6244	6483 ± 4408	0.056
8 weeks postpartum (µmol/L), mean ± SD	8493 ± 6199	7739 ± 5953	8055 ± 6586	0.763
Serum Calcium				
Baseline (mmol/L), mean ± SD	2.21 ± 0.18	2.26 ± 0.18	2.21 ± 0.24	0.210
8 weeks postpartum (mmol/L), mean ± SD	2.37 ± 0.17	2.42 ± 0.17	2.43 ± 0.20	0.184
Urine Calcium				
Baseline (mmol/L), mean ± SD	4.63 ± 3.33	3.58 ± 2.44	3.78 ± 2.73	0.060
8 weeks postpartum (mmol/L), mean ± SD	2.72 ± 2.36	2.71 ± 2.44	2.27 ± 1.94	0.420
Serum Phosphate				
Baseline (mmol/L), mean ± SD	1.22 ± 0.16	1.22 ± 0.18	1.22 ± 0.20	0.967
8 weeks postpartum (mmol/L), mean ± SD	1.36 ± 0.206	1.34 ± 0.17	1.38 ± 0.21	0.432
Urine Phosphate				
Baseline (mmol/L), mean ± SD	19.84 ± 15.24	13.55 ± 9.05	13.73 ± 8.59	0.001
8 weeks postpartum (mmol/L), mean ± SD	17.53 ± 10.93	17.17 ± 12.77	15.99 ± 11.3	0.712
Urine Calcium Creatine Ratio				
Baseline (mg/dL), mean ± SD	0.238 ± 0.155	0.251 ± 0.160	0.251 ± 0.186	0.860
8 weeks postpartum (mg/dL), mean ± SD	0.134 ± 0.117	0.153 ± 0.136	0.126 ± 0.089	0.384
Change from baseline to 8 wks postpartum (mg/dL) mean ± SD	0.108 ± 0.173	0.094 ± 0.116	0.133 ± 0.185	0.000

ITT, Intent to Treat Model used

Table 3.20 – Serum and Urine Calcium, Creatinine and Phosphate at Baseline and 8 Weeks Postpartum (AT)

Measure	400 IU (n=52)	1000 IU (n=52)	2000 IU (n=51)	<i>p</i> Value
Serum Creatinine				
Baseline (µmol/L), mean ± SD	49.7 ± 7.8	49.2 ± 7.4	48.9 ± 8.0	0.842
8 weeks postpartum (µmol/L), mean ± SD	72.3 ± 10.4	76.4 ± 53.5	72.9 ± 13.7	0.762
Urine Creatinine				
Baseline (µmol/L), mean ± SD	8531 ± 5846	6706 ± 6244	6483 ± 4408	0.056
8 weeks postpartum (µmol/L), mean ± SD	8778 ± 6440	7768 ± 6037	8155 ± 6898	0.698
Serum Calcium				
Baseline (mmol/L), mean ± SD	2.21 ± 0.18	2.26 ± 0.18	2.21 ± 0.24	0.210
8 weeks postpartum (mmol/L), mean ± SD	2.43 ± 0.14	2.43 ± 0.14	2.49 ± 0.17	0.126
Urine Calcium				
Baseline (mmol/L), mean ± SD	4.63 ± 3.33	3.58 ± 2.44	3.78 ± 2.73	0.060
8 weeks postpartum (mmol/L), mean ± SD	2.38 ± 1.82	2.02 ± 1.26	1.98 ± 1.59	0.347
Serum Phosphate				
Baseline (mmol/L), mean ± SD	1.22 ± 0.16	1.22 ± 0.18	1.22 ± 0.20	0.967
8 weeks postpartum (mmol/L), mean ± SD	1.40 ± 0.20	1.37 ± 0.18	1.44 ± 0.18	0.194
Urine Phosphate				
Baseline (mmol/L), mean ± SD	19.84 ± 15.24	13.55 ± 9.05	13.73 ± 8.59	0.001
8 weeks postpartum (mmol/L), mean ± SD	17.37 ± 10.50	15.80 ± 10.90	16.31 ± 11.78	0.739
Urine Calcium Creatine Ratio				
Baseline (mg/dL), mean ± SD	0.238 ± 0.155	0.251 ± 0.160	0.251 ± 0.186	0.860
8 weeks postpartum (mg/dL), mean ± SD	0.109 ± 0.935	0.108 ± 0.066	0.194 ± 0.550	0.300
Change from baseline to 8 wks postpartum (mg/dL) mean ± SD	0.126 ± 0.169	0.121 ± 0.125	0.064 ± 0.618	0.001

AT, As-Treated Model used

Table 3.21 – Elevated Serum Calcium by Treatment Group

Supplement Dose	Elevated Serum Calcium (>2.7 mmol/L) n (%)
400 IU (n=73)	4 (5.5)
1000 IU (n=74)	3 (4.1)
2000 IU (n=71)	7 (9.9)
<i>p</i> value	0.471

Table 3.22 – Elevated Urine Calcium Creatinine Ratio by Treatment Group

Supplement Dose	Elevated Urine Calcium Creatinine Ratio (> 0.20) n (%)
400 IU (n=73)	13 (17.8)
1000 IU (n=74)	14 (23.0)
2000 IU (n=71)	17 (20.0)
<i>p</i> value	0.198

Chapter 4: Discussion

Maternal vitamin D status during pregnancy and lactation is directly related to infant vitamin D concentration [13]. Inadequate maternal vitamin D concentrations resulting from lack of sun exposure or low intake of vitamin D from diet or supplement contributes to low breast milk vitamin D content and infant vitamin D deficiency [13, 46, 75]. Currently the Institute of Medicine (endorsed by Health Canada) recommends 600 IU/d of vitamin D for pregnant and lactating women based on evidence for bone health [2, 14]. However, roles of vitamin D beyond bone health including immune functions, T1DM, and asthma have been suggested and higher vitamin D intake may be needed to support these other non-bone health related outcomes [3, 30, 61, 103]. In addition, it is uncertain whether mothers and their infants may benefit from higher levels of vitamin D and cut-offs for vitamin D sufficiency is debated. The IOM recommends a serum 25OHD concentration of 40 nmol/L as sufficient for a population and 50 nmol/L as a target for an individual; however, other experts have suggested for higher concentrations of up to 100 nmol/L [2, 3]. Breast milk is generally low in vitamin D and as a result Health Canada recommends supplementing breastfed infants with 400 IU/d of vitamin D per day [15]. However, there have been concerns of overdose with infant vitamin D supplementation and supplementation may raise concerns among mothers about the adequacy of their breast milk to provide optimal nutrition to their infants [13, 16]. Telling mothers that they must supplement their breastfed infant with vitamin D gives the impression that breast milk is inadequate for the infant [13]. Since maternal vitamin D supplementation has been shown to improve both mother and infant vitamin D status and previous research in Canada has shown that over 80% of pregnant women already take a prenatal supplement, supplementing mothers with vitamin D during pregnancy and lactation

may be the safest, most practical and cost-effective way to reach optimal vitamin D status in mothers and infants without interfering with recommendations for exclusive breastfeeding [12]. Currently, there are gaps in evidence regarding adequate maternal vitamin D supplement dosage and corresponding serum 25OHD concentrations and its relation to breast milk vitamin D content and infant 25OHD concentrations. To my knowledge, our study is the first randomized control trial to examine vitamin D supplementation from the first trimester through lactation and its effects on maternal and infant 25OHD concentrations. The primary aim of my research is to evaluate the effect of maternal vitamin D supplementation at one of three doses during pregnancy and lactation on maternal and infant serum 25OHD concentrations at 8 weeks postpartum.

4.1 25OHD Outcomes at 8 Weeks Postpartum

4.1.1 Maternal 25OHD Outcomes

The results of my research indicate that maternal vitamin D supplementation throughout pregnancy and lactation increases serum 25OHD concentration in a dose response manner, with the women in the 2000 IU/d treatment group having the highest serum 25OHD concentration, followed by the 1000 IU/d treatment group and then the 400 IU/d group. At 8 weeks postpartum maternal 25OHD concentration was found to be significantly different from each other in both intent to treat [mean (95%) 400 IU/d: 69 (66, 73) nmol/L, 1000 IU/d: 78 (74,81) nmol/L, 2000 IU/d: 87 (83, 90) nmol/L] and as-treated analysis [mean (95% CI) 400 IU/d: 71 (67, 75) nmol/L, 1000 IU/d: 79 (75,83) nmol/L, 2000 IU/d: 91 (87, 95) nmol/L] for all three treatment groups. Interestingly, in the analysis that only included women who were over 80% compliant to study regimen, 25OHD concentration differed significantly between the 2000 IU/d and 1000 IU/d group, and the 2000 IU/d and 400 IU/d group but did

not differ significantly between the 400 IU/d and 1000 IU/d group. Furthermore, after adjusting for baseline 25OHD values, women in the 1000 IU/d group had 25OHD concentration that was 8 (95% CI: 2, 15) higher than the 400 IU/d and women in the 2000 IU/d group had 25OHD concentration that was 17 (95% CI: 11, 24) higher in ITT analysis. This difference was also observed in the AT analysis, but not in the >80% Compliant analysis. An explanation for this could be that most women were already taking a prenatal supplement containing 300-400 IU/d of vitamin D at the beginning of the study, and thus higher vitamin D dosages would be needed to increase baseline serum 25OHD concentrations. This might also suggest that although oral vitamin D supplementation does increase serum 25OHD concentration, the relationship may not be linear.

At 8 weeks postpartum, there were fewer women with serum 25OHD concentration below 50 nmol/L and 75 nmol/L in the 2000 IU/d treatment group compared to the 400 IU/d and 1000 IU/d ($p=0.045$). As reported earlier, although the mean serum 25OHD concentration is higher in the 1000 IU/d group, there were no significant differences observed between the two groups in those who did not achieve the 50 nmol/L and 75 nmol/L cutoffs. Regardless of treatment, it was observed that although 22 (9.7%) did not achieve the 50 nmol/L cutoff for sufficiency, only 7 (2.6%) were below the 40 nmol/L cutoff. In the AT and >80% Compliant analysis, all women in the 2000 IU/d treatment group were above the 50 nmol/L cutoff and approximately 80% were above the 75 nmol/L cutoff. Our results also show that all women in the 2000 IU/d group who were >80% compliant to study regimen were above 40 nmol/L – the level of sufficiency set for the Estimated Average Requirement (EAR), the median daily intake value estimated to meet the requirement for half the healthy individuals and suggested by Health Canada to be the best reference value to use for

determining adequacy of estimated nutrient intakes for groups [14, 117]. Although our study is the first to examine the effects of vitamin D supplementation during pregnancy and lactation on maternal and infant status there are a few studies that have examined vitamin D supplementation during lactation. Most notably, Hollis and his colleagues conducted a pilot study to determine the effects of high dose vitamin D supplementation on the vitamin D status of mothers and their infants [91]. 18 lactating mothers were randomized at one month to one of two treatment arms (1600 IU/d or 3600 IU/d vitamin D₂) and both groups were given 400 IU/d of vitamin D₃ as part of a prenatal supplement. At the end of three months, serum 25OHD₃ in women who were given 1600 IU/d of vitamin D₂ actually decreased whereas the serum 25OHD₃ in women who were given 3600 IU/d of vitamin D₂ increased slightly. Total circulating 25OHD did however increase in both groups, the 1600 IU/d group increased from 69 ± 8 to 90 ± 6 nmol/L ($p < 0.05$) and the 3600 IU/d group increase from 82 ± 6 to 111 ± 10 nmol ($p < 0.04$). We found a similar maternal 25OHD concentration in the 2000 IU/d treatment group at 8 weeks post-partum compared to the 1600 IU/d vitamin D₂ + 400 IU/d vitamin D₃ Hollis treatment group at 4 months [Our study: 87 (95% CI: 83, 90) nmol/L, Hollis study: 90.3 ± 5.8 nmol/L ($p < 0.05$)]. Again there are many differences in the study including sample size, participant characteristics, timing and duration of supplementation. In addition, vitamin D₂ may not be as potent as vitamin D₃ and so it may not be reasonable to compare the 25OHD concentration in both studies [17]. In addition, we did not use a supplement dosage greater than 2000 IU/d as the Tolerable Upper Level (UL) for vitamin D was set at 2000 IU/d when our study first commenced. Subsequently, the IOM has increased the UL to 4000 IU/d.

This group of researchers conducted a second study where 19 fully lactating women and their infants were randomized from one month postpartum to 2 different dosages of vitamin D₃ (400 IU/d vs. 6400 IU/d) for 6 months [109]. Infants of mothers who were in the placebo group also received 300 IU/d of vitamin D₃ per day. Significant differences were found among serum 25OHD concentration from baseline to end of study between the 6400 IU/d group compared to the 400 IU/d group ($p < 0.0028$ and 0.0043 , respectively). Although the highest supplement dose used in our study was 2000 IU/d, significant differences were also found between the 400 IU/d and 2000 IU/d group at 8 weeks postpartum. In addition to using a much higher vitamin D supplement dose, the Hollis study had a much smaller sample size compared to our study ($n=19$ vs. $n=226$) and did not supplement mothers throughout pregnancy.

Recently, Saadi et. al examined vitamin D supplementation in a large group of breastfeeding mothers ($n=90$) and their infants ($n=92$) living in the United Arab Emirates for 3 months. Mothers were assigned to 2000 IU of vitamin D₂ per day or 60 000 IU of D₂ per month and all infants received 400 IU of vitamin D₂ per day [110]. Both daily and intermittent supplementation was reported to improve maternal serum 25OHD concentration, with the daily group having achieved slightly higher serum 25OHD compared to the monthly group (41.7 ± 14.0 vs 35.8 ± 9.9 nmol/L) [110]. Saddi et al. did find significantly improved vitamin D concentration in breastfeeding women with both daily and monthly supplementation in this group of women with low vitamin D status [110]. Compared to our study, the ethnic makeup of their study participants was very different. Most women in the Sadddi et al. study were veiled women with vitamin D deficiency at baseline with a serum 25OHD of approximately 14 nmol/L. In our study, baseline 25OHD was 66 nmol/L. In

addition, the authors reported that 95% of infants were deficient in vitamin D when beginning the study (serum 25OHD of <37.5 nmol/L). It is possible that it takes more vitamin D to correct a severe deficiency than increase 25OHD in the moderate range. Moreover, the participants in the study were not blinded to the treatments, which could introduce bias.

As there are limited studies that have examined vitamin D supplementation during lactation and their results are not quite comparable to ours due to many differences in study duration, sample size, supplement dosage etc., I will also compare our findings to a recent RCT study on vitamin D supplementation during pregnancy. Hollis et al. randomized 350 pregnant women to three treatment groups (400 IU/d, 2000 IU/d or 4000 IU/d) from 12-16 weeks gestation to delivery. At delivery, significant differences were found between cord blood serum 25OHD concentrations in 400 IU/d (79 ± 37 nmol/L), 2000 IU/d (98 ± 34 nmol/L) and 4000 IU/d group (111 ± 40 nmol/L) [$p < 0.0001$] [114]. In comparison to our results of the 107 cord blood samples collected, mean (95% CI) cord blood 25OHD was 89 (68, 110), 73 (65, 82), 95 (87, 102) in the 400 IU/d, 1000 IU/d and 2000 IU/d treatment groups respectively with no significant differences between treatment groups ($p = 0.135$). Although the mothers in our 2000 IU/d group had similar 25OHD concentration at delivery to the women in Hollis' 2000 IU/d group, we did not see the trends in our study that were observed in his. However, these differences could be attributed to many factors, the Hollis' study began supplementation between 12-16 weeks gestation, while supplementation in our study began between 13-24 weeks gestation. Although there is some overlap in the time frame, at the time of delivery, participants in the Hollis' study had been taking the supplements for a longer period of time, which could contribute to bigger differences

between treatment groups. In addition, at baseline, women in the Hollis' study had a mean 25OHD of 60 nmol/L in those who completed the study vs. 51 nmol/L in those who did not complete the study with only a rate of 12.4% of prenatal supplementation at the time of randomization. In our study at baseline, mean serum 25OHD concentration was 66 nmol/L and over 90% of women on a prenatal multivitamin containing vitamin D. The high rate of supplementation prior to baseline and a higher 25OHD concentration to begin with could attribute to the fact that a higher 25OHD concentration was found in the 400 IU/d group at delivery in our study as compared to the 400 IU/d group in the Hollis study (Our study: 89 nmol/L vs. Hollis: 79 nmol/L), providing an explanation for why there were no significant differences observed between our 400 IU/d and 2000 IU/d groups as was reported by the Hollis study. Furthermore, the participants in the Hollis' study included a much higher proportion of African American and Hispanic women, women of younger age, lower education and employment rate, contributing to a lower baseline serum 25OHD concentration as compared to our study. A previous study examining maternal 25OHD status during the third trimester found that maternal socioeconomic status (SES) and level of education were predictors of 25OHD status [118]. Nevertheless, differences in study population, ethnicity, compliance, age, latitude, supplement dosage, and time on supplement are some of the many factors that contribute to the differences in results between the studies compared here and gaps in evidence still remain.

4.1.2 Infant 25OHD Outcomes

To our knowledge, our research is the first randomized control trial to look at the effects of maternal supplementation throughout pregnancy and lactation on infant serum

25OHD concentration. At 8 weeks postpartum, significant differences were observed in 25OHD concentration of infants whose mothers were assigned to the 400 IU/d [45 (95% CI: 38, 52)] nmol/L), or 1000 IU/d [52 (95% CI: 38, 58)] treatment groups compared to the 2000 IU/d treatment group [75 (95% CI: 67, 83)] ($p<0.05$). Infant 25OHD was also found to be significantly higher in the 2000 IU/d group compared to the two lower supplement doses in the analysis of infants born to mothers who were over 80% compliant to study regimen and infants who were not given vitamin D supplementation.

In infants who are below 30, 40, 50 and 75 nmol/L cutoffs, significant differences were observed among treatment groups across all analysis (Overall, >80% Compliant, Non-Supplement). At 8 weeks postpartum, there were 22 infants in the 400 IU/d group, 7 infants in the 1000 IU/d group, and 1 infant in the 2000 IU/d group who had 25OHD concentrations indicative of vitamin D deficiency (<30 nmol/L). Similar trends were also observed in the over 80% Compliant analysis and Non-Supplement analysis. Moreover, the majority of infants in the 2000 IU/d treatment group were above the 50 nmol/L cutoff (40 out of 45) suggesting that maternal supplementation can effectively raise infant 25OHD concentration to adequate levels. The results show that increased maternal vitamin D supplementation is correlated with increased infant serum 25OHD concentration. This finding is supported by the study done by Hollis et al on lactating mothers [91]. Infants whose mothers were given 1600 IU/d of vitamin D₂ and 400 IU/d of vitamin D₃ had significantly lower 25OHD concentrations compared to infants whose mothers were supplemented with 3600 IU/d of vitamin D₂ and 400 IU/d of vitamin D₃ (infants in 2000 IU treatment group: 69.5 ± 7.8 nmol/L vs. infants in the 4000 IU/d treatment group 77.0 ± 12.5 nmol/L) [91]. In addition, Wagner et al also suggested that maternal supplementation with 6400 IU/d during lactation was able to

achieve similar 25OHD concentrations in infants as a 300 IU/d infant vitamin D supplement [109]. However, both these studies did not begin until 1 month postpartum, and would not be able to see any possible effects of in-utero accumulation of vitamin D during pregnancy. In addition, the small sample size in both studies may not be representative of a larger population.

Recently, Gallo et al conducted a RCT to determine the infant supplement dose required to achieve 75 nmol/L or greater in 97.5% of infants at 3 months [119]. 132 were randomized to one of four treatment groups: 400 IU/d (n=39), 800 IU/d (n=39), 1200 IU/d (n=38), 1600 IU (n=16). They reported that overall, 97% of infants were able to achieve a 25OHD of 50 nmol/L or greater at 3 months regardless of treatment with no significant differences among treatment groups and infants sustained this concentration of 12 months with continued supplementation. Results from our study show that maternal supplementation at 2000 IU/d allowed almost 90% (40/45) of infants achieve a serum 25OHD of over 50 nmol/L at 8 weeks postpartum and this proportion was increased to 94% (32/34) in infants whose mothers who were over 80% compliant to study regimen and also similar (89%, 32/36) in infants who were not supplemented with vitamin D after birth. This suggests that maternal supplementation of 2000IU/d during pregnancy and lactation has the potential to help breastfed infants achieve adequate vitamin D concentrations for two months after delivery and in mothers who are compliant to treatment appears to be as effective as a 400 IU/d oral vitamin D supplement given to breastfed infants.

Results from our study show that a vitamin D supplementation of 2000 IU/d in mothers during pregnancy and lactation seem to be protective against vitamin D deficiency in infants for the first two months after birth. It is unsure however whether this serum 25OHD

concentration is maintained after 2 months. Also it is not clear whether the high 25OHD in infants whose mothers received 2000 IU/d versus lower doses is a residual effect of increased vitamin D accrual during pregnancy or results from providing greater vitamin to mother has increased her breast milk vitamin D content. Further research from our study will analyze breast milk samples collected at 8 weeks postpartum for 25OHD content and examine the relationship between maternal vitamin D supplementation, breast milk vitamin D content and infant serum 25OHD concentration.

4.2 Dietary Vitamin D Intake

Previous Canadian literature from the 2004 Canadian Community Health Survey (CCHS) reported mean vitamin D intake among 19-50 year old Caucasian women to be 208 IU/d and 180 IU/d in non-Caucasians [120]. Our findings for dietary vitamin D intake are consistent with the CCHS. At baseline the mean \pm SD dietary vitamin D intake was 193 ± 106 in the 400 IU/d group, 191 ± 111 in the 1000 IU/d group and 207 ± 127 in the 2000 IU/d group. There was a slight decrease in mean vitamin D intake (mean \pm SD) at 8 weeks postpartum to 180 ± 104 in the 400 IU/d treatment group, 197 ± 103 in the 1000 IU/d treatment group and 190 ± 126 in the 2000 IU/d treatment group. However, no significant differences were found between groups at baseline ($p=0.641$) or 8 weeks postpartum using ITT analysis ($p=0.650$).

Health Canada currently recommends 600 IU/d of vitamin D per day for pregnant and lactating women. At baseline, women in our study had a vitamin D supplement intake of 456 ± 335 in the 400 IU/d group, 419 ± 228 in the 1000 IU/d group and 449 ± 227 in the 2000

IU/d group. Combined with their dietary vitamin D intake of approximately 200 IU per day across treatment groups, the women in our study are meeting the current RDA of 600 IU/d.

4.3 BSAP Concentrations

Bone specific alkaline phosphatase (BSAP) is present in serum as a functional marker of osteoblast activity [121]. It is known for its role in bone mineralization and used clinically as a biomarker of bone turnover. In women, the normal reference range for BSAP is 6.5-20.1 µg/L. Baseline BSAP concentration (mean ± SD) was not significantly different between treatment groups (400 IU/d: 8.3 ± 2.5 µg/L, 1000 IU/d: 8.1 ± 2.4 µg/L, 2000 IU/d: 8.0 ± 2.5 µg/L [$p=0.687$]). After adjusting for baseline values, BSAP concentration at 8 weeks postpartum increased in all treatment groups from baseline, although there were no significant differences observed between groups (400 IU/d: 14.7 (95% CI: 13.1, 16.3) µg/L, 1000 IU/d: 14.5 (95% CI: 12.8, 16.1) µg/L, 2000 IU/d: 8.0 (95% CI: 14.4, 17.7) µg/L [$p=0.372$]). It does not appear that the differences observed in serum 25OHD concentrations between treatment groups were found in BSAP concentration. The overall increase in BSAP from baseline to 8 weeks postpartum across treatment groups could be attributed to placenta production of alkaline phosphatase during pregnancy, which would increase serum concentrations. In addition, bone turnover is also increased in lactating women as compared to mothers who formula fed their infants [122].

Our research did not find significant differences between treatment groups in maternal vitamin D supplementation during pregnancy and lactation on infant BSAP concentration at 8 weeks postpartum (400 IU/d: 116.2 ± 67.9 µg/L, 1000 IU/d: 8.1 ± 2.4 µg/L, 2000 IU/d: 8.0 ± 2.5 µg/L [$p=0.653$]). Similar to the mothers, there were no significant

trends observed between infants on different dose allocations in 25OHD concentration and BSAP concentration. Zhang et al measured serum BSAP in infected (n=33) and non-infected infants (n=30) and found that changes BSAP was not significantly associated with PTH or 25OHD concentration [123]. In addition, bone mineral concentration was measured in a sample of Montreal infants (n=132) supplemented from one month after birth with one of four dosages of vitamin D [119]. Gallo et al found that although bone mineral concentration increased over time, it did not differ by group despite differences in serum 25OHD concentration [119]. Similar to findings from our study, results from Gallo et al also reported no significant association between bone biomarkers at this stage of infancy with serum 25OHD concentration. Furthermore, infants at 2 months are undergoing a period of rapid bone development and formation and there are no established cut-offs for BSAP concentrations in this population, although their concentrations are usually much higher than adult BSAP concentrations [124, 125].

4.4 Safety

We measured for serum 25OHD concentration, serum calcium, creatinine, phosphate, and urine calcium, creatinine and phosphate as safety measures. Using ITT analysis, 14 women in total had a serum calcium concentration above 2.7 mmol/L at 8 weeks postpartum with no significant differences between groups ($p=0.471$). There were 13, 14, and 17 women in 400 IU/d, 1000 IU/d, 2000 IU/d treatment groups respectively with calcium/creatinine ratios that exceeded 0.20 mg/dL. However there were no significant differences between treatment groups ($p=0.198$), indicating that there might be other explanations for elevated calcium/creatinine ratios. It has been reported that calcium/creatinine ratios can be elevated

during pregnancy as a result of physiological increases in glomerular filtration rate and does not indicate hypervitaminosis D or hypercalciuria [126].

4.5 Limitations

Although our study has many strengths including rigorous methods, there are a few limitations. Participants were enrolled in the study from 13-24 weeks of gestation. Ideally, we would like to recruit women pre-conception and begin supplementation at the start of pregnancy and have the participants take the supplements for a longer period of time and allow researchers to measure the full effects of maternal vitamin D supplementation throughout pregnancy. In addition, women in our study had good vitamin D status to start since they were mostly already taking 300-400 IU/d of vitamin D prior to entering the study. Our results may not be generalizable to the Canadian population since the majority of our participants were Caucasian women, had at least college or undergraduate education and a family income of over \$50 000 a year. Lower education and lower income levels may be risk factors for vitamin D deficiency in pregnant women, and these women would thus benefit more from vitamin D supplementation. Currently it is not known whether women of lower socioeconomic status (SES) in Vancouver had higher or lower baseline 25OHD concentration compared to the women in our study. Although our study does include a small number of women of lower SES, it is not a representative sample of women with lower SES in Greater Vancouver.

All the women from our study were recruited from the Greater Vancouver area and the results may not be extrapolated to all women during pregnancy in the rest of Canada due to varying differences in latitude, and location. Our study was also a convenience sample,

which included women who were interested in vitamin D supplementation and nutrition during pregnancy. This could have biased our sample since these women may have potentially been more health conscious compared to the general population. Furthermore, the women self-reported their pre-pregnancy weight and in some cases made estimations which could have resulted in inaccurate self-reported data. In addition, dietary intake was recorded using semi-quantitative food frequency questionnaire (FFQ). Although self-reported food intake may be inaccurate at times, the FFQ was validated and provides the best estimate of dietary vitamin D intake, and would be more realistic to use in this setting compared to a 7-day weighed food record. We also found that the dietary vitamin D intake reported in our study was consistent with results from the Canadian Health Measures Survey (CHMS).

Our exclusion criteria excluded women from the study if they had pre-eclampsia, diabetes, renal, cardiac disease, communicable diseases and other co-morbidities. Women self-reported these conditions it may be possible if a participant had developed a condition such as gestational diabetes during the course of the study and failed to report this information.

4.6 Future Directions

To our knowledge, this study is the first RCT to supplement mothers with vitamin D throughout pregnancy and lactation and examine the effects on maternal and infant 25OHD at 8 weeks postpartum. This will answer the all-important question as to whether maternal vitamin D supplementation at as little as 2000 IU/d from early pregnancy increases breast milk vitamin D content and infant 25OHD concentration to adequate levels. Future analysis of the study will also measure for vitamin D content of breast milk samples collected from mothers at 8 weeks postpartum. In addition, samples collected from the study will also be

analyzed for vitamin B12, folate and fatty acids. The data set can also be further analyzed for other outcome variables such as PTH concentration in mother and infants and other bone biomarkers such as calcitonin and osteocalcin and allow us to better understand bone resorption and formation during pregnancy and lactation.

The optimal serum 25OHD concentration for women during pregnancy and lactation is still uncertain and more long-term RCTs with large sample sizes that carry on from beginning of pregnancy throughout lactation will be required to determine further effects of vitamin D supplementation on maternal and infant health and additional health outcomes such as bone health, immune functions and adverse pregnancy outcomes.

Chapter 5: Conclusion

To my knowledge our study is the first study to examine maternal vitamin D supplementation during pregnancy and lactation and explore its effects on maternal and infant 25OHD concentration. At baseline (13-24 weeks gestation), 21.2% of women had insufficient 25OHD concentrations (<50 nmol/L). At the end of study at 8 weeks postpartum, a significant difference was found in maternal 25OHD concentration between all treatment groups using ITT analysis (400 IU/d and 1000 IU/d: $p=0.004$, 400 IU and 2000 IU/d: $p=0.000$, 1000 IU/d and 2000 IU/d: $p=0.002$) with women in the 2000 IU/d group having the highest 25OHD concentration followed by the 1000 IU/d and 400 IU/d treatment groups. In comparison with the 400 IU/d group, the 1000 IU/d treatment group had a mean 25OHD concentration that was 8 nmol/L higher, and the 2000 IU/d treatment group had a mean 25OHD concentration that was 17 nmol/L higher. In mothers who were in the 2000 IU/d treatment group, only 2 out of 226 did not achieve serum 25OHD of 50 nmol/L using ITT analysis, and no women in the 2000 IU/d treatment group were below 50 nmol/L in the AT and >80% Compliant analysis. This suggests that maternal vitamin D supplementation of 2000 IU/d is sufficient for all women to achieve 25OHD concentrations above 50 nmol/L.

In infants, significant differences were observed between those whose mothers were in the in 400 IU/d and 2000 IU/d group ($p=0.000$) and 1000 IU/d and 2000 IU/d ($p=0.000$) but not between the 400 IU/d and 1000 IU/d group ($p=0.499$). Infants whose mothers were supplemented with the highest dose of supplemental vitamin D had the highest 25OHD concentration at 75 (95% CI: 67, 83) nmol/L. Compared to the 400 IU/d treatment group, the 1000 IU/d treatment group had a mean 25OHD concentration that was 7 nmol/L higher, and the 2000 IU/d treatment group had a mean 25OHD concentration that was 30 nmol/L higher.

Our study found no differences in BSAP between treatment groups in mothers or infants at 8 weeks postpartum. Consistent with Health Canada recommendations of 600 IU/d, our study found that women in our study had a combined intake of vitamin D from dietary (mean \pm SD: 187 \pm 110 IU/d) and supplement intake of approximately 600 IU/d.

Results from our study suggest that maternal supplementation of 2000 IU/d during pregnancy and lactation is effective in raising infant 25OHD concentration and may be protective against infant vitamin D deficiency at 8 weeks postpartum, however it is uncertain whether this concentration is maintained after 2 months. Further research is recommended to evaluate further health outcomes for maternal vitamin D supplementation in mothers and infants.

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Appendix

Appendix A



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THE UNIVERSITY
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Vitamin D in Pregnancy and Lactation

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Site

Children's and Women's Health Centre of British
Columbia

Background

Pregnant and breastfeeding women need to have adequate blood levels of vitamin D because vitamin D is important for health of the mother and baby. Scientists have observed that some Canadian women have low levels of vitamin D, especially women who do not take extra vitamin D from pills (a vitamin supplement). Humans get vitamin D from the foods we eat and we also make vitamin D in our skin when it absorbs sunlight. In winter in Canada, there is not enough sunlight to make this happen. Even in the summer some women may not get enough vitamin D if they spend little time outside or cover up with clothing and sunscreen. During pregnancy and breastfeeding, women need extra vitamin D so it is important that extra vitamin D from a supplement is taken. However, scientists do not yet know how much extra vitamin D pregnant and breastfeeding women need to ensure good health for themselves and their babies.

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Who Is Conducting This Study?

The study is being conducted by researchers at the Nutrition Research Program of the Child and Family Research Institute at the University of British Columbia. The Canadian Institutes of Health Research is funding the study.

What Is the Purpose of the Study?

We want to find out how much extra vitamin D pregnant and breastfeeding women need to take in order to achieve a healthy blood level of vitamin D in themselves and their babies.

Summary of Study

You are being invited to take part in this study because you are pregnant (less than 6 months) and between 18 and 45 years old. This study will only include women who want to take part. Please take time with your decision. This is a study in which one of three different amounts of vitamin D will be given. All of the amounts of vitamin D (400 IU, 1000 IU and 2000 IU) are safe and in amounts that have been suggested for pregnant women. There is no placebo (like a sugar pill) and all women will receive some vitamin D.

Who Can Participate?

Women who are 18-45 years old, who are between 13 and 22 weeks of pregnancy, expecting one baby, and planning to breast feed can take part in this study.

Who Should not Participate in This Study?

Women should not participate in this study if they:

have any other medical condition such as diabetes, cardiac or renal disease, HIV/AIDS, chronic high blood pressure, inflammatory bowel disease (i.e. Crohn's Disease and Ulcerative Colitis), autoimmune disease (i.e. Lupus), liver disease, epilepsy, celiac disease, or gastric bypass surgery (stomach stapling);
have had pregnancy complications before such as preterm delivery (<37 weeks), stillbirth, severe pre-eclampsia (hypertension of pregnancy), eclampsia, HELLP syndrome (hemolytic anemia, elevated liver enzymes, and low platelet count).
drugs known to interfere with vitamin D metabolism (i.e. corticosteroids).

Study Procedures

If you agree to participate, you will be asked to attend three appointments at the Children's and Women's Health Centre of British Columbia. The first appointment will last approximately 1 hour and the other two will last 30 to 45 minutes.

Clinic 1 (13-22 weeks of pregnancy; time 1- hour)

You will be asked to complete a questionnaire which includes questions on past pregnancies, ethnicity and vitamin and mineral supplement use. You will also be asked questions on how much sunlight exposure you get, including time spent outdoors, how much you cover up (i.e. clothes) and whether you use sunscreen.

You will be weighed and your height recorded and your blood pressure will be taken.

You will be asked to complete a food questionnaire to determine your intake of calcium and vitamin D over the previous month.

You will also have your skin color measured on your forearm and upper inner arm.

You will allow a nurse or other certified person to take a small blood sample of 14 mL (3 teaspoons). The blood will be used to measure vitamin D and other related factors.

You will provide a urine sample

You will stop taking any vitamin and mineral supplements you are currently taking. Instead you will take a standard pregnancy vitamin and mineral supplement (available in stores) provided to you.

You will also be assigned without bias to take supplements that contain one of three amounts of vitamin D.

You will be asked to take the supplement that you are assigned to until your baby is 8 weeks old.

Clinic 2 (36 weeks of pregnancy; Time 30 minutes)

You will have your skin color measured on my forearm and upper inner arm

You will allow a nurse or other certified person to take a small blood sample of 14 mL (3 teaspoons).

You will provide a urine sample (36 weeks only)

You will be weighed and blood pressure will be taken.

You will complete the same questionnaire regarding food intake and sun exposure over the last month as at the previous visit.

At this visit you will receive tubes, which will be used for your breast milk sample at the third and final visit.

Birth

A cord sample will be collected at birth

Clinic 3 (8 weeks after baby is born; Time 45 minutes)

You will collect a complete breast milk sample using an electric pump (either at home or provided during the visit) to completely express the milk from one breast ≥ 2 h after the previous feed. You may feed your baby from the alternate breast while you are collecting a sample of milk. If collection completed at home you will put the container in my freezer and bring it to clinic on my next appointment.

You will have your skin color measured on my forearm and upper inner arm

You will allow a nurse or other certified person to take a small blood sample of 14 mL (3 teaspoons).

You will allow a small sample of blood of about 2 ml (1/3rd teaspoon) to be collected from your baby by venipuncture.

You will be weighed, and the length and weight of your baby will be measured.

You will complete a questionnaire on the foods that you eat when your baby is 8 weeks old.

Other

You allow the investigators to access your health records solely to collect relevant information on your baby's birth, such as birth weight, and length.

Risks

It is felt that there are little known risks to participating in this study. There is no placebo and all women will receive at least the amount of vitamin D currently recommended by Health Canada. Very high intakes of Vitamin D (much higher than amounts used in this study) may cause an increase in blood calcium levels, which can cause mental confusion and heart arrhythmias. We will measure blood calcium levels at each visit to make sure it is not high.

A certified technologist, nurse, or other qualified person will draw the small amount of blood. Taking a blood sample is felt to have very low risks. The needles used to take blood might be uncomfortable and you might get minor bruising or, very rarely, an infection at the site of the needle poke. Taking a blood sample occasionally causes one to feel light-headed, faint and/or dizzy. The blood samples provided by you/you will not be used for any purposes other than this study. Very low vitamin D levels in infants causes rickets. This usually only occurs in mother who have not taken supplements. Within 7 days of collecting blood from your infant we will measure vitamin D. If the blood level is low we will contact you and provide you with free vitamin D supplements to give to your child.

Benefits

If you agree to take part in this study, there may or may not be a direct benefit to you. We will provide you with free pregnancy supplements. Also, we will monitor you and your infant's vitamin D blood levels. If we find the best amount of vitamin D, this information will be used to develop better vitamin supplements for use in pregnancy and lactation. Your parking and transportation costs to and from the hospital will be compensated for. You will be given gift certificates in the amount of \$25 at each visit in appreciation of the time it takes to complete the assessments in this study.

Confidentiality

In this study you/your child will be identified by a study code and any identifying information will be kept behind locked doors. No records which identify you/your child by name, initials or date of birth will be allowed to leave the Investigators' offices. You/ your child's confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada, and the UBC

Research Ethics Boards for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

Your/ your child's rights to privacy are protected and guaranteed by the "Freedom of Information and Protection of Privacy Act of British Columbia". This act lays down the safeguards respecting your privacy, and also gives you the right of access to the information about you that has been provided to the study, and if needed, you have the chance to correct any errors in this information. Further details about this act are available on request.

Consent

This study has been explained to you and you have been given the chance to ask questions about taking part in this study. If you have questions you can ask Dr. Tim Green or one of his associates.

Participation and Withdrawal from this Study

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time and do not have to give a reason for your decision. If you decide not to take part or decide to leave the study, you will continue to receive the best medical care available. You will be given a copy of this signed and dated consent form.

If you have any concerns about you/ your child's treatment or rights as a research subject, you may telephone the Research Subject Information Line, in the UBC Office of Research Services.

Compensation for Injury

Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

Your signature on the consent form means the following:

The study has been explained to you and all of your questions have been answered.
You understand what the study requires and the risks of the study; and
You agree to take part in this study.

**Subject Informed Consent
Vitamin D in Pregnancy and Lactation**

I have read and understood the information concerning this study.
I have had sufficient time to consider the information provided and to get advice and ask questions if necessary.
I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.
I understand that my/my child's participation in this study is voluntary and I do not have to take part in this study, and my refusal to participate or my decision to withdraw from the study will not change in any way the quality of care that I or my child receive(s).
I understand that signing this consent form in no way limits my legal rights against the sponsor, investigators, or anyone else.
I freely consent for my/my child's participation in this study. I understand that there is no guarantee that this study will provide any benefits to myself.
I am satisfied that the information contained in this consent form was explained to myself, that all questions have been answered.
I have been told that I will receive a dated and signed copy of this form.
I understand that if I have any questions or desire further information, I should contact Dr. Tim Green or one of his associates.
If I have any concerns about my/my child's rights as a research subject and/or my/my child's experiences while participating in this study, I may contact the 'Research Subject Information Line' ² in the University of British Columbia Office of Research Services.

I, _____ voluntarily give consent for my/my child's participation in the research study on vitamin D.

_____	_____	_____
<i>Signed</i>	<u>Tim J Green</u>	<i>Date</i>
_____	_____	_____
<i>Investigator Signature</i>	<i>Printed Name</i>	<i>Date</i>



Vitamin D in Pregnancy & Lactation Study



Are you pregnant and planning on breastfeeding? We need pregnant women in Vancouver and surrounding regions that are interested in helping us learn more about vitamin D!

Who Can Participate in the Study?

- Pregnant women between 18–42 years of age who plan to breastfeed
- Single pregnancy (not twins or multiples)
- No history of eclampsia, diabetes, kidney diseases, or cardiac disease
- No conditions affecting nutrient absorption
- No communicable diseases (AIDS etc.)



What would be required of you?

- 3 visits to BC Women and Children's Hospital
- At each visit:
 - Blood Sample & Urine Sample
 - Skin colour measurement
 - Fill out a food and lifestyle questionnaire
 - At Final visit a breast milk sample and a baby blood sample

What will YOU get from the study?

- You will learn about you and your infants vitamin D status
- You will receive pre-natal vitamins as part of the study
- Compensation and transportation costs will be covered for each visit

Appendix C

ARE YOU PREGNANT?

PLANNING ON BREASTFEEDING?

You may be eligible to participate in a research study to determine maternal and infant response to Vitamin D.

We are looking for participants that are between 13 & 22 weeks pregnant and do not have a history of pregnancy complications.

If eligible, you will receive maternal vitamin supplements and a vitamin D supplement.

You will be asked to give a blood sample, urine sample, and complete questionnaires about dietary vitamin D and sun exposure.

For more information please contact,

Principle Investigator:
Dr. Tim Green, Dept. Human Nutrition



What will YOU get from the Study?

- ✓ You will learn about you and your infants vitamin D status
- ✓ You will receive pre-natal vitamins
- ✓ You will be compensated for each of your visits
- ✓ You will be compensated for transportation/parking costs at BC Women and Children's Hospital
- ✓ You will aid scientific research and help set new guidelines for Vitamin D intake throughout pregnancy

CONTACTS:

Please contact us if you are interested or have any questions

Research Coordinator: Kaitlin March

Research Coordinator: Nancy Chen

OR checkout our website:
<http://blogs.landfood.ubc.ca/vitaminD>

Principal Investigator:
Tim Green, PhD.

Are you pregnant and interested in optimal health for you and your baby?



You are invited to participate in....

Vitamin D in Pregnancy and Lactation Study



Are you getting enough Vitamin D?

- Most Canadian women don't get adequate vitamin D all year long and may be deficient.
- Low vitamin D can lead to negative consequences for you and your baby
- Low vitamin D can increase the risk of pre-eclampsia and osteomalacia (bone loss) for mothers, it can also increase the risk of rickets, diabetes, asthma and poor dental health in infants
- Having an adequate vitamin D status can reduce the risk of these illnesses in your baby and lead to better life-long health

Are you pregnant and interested in Vitamin D?

We need pregnant women in Vancouver and surrounding regions that are interested in helping us learn more about Vitamin D throughout pregnancy and breast-feeding

Who can participate?

- ✓ Pregnant women between 18-42 years of age
- ✓ Single pregnancy (no twins)
- ✓ 13-22 weeks pregnant
- ✓ No history of eclampsia, diabetes, kidney disease or heart disease
- ✓ No conditions affecting nutrient absorption
- ✓ No communicable disease

The Study

- ✓ 3 ONE-HOUR long visits to BC Women and Children's Hospital (over 9 months)
- ✓ At the first two visits (during pregnancy):
 - ✓ Blood sample
 - ✓ Urine sample
 - ✓ Skin colour measurement
 - ✓ Fill out food and lifestyle questionnaire
- ✓ At the final visit (after delivery):
 - ✓ Breast milk sample
 - ✓ Baby blood sample



Appendix E: Inclusion/Exclusion Criteria

Inclusion/Exclusion Criteria:

DATE: _____

ID: _____

Participant:

Yes

No

Has a low risk pregnancy

Is 18-42 years of age

Is 13-22 weeks of gestation at randomization

Is expected to deliver a single, full-term

(37-42 weeks) infant

Does not have diabetes

Does not have cardiac disease

Does not have renal disease

Does not have any communicable disease

Answers to these questions must all be “yes” to be eligible to participate

Signature: _____

Date: _____

Appendix F

Patient Socio-Demographic and Pregnancy History

DATE: _____

ID: _____

Section 1: Socio-demographic Information

Date of birth? Day () Month () Year ()

Country of birth _____

Ethnicity

- White
- First Nations
- Black
- Chinese
- East Indian
- Other Asians (specify) _____

Infants Ethnicity

- White
- First Nations
- Black
- Chinese
- East Indian
- Other Asians (specify) _____

Education

- | | |
|--|------------------------------------|
| <input type="checkbox"/> High School | <input type="checkbox"/> Completed |
| <input type="checkbox"/> College | <input type="checkbox"/> Completed |
| <input type="checkbox"/> Undergraduate | <input type="checkbox"/> Completed |
| <input type="checkbox"/> Graduate | <input type="checkbox"/> Completed |

What is your usual occupation? _____

What is your total family income?

- <\$20,000
- \$20,000-\$50,000
- \$50,000-\$100,000
- >\$100,000

How many people are supported by the family income? _____

Section 2: Health Information

Height: _____ Ft. _____ Inches

Weight before pregnancy: _____

Is this your first pregnancy?

Yes

No

If no, how many times have you been pregnant? _____

Due date? ____/____/_____
Month / Day / Year

Have you ever had a miscarriage?

Yes

No

If yes, how many times have you miscarried? _____

Have you ever had preterm labour?

Yes

No

How many live births have you had? _____

Have you ever had twins or triplets?

Yes

No

Have you smoked cigarettes during this pregnancy?

Yes

No

If yes, what is the average number of cigarettes you smoke per week? _____

Have you consumed alcoholic beverages during this pregnancy?

- Yes
- No
- If yes, what is the average number of drinks per week? _____

During this pregnancy have you taken any vitamin and/or mineral supplements?

1. Supplements name _____
How often do you take them? _____
When did you start taking them? _____
Why did you start taking them? _____

2. Supplements name _____
How often do you take them? _____
When did you start taking them? _____
Why did you start taking them? _____

3. Supplements name _____
How often do you take them? _____
When did you start taking them? _____
Why did you start taking them? _____

During this pregnancy did you follow any particular diet?

- Yes
- No

If yes, what diet did you follow?

- Lacto-ovo vegetarian (eats all milk and milk products and eggs)
- Semi-vegetarian (eats all milk and milk products, eggs, poultry and fish)
- Vegan (avoids ALL animal products)
- Other, specify: _____

During this pregnancy have you taken any medications? (include current use)

Yes

No

If yes:

1. Medications: _____

Dose: _____

Condition: _____

Time period: _____

2. Medications: _____

Dose: _____

Condition: _____

Time period: _____

3. Medications: _____

Dose: _____

Condition: _____

Time period: _____

Appendix G: Sun Exposure and Lifestyle Questionnaire

Vitamin D During Pregnancy Study Questionnaire ID { }

Please note that all information on this questionnaire is labelled with an ID number only, and stored separately from your name and contact details. All information collected will be stored securely, and personally identifiable information will be accessible to no one except the investigators.

SUN EXPOSURE INFORMATION

Since we make most of our vitamin D from the action of sunlight on skin the next few questions focus on how much sunlight exposure you receive. The following questions refer to the last two months.

1. Do you cover up when outside?

YES NO

If yes what is covered,

- Head Legs (pantyhose, long dresses, trousers)
 Face Arms
 Hands

2. When you are in the sun, do you wear a hat or scarves:

1 never 2 sometimes 3 frequently

3. When you are in the sun, do you wear long sleeves/ long pants, pantyhose or skirt:

1 never 2 sometimes 3 frequently

4. A) Over the last two months during your work days are you:

Mostly indoors / mixture of in and outdoors / mostly outdoors? (please circle)

B) Over the last two months, during your workdays, how long have you spent outdoors, on average, each day?

- 1 less than 15 minutes/day 2 15 to 30 minutes/day
3 30 minutes to 1 hour/day 4 1 to 2 hours/day
5 2 to 4 hours/day 6 more than 4 hours/day

C) Do you ever work night shift? YES NO

Occasionally / fairly often / most of the time

5. A) Over the last two months during your leisure days are you:

Mostly indoors / mixture of in and outdoors / mostly outdoors? (please circle)

B) Over the last two months, during your leisure days, how long have you spent outdoors, on average, each day?

1 less than 15 minutes/day 2 15 to 30 minutes/day

3 30 minutes to 1 hour/day 4 1 to 2 hours/day

5 2 to 4 hours/day 6 more than 4 hours/day

6. Have you traveled outside Canada in the last 2 months? YES No

Where? _____ When? _____ For how long? _____

Where? _____ When? _____ For how long? _____

7. Have you sunbathed regularly over the last two months to try to get a suntan? (By sunbathe we mean that you stayed out in the sun because you wanted your skin to go browner or more golden in color)

YES

NO

8. Have you used a sunbed in the last 3 months?

NO

Yes, once every 2 weeks

Yes, more than twice a week

Yes, monthly

Yes, once a week

Yes, every 2 or 3 months

9. Over the last two months how often did you use sunscreen (or moisturizer, make-up etc. that contains sun protection) when you were out in the sun?

(please check only one box).

Never

Sometimes

Most of the time

Always

10. What was the Sun Protection Factor (or SPF) of the sunscreen you usually or mainly used?

(please check only one box).

Didn't usually use a sunscreen

- Less than SPF 15
- SPF 15
- SPF 30
- Greater than SPF 30
- Don't know

11. Did you ever put sunscreen on some parts of your body that were exposed to the sun, but not on other exposed parts? For example, you put sunscreen on your face but did not put it on your arms or legs. (Please Indicate)

PERSONAL INFORMATION

The next few questions are taken mainly from last Canadian Census in 2006. The answers to these questions will allow us to compare the people in our survey to the wider Vancouver population. Remember you information will be kept strictly confidential

1. Date of Birth (Day/Month/Year)

2. In what country were you born?

If you were not born in Canada what year did you come to Canada to live?

.....

3. What were the ethnic or cultural origins of your ancestors? An ancestor is usually more distant than a grandparent. For example, Canadian, English, French, Chinese, Italian, German, Scottish, East Indian, Irish, Cree, Mi'kmaq (Micmac), Métis, Inuit (Eskimo), Ukrainian, Dutch, Filipino, Polish, Portuguese, Jewish, Greek, Jamaican, Vietnamese, Lebanese, Chilean, Salvadorean, Somali, etc. Specify as many origins as applicable

.....
.....

4. Are you an Aboriginal person, that is, North American Indian, Métis or Inuit (Eskimo)?

No, Continue with next question

Yes, North American Indian

Yes, Metis

Yes, Inuit (Eskimo)

5. Are you (check all that apply)

White

Southeast Asian (e.g., Vietnamese, Cambodian, Malaysian, Laotian, etc.)

Chinese

South Asian (e.g., East Indian, Pakistani, Sri Lankan, etc.)

Black

Filipino

- Latin American
- Arab
- West Asian (e.g., Iranian, Afghan, etc.)
- Korean
- Japanese
- Other — Specify

6. How many people live in your household?

7. What is your best estimate of the total income, before taxes and deductions, of all household members from all sources in the past 12 months?

\$.....

- Don't know
- Don't want to say

1. What is the highest level of education you have completed?

- | | |
|--|---|
| <input type="checkbox"/> No schooling | <input type="checkbox"/> Completed trade/ vocational training |
| <input type="checkbox"/> Some Elementary | <input type="checkbox"/> Some University |
| <input type="checkbox"/> Completed elementary | <input type="checkbox"/> Completed University |
| <input type="checkbox"/> Some trade/ vocational training | |

TYPE of Food or DRINK	Never or less than 1 per month	1 per month	2 - 3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Serving Size		
											S	M	L
Milk: whole, 2%, 1% or skim										1 cup (8 oz or 250 ml)			
Chocolate Milk										1 cup (8 oz or 250 ml)			
Soy Milk Beverage: Fortified										1 cup (8 oz or 250 ml)			
Soy Drink: Plain (not fortified)										1 cup (8 oz or 250 ml)			
Other plant milks (rice, potato, etc)										1 cup (8 oz or 250 ml)			
Milk in coffee or tea										1 tablespoon			
Milk on cereal (if not included above)										1/2 cup			
Milk shake										1 cup (8 oz or 250 ml)			
Milk dessert (ice cream, pudding)										1/2 cup (one scoop, 1 container)			
Yogurt (milk or soy)										1/2 cup (125 g, 1 container)			
Soft Cheese										1 tablespoon			
Hard Cheese										1 cube 2" (2 slices)			
White bread, roll, bun, biscuit, bagel, nan, tortilla										1 slice, 1 small roll, 1/2 bagel			
Dark bread, roll, bagel										1 slice, 1 small roll, 1/2 bagel			
Taco chips, nacho chips										1 cup (28g)			
Waffle, pancake, French toast										1 piece (4" round)			
Butter (in any foods eaten)										1 pat 1 teaspoon			
Margarine (in any foods eaten)										1 pat 1 teaspoon			

TYPE of Food or DRINK	Never or less than 1 per month	1 per month	2 -3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	Serving Size		
											S	M	L
Tofu	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cube (2")	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Macaroni with cheese	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned Salmon	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	2 tablespoons or 1 cup of casserole	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned Tuna	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	2 tablespoons or 1 cup of casserole	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned Sardines	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	2 fish (1/2 can)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salmon Steak	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	90 g (3 oz)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other fish: white	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	90 g (3 oz)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other fish: oily	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	90 g (3 oz)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cream soups made with milk	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cup (8oz or 250 ml)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Taco or burrito made with cheese	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 regular taco: 1/2 burrito	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pizza made with cheese	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lentils, beans, peas	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1/2 cup cooked	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Eggs: eaten alone or in other foods	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 large egg	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Potatoes: mashed with milk & margarine	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1/2 cup (1scoop)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Orange Juice: not fortified with calcium, vitamin D	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cup (8 oz or 250 ml)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Orange Juice: with calcium, vitamin D	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cup (8 oz or 250 ml)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Broccoli, kale, greens	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cup raw or 1/2 cup cooked	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Seafood: e.g shrimp, crab	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1 cup meat	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Shellfish: e.g Mussels	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	1/2 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Appendix I:

Supplement Usage Throughout Pregnancy

Study ID: _____ **Date:** _____

Vitamin D

Approximately how many times did you take your vitamin D supplement in the last week?

Approximately how many times did you take your vitamin D supplement in the last two months?

Multivitamin

Did you take the multivitamin provided?

If yes, how many times did you take the multivitamin in the last week?

And approximately how many times did you take the multivitamin in the last two months?

Last Visit

Have you been supplementing your infant?

If yes, with what?
