THE EFFECT OF THREE DOSAGES OF SUPPLEMENTAL VITAMIN D (400, 1000 AND 2000 IU) ON MATERNAL AND NEWBORN 25-HYDOXYVITAMIN D CONCENTRATIONS

by

Kaitlin March

B.Sc., Queen’s University, 2009

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE in

THE FACULTY OF GRADUATE STUDIES (Human Nutrition)

THE UNIVERSITY OF BRITISH COLUMBIA (Vancouver)

August 2012

© Kaitlin March, 2012
Abstract

BACKGROUND: Attaining adequate vitamin D throughout pregnancy is important for maternal and infant health. Current Canadian guidelines for vitamin D intake vary; the Recommended Dietary Allowance (RDA) set by Health Canada is 600IU/d whereas the Canadian Pediatric Society recommends 2000IU/d. At present, there is a lack of evidence as to the most appropriate intake.

OBJECTIVE: To determine the effect of each of three dosages of supplemental vitamin D (400, 1000 and 2000IU) throughout pregnancy on the percentage of women achieving sufficient serum 25-hydroxyvitamin D (25OHD) defined as > 50 nmol/L and the maternal and newborn 25OHD concentrations in response to each supplementation dose.

METHODS: In a randomized, double-blind, controlled trial pregnant women (n=110) were randomized to one of three doses of supplemental vitamin D₃ (400, 1000 or 2000 IU) at 13-22 weeks (baseline). Participants attended a study visit at 36 weeks and infant cord blood was collected at delivery. Blood was collected for analysis for serum 25OHD at baseline and 36 weeks gestation.

RESULTS: At baseline 18.2% of participants were classified as vitamin D insufficient (serum 25OHD < 50 nmol/L) with 10.9% vitamin D insufficient at 36 weeks. There were no significant differences among treatment groups in the percentage of women classified as vitamin D insufficient at 36 weeks gestation [400IU: 11%, 1000IU: 18.4%, 2000IU: 5.6%, p=0.227] or in serum 25OHD concentrations [400IU: 70.4±19.5 nmol/L, 1000IU: 76.5±25.7 nmol/L, 2000IU 80.1±20.3 nmol/L, p=0.178]. The change in serum 25OHD concentrations from baseline to 36 weeks was significantly greater in the 1000IU and 2000IU group versus the 400IU group [400IU: -0.08±15.5 nmol/L, 1000IU: 10.5±20.8 nmol/L, 2000IU:12.4±13.7...
Newborn serum 25OHD concentrations did not differ across treatments [400IU: 72.2±21.4, 1000IU: 65.5±22.6, 2000IU: 91.5±19.8, \( p=0.111 \)].

**CONCLUSION:** There were no significant differences among three dosages of supplemental vitamin D (400, 1000 and 2000IU) in the percentage of women achieving serum 25OHD > 50 nmol/L. Maternal and newborn 25OHD concentrations were not significantly different among women on the three dosages of supplemental vitamin D.
Preface

This thesis contains the work of a research study conducted by myself, the candidate, Kaitlin March, under the supervision of Dr. Tim Green with guidance from Dr. Peter Von Dadelszen, Dr. Sheila Innis, Dr. Hope Weiler and Dr. Susan Whiting. Dr. Tim Green, Dr. Sheila Innis, Dr. Antonia Shand and Dr. Peter Von Dadelszen completed the study design. The research presented in this thesis is part of a larger research project examining maternal and infant responses to maternal vitamin D supplementation throughout pregnancy and lactation. The research team for this study was comprised of graduate students, other research assistants, volunteers and myself.

I was responsible for starting the study in combination with Dr. Green 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 manuscript were primarily my work. This study is a large randomized controlled trial with 225 participants each seen over a 9 month time period. As the study is ongoing at the time of publication of this thesis, my manuscript only focuses on the first two clinic visits of the first 110 participants, concentrating on maternal 25OHD outcomes throughout pregnancy. Further research from this study will emphasize infant outcomes and breast milk 25OHD content. 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 (NCT01112891).
Table of Contents

Abstract ................................................................................................................................................ i
Preface .................................................................................................................................................... iii
Table of Contents ................................................................................................................................. iv
List of Tables ........................................................................................................................................ vi
List of Figures ....................................................................................................................................... vii
List of Abbreviations ........................................................................................................................... viii
Acknowledgements .............................................................................................................................. ix

Chapter 1: Literature Review ............................................................................................................... 1
  1.1 Introduction ..................................................................................................................................... 1
  1.2 Vitamin D metabolism .................................................................................................................. 4
    1.2.1 Physiological roles of vitamin D ......................................................................................... 5
    1.2.2 Consequences of low vitamin D ....................................................................................... 6
  1.3 Assessment of vitamin D status ...................................................................................................... 7
    1.3.1 Factors affecting 25-hydroxyvitamin D .......................................................................... 8
    1.3.2 Defining adequate vitamin D status using 25OHD ......................................................... 9
    1.3.3 Vitamin D deficiency ......................................................................................................... 9
    1.3.4 Vitamin D insufficiency and sufficiency ......................................................................... 9
  1.4 Vitamin D metabolism in pregnancy ............................................................................................ 10
  1.5 Consequences of inadequate vitamin D in pregnancy ................................................................. 12
    1.5.1 Maternal consequences of low vitamin D ...................................................................... 13
      1.5.1.1 Musculo-skeletal health ......................................................................................... 13
      1.5.1.2 Preeclampsia .......................................................................................................... 14
      1.5.1.3 Gestational diabetes mellitus ............................................................................... 15
    1.5.2 Infant consequences of low maternal vitamin D ............................................................. 16
      1.5.2.1 Musculo-skeletal health ......................................................................................... 16
      1.5.2.2 Asthma .................................................................................................................. 17
      1.5.2.3 Type 1 diabetes mellitus ....................................................................................... 18
  1.6 Current recommendations for vitamin D ...................................................................................... 19
    1.6.1 Adverse outcomes of high vitamin D ............................................................................. 21
  1.7 Vitamin D status of pregnant and lactating women ...................................................................... 22
  1.8 Vitamin D supplementation during pregnancy ............................................................................ 24
    1.8.1 Vitamin D supplementation during pregnancy on maternal 25OHD and neonatal outcomes ............................................................................................................................... 24

Chapter 2: Research Study ................................................................................................................... 34
  2.1 Purpose .......................................................................................................................................... 34
  2.2 Objectives and hypothesis ............................................................................................................. 34
  2.3 Methods ......................................................................................................................................... 35
    2.3.1 Overview ............................................................................................................................ 35
    2.3.2 Sample size ........................................................................................................................ 35
    2.3.3 Recruitment and participant selection .............................................................................. 36
    2.3.4 Procedures .......................................................................................................................... 37
    2.3.5 Supplements ....................................................................................................................... 38
    2.3.6 Dietary assessment ............................................................................................................ 38
    2.3.7 Blood and urine samples ................................................................................................... 39
List of Tables

Table 1.1  25OHD status of Canadian pregnant women……………………………………30
Table 1.2  Controlled trials of vitamin D supplementation throughout pregnancy……31
Table 1.3  Hollis et al. supplementation during pregnancy: double-blind, randomized, clinical trial of safety and effectiveness………………………………………33
Table 2.1  Natural Factors prenatal vitamin product monograph………………………44
Table 2.2  Vitamin D supplement dosage quality control (IU)……………………………45
Table 3.1  Baseline participant characteristics……………………………………………54
Table 3.2  Compliance by supplement dosage…………………………………………….55
Table 3.3  Serum 25OHD concentrations (nmol/L) during pregnancy and in cord blood (ITT)………………………………………………………………………56
Table 3.4  Serum 25OHD concentrations (nmol/L) during pregnancy and in cord blood (AT) …………………………………………………………………………………56
Table 3.5  Serum 25OHD concentrations (nmol/L) during pregnancy and in cord blood (>80% compliant)……………………………………………………………57
Table 3.6  The percentage of women achieving different cut-offs of serum 25OHD at 36 weeks gestation (ITT)……………………………………………………………58
Table 3.7  The percentage of women achieving different cut-offs of serum 25OHD at 36 weeks gestation (AT)……………………………………………………………58
Table 3.8  The percentage of women achieving different cut-offs of serum 25OHD at 36 weeks gestation for those over 80% compliant……………………………………58
Table 3.9  Estimated difference in 25OHD concentrations (nmol/L) according to selected variables (ITT)……………………………………………………………………59
Table 3.10 Estimated difference in 25OHD concentrations (nmol/L) according to selected variables, as-treated…………………………………………………………59
Table 3.11 Dietary vitamin D and calcium intake throughout pregnancy (ITT)……………60
Table 3.12 Dietary vitamin D and calcium intake throughout pregnancy, as-treated…..60
Table 3.13 Total circulating bone-specific alkaline phosphatase (BSAP) concentrations (ug/L) during pregnancy (ITT)…………………………………………………61
Table 3.14 Total circulating bone-specific alkaline phosphatase (BSAP) concentrations (ug/L) during pregnancy, as-treated…………………………………………………61
Table 3.15 Serum and urine creatinine calcium and phosphate at baseline and 36 weeks gestation (ITT)………………………………………………………………………62
Table 3.16 Serum and urine creatinine calcium and phosphate at baseline and 36 weeks gestation, as-treated………………………………………………………………………63
List of Figures

Figure 1.1 Hydroxylation of vitamin D3 ................................................................. 29
Figure 1.2 The vitamin D endocrine system ......................................................... 29
Figure 3.1 Flow diagram of pregnancy study, ....................................................... 53
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25OH₂D</td>
<td>1,25-hydroxyvitamin D</td>
</tr>
<tr>
<td>25OHD</td>
<td>25-hydroxyvitamin D</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>AT</td>
<td>As-Treated</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BSAP</td>
<td>Bone Specific Alkaline Phosphatase</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Cell Count</td>
</tr>
<tr>
<td>CHMS</td>
<td>Canadian Health Measures Survey</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D Binding Protein</td>
</tr>
<tr>
<td>DBRCT</td>
<td>Double-blind Randomized Controlled Trial</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>GDM</td>
<td>Gestational Diabetes Mellitus</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention to Treat</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>ND</td>
<td>Not Determined</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic Status</td>
</tr>
<tr>
<td>T1DM</td>
<td>Type 1 Diabetes Mellitus</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable Upper Level of Intake</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
</tr>
</tbody>
</table>
Acknowledgements

I would first and foremost like to acknowledge and thank my supervisor, Dr. Tim Green, for his guidance, encouragement, mentorship and support over the last three years and most importantly for allowing me countless opportunities and experiences that have developed my interest and passion for research in the field of nutrition. I would also like to thank my committee members, Dr. Sheila Innis and Dr. Peter von Dadelszen for their expertise, support and valuable insights into the project and the use of their resources both within the Child and Family Research Institute and BC Women’s Hospital.

I am grateful for the sources of funding provided to me throughout this project from Dr. Tim Green, The University of British Columbia, the Canadian Institutes of Health Research and the Child and Family Research Institute. I would like to acknowledge the Children and Women’s Health Centre of British Columbia for their support in this research and the use of the hospital facilities and resources. Many thanks to John Bhullar and the clinical laboratory staff for their phlebotomy expertise and sample analyses. Sincere thanks to Dr. Susan Whiting for the food frequency questionnaire analyses and Dr. Hope Weiler for her guidance on the project, expertise in the field and analysis of our 25-hydroxyvitamin D and bone-specific alkaline phosphatase samples. I would also like to thank the participants who have volunteered their time and the excitement they brought to the project.

Lastly I would like to thank my amazing lab mates and fellow graduate students, as I couldn’t have done it without them. A special thank you to Nancy Chen, the other graduate student on this project and to Andrea Magwood, Russell Friessen, Nicole Curtis, Samira Dadgar and Debbie van den Brink for the knowledge and time they dedicated to the study. I would also like to thank fellow graduate students Sarah Harvey, Kelly Mulder, Kirstin
Wingate and Sarah Neil for their support and friendship during this time. Special thanks are owed to my parents, who have supported me throughout my years of education, both emotionally and financially.
Chapter 1: Literature Review

1.1 Introduction

Vitamin D is a fat-soluble vitamin that functions as a steroid hormone when activated and is best known for its role in regulating serum calcium and phosphorous concentrations [1]. Vitamin D has been brought to the forefront of nutrition research in recent years as it has been recognized that vitamin D has roles beyond calcium and phosphorous metabolism; indeed vitamin D receptors have been found in many different types of cells throughout the body [1, 2]. Inadequate vitamin D status has been associated with cancer, cardiovascular disease, infections and several autoimmune conditions such as Type 1 Diabetes [2, 3].

Vitamin D is naturally present in very few foods but is produced endogenously in the skin following ultra violet (UV) exposure from sunlight. Provided there is adequate UV light, most people can achieve sufficient vitamin D blood concentrations; however, many factors influence exposure to adequate UV light such as season, latitude, weather, clothing, skin melanin content, time of day, and indoor work and lifestyles [4]. People who cannot achieve adequate vitamin D through UV exposure may improve their status through increasing consumption of foods rich in or fortified with vitamin D, or through supplementation. Major natural food sources of vitamin D are limited to fatty fish and organ meats [5]. Circulating serum 25-hydroxyvitamin D (25OHD) is currently accepted as the best indicator of vitamin D status as it represents both dietary intake and vitamin D obtained through skin synthesis [6]. The optimal serum 25OHD concentration is unknown, however, the United States Institute of Medicine has stated that maintaining a blood serum concentration of 25OHD > 50 nmol/L is suitable for bone health outcomes in adults and infants including pregnant and
lactating women. However, the Canadian Pediatric Society recommends concentrations of 25OHD > 75 nmol/L as “sufficient” for pregnant and lactating women and infants [7, 8].

Adequate vitamin D status is important at all life stages, but attaining adequate concentrations throughout pregnancy and lactation may be especially important for maternal and infant health. Low vitamin D concentrations in mothers and infants can result in poor fetal and infant bone mineralization, which may persist into later life [9, 10]. For instance, vitamin D deficiency throughout pregnancy is linked to infantile rickets, which is found mainly in exclusively breastfed infants born to vitamin D deficient mothers [11]. Moreover, low vitamin D concentrations in pregnancy and infancy have been associated with increased risk of maternal preeclampsia and increased incidence of asthma and Type 1 Diabetes in infants and children, which can persist into later life [12-14].

Vitamin D insufficiency is prevalent in Canadian women of childbearing age and pregnant women [15, 16]. Women in Canada receive little sun exposure in the winter months and those of childbearing age receive less than 400 IU/d of vitamin D between diet and supplements combined [3, 5]. Research has fortunately shown that over 80% of women take a prenatal supplement during pregnancy, and vitamin D supplementation would be the safest and most cost-effective way to improve vitamin D status [17]. Current recommendations for vitamin D intake during pregnancy vary considerably; Health Canada’s DRI (dietary reference intake) is 600 IU/d whereas the Canadian Pediatric Association recommends 2000 IU/d [8, 18]. At present, we have neither clear guidelines about how much vitamin D pregnant should be taking, nor whether current dosages and recommendations are adequate for optimal health of mother and child. This priority health issue was addressed by Health
Canada in a call for research to “determine optimal vitamin D intakes for pregnant and lactating women as a function of latitude and race” [19].

Given the lack of knowledge regarding the vitamin D intake during pregnancy necessary to achieve a serum 25OHD > 50 nmol/L and the scarcity of Canadian data regarding vitamin D led to this research. The objectives are in a diverse group of Vancouver pregnant women to determine:

1. The effect of three doses of supplemental vitamin D on maternal and neonate serum 25OHD concentrations.

2. The percentage of women taking different supplemental dosages of vitamin D that achieve serum 25OHD concentrations of > 50, 75 and 100 nmol/L.

3. Determine the effect of three doses of supplemental vitamin D on maternal and neonate serum BSAP, a marker of bone formation.

4. Factors that predict vitamin D status among pregnant women at 36 weeks gestation, including the importance of dietary vitamin D intake, season and ethnicity.

The first sections of this literature review summarize vitamin D metabolism, methods for assessment of vitamin D status and criteria used to define vitamin D deficiency and sufficiency. These are followed by discussions of the consequences of vitamin D inadequacy during pregnancy for both mother and infant. Current knowledge regarding vitamin D status of Canadian pregnant women is then summarized and finally information on vitamin D supplementation during pregnancy and its impact on maternal serum 25OHD concentrations and neonatal outcomes are reviewed.
1.2 Vitamin D metabolism

Vitamin D and its metabolites are classified into two sterol families based on their precursors. The first form is animal-derived cholecalciferol (vitamin D$_3$), which is produced endogenously within the body. Endogenous vitamin D production begins when sunlight interacts with 7-dehydrocholesterol found in the cutaneous layer of the epidermis. Ultraviolet blue (UVB) wavelengths between 290-320 nm penetrate the skin and this leads to synthesis of previtamin D$_3$ by breaking the β-steroid ring in 7-dehydrocholesterol [20]. The second form of vitamin D is ergocalciferol (vitamin D$_2$), which is derived from fungi or yeast sterols by exposing food sources or yeast to UV radiation [21]. It differs from vitamin D$_3$ molecularly in its side chain structure as it has an additional double bond between carbons 22 and 23 as well as a C$_{24}$-methyl group. Vitamin D$_2$ is not produced by the body but can be found in supplements and fortified food products. There is evidence that dietary or supplemental vitamin D$_3$ is more bioavailable than vitamin D$_2$ [21].

Two hydroxylation events occur in the conversion of vitamin D to its active form; the first occurs in the liver and the second in the kidney (Figure 1.1). Vitamin D metabolites are transported throughout the body via the blood stream bound to a vitamin D-binding protein (DBP), which stabilizes and protects them from photo degradation [22]. In the liver it is believed that the 25-hydroxylation event is carried out by the 25-hydroxylase enzyme CYP2R1, which is not liver specific but is most active within the liver [1]. The resulting 25-hydroxyvitamin D (25OHD) is the main form of vitamin D circulating in the blood [23]. 25OHD bound to vitamin D binding protein is transported through the blood and is again hydroxylated in the kidneys by the enzyme 1α-hydroxylase (CYP27B1). The renal conversion of 25OHD to 1,25-hydroxyvitamin D (1,25OH$_2$D), also known as calcitriol, is
tightly regulated by parathyroid hormone (PTH), which in turn is controlled by blood calcium and phosphate concentrations. The enzyme, 1α-hydroxylase has also been found in other tissues where vitamin D may play a role, such as the intestine, lymphocytes, brain, heart, bone and kidneys [22, 24].

1.2.1 Physiological roles of vitamin D

The active 1,25OH₂D exerts its biological effects by binding to intracellular vitamin D receptors (VDR’s) and acting as a transcription factor as the receptor has both DNA binding and hormone binding domains. Vitamin D’s most well described role is in calcium regulation and phosphorous homeostasis resulting in normal bone mineralization and prevention of rickets and osteoporosis. Calcium concentrations within the blood are sensed by the parathyroid glands. In response to low serum calcium, the parathyroid glands release parathyroid hormone (PTH), which stimulates the conversion of 25OHD to 1,25OH₂D in the kidney. 1,25OH₂D and PTH cause bone resorption, which in turn releases calcium and phosphorous. 1,25OH₂D also acts in the small intestine causing increased absorption of calcium, and in the kidney it promotes calcium reabsorption. The net result of these processes is to increase serum calcium concentrations. The reverse happens when serum calcium concentrations are high, resulting in decreased calcium absorption and increased bone absorption of calcium and phosphorous which together lower serum calcium concentrations. Vitamin D receptors (VDRs) are found in bone and throughout the GI tract, as well as in various tissues not associated with phosphorous and calcium homeostasis, such as skeletal muscle [25]. The ubiquitous expression of VDRs has created interest in other roles for vitamin D; it has been hypothesized that 1,25OH₂D has diverse functions in the body, or other ligands may bind VDRs.
1.2.2 Consequences of low vitamin D

Vitamin D inadequacy exerts it’s effects on bone mineralization via secondary hyperparathyroidism. Secondary hyperparathyroidism occurs when chronically low serum calcium concentrations result in increased serum concentrations of PTH. PTH and 25OHD are inversely correlated, as PTH concentration increases this stimulates kidney conversion of 25OHD to active 1,25OH$_2$D. PTH also directly acts upon the bone itself causing calcium resorption to increase serum calcium concentrations. Active 1,25OH$_2$D also causes bone resorption of calcium and causes increased intestinal calcium absorption. When vitamin D is inadequate, PTH concentrations remain elevated, however there is no increase in intestinal calcium absorption and PTH continues to act directly on bone to promote resorption (Figure 1.2). Therefore, chronic inadequate vitamin D status directly affects bone health by weakening bones over time.

Severe vitamin D deficiency is associated with poor skeletal mineralization, in particular the classical diseases of rickets and osteomalacia. Rickets is found in children and characterized by soft bones and skeletal deformities when skeletal mineralization doesn’t occur. The prominent clinical manifestations of severe rickets are rachitic rosary, bowed legs, bone pain, muscle weakness, growth disturbances and an increased risk for fractures [3]. Osteomalacia is the adult form of rickets. It is also a result of poor skeletal mineralization and can cause aches and pains in bone, increased fracture risk, impaired muscle function and can lead to waddling gait and physical deformities [26].

Osteoporosis is a disease characterized by low bone mass and the deterioration of bone tissue leading to loss of independence, injury from falls and fractures, and potentially death [27]. Cross-sectional studies have shown that low vitamin D status is associated with
lower bone mineral density [28]. It has been shown that vitamin D supplementation, along with calcium can increase bone mineral density and reduce the risk of fractures, although results have not been entirely consistent [27, 29]. Low vitamin D status is also associated with non-skeletal effects on health. There is currently evidence for a biological/mechanistic role for vitamin D in cancer, but findings regarding vitamin D’s protective effect have been inconsistent [30]. Other emerging roles for vitamin D include the prevention and treatment of type 1 diabetes mellitus, multiple sclerosis, cardiovascular disease and other medical conditions [20]. A discussion of these conditions is beyond the scope of this thesis.

1.3 Assessment of vitamin D status

A number of blood biomarkers have been used to assess vitamin D status. The three most common biomarkers include serum PTH, 1,25OH₂D and 25OHD concentrations. Serum PTH is inversely associated with serum 25OHD concentration; in vitamin D deficiency serum PTH synthesis is increased and calcium absorption decreases, thus serum PTH concentration is not a good indicator of vitamin D status as PTH concentrations plateau at a threshold concentration of 25OHD, further the concentration of 25OHD at which PTH will no longer increase has varied considerably among studies [31, 32]. Other factors such as calcium and phosphorus intake, stage of life, ethnicity, physical activity and serum phosphate concentration can affect serum PTH concentration further limiting its usefulness as an assessment tool for vitamin D status [33]. Circulating 1,25OH₂D concentrations are tightly regulated by serum calcium, phosphorus and PTH. Serum 1,25OH₂D can remain normal in mild vitamin D deficiency and may rise and then fall only in more severe vitamin D deficiency, making it a poor indicator of vitamin D status [34]. 1,25OH₂D only represents recent vitamin D production or intake and has a short half-life of around 15 hours, making it
more difficult to use as a measure of vitamin D status [23]. Currently, the best indicator of vitamin D status is considered to be circulating serum 25OHD concentration. Serum 25OHD has a longer half-life of around 15 days compared to 1,25OH$_2$D and circulating 25OHD represents vitamin D from both endogenous and exogenous sources [6, 23]. 25OHD concentrations can be measured in both plasma and serum, however in this thesis we have used serum 25OHD and when 25OHD concentrations are mentioned it is assumed it is within the serum.

1.3.1 Factors affecting 25-hydroxyvitamin D

Ultraviolet light exposure is a major determinant of 25OHD, which in turn is dependent on climate, season, latitude, sunscreen use, clothing, tanning, ethnicity/skin colour, and age. For example, African Americans generally have lower 25OHD concentrations than Whites living in the same geographical area, presumably because of their darker skin colour [35]. In the winter months at higher latitudes, such as Canada, there is little or no UV light and thus people in these geographic regions require an exogenous source of vitamin D, either dietary or supplements. Food sources of vitamin D are usually of animal origin and include fatty fish, cod liver oil, liver, organ meats and egg yolks. Some countries fortify the food supply with vitamin D; foods commonly fortified include milk, margarine and spreads, yogurt and cereal products. In Canada fluid milk products are fortified with ~100 IU of vitamin D per 250 mL serving and all margarines are fortified with 530 IU/100g [5]. Individuals in Canada who consume milk products have higher 25OHD concentrations than those who do not [5].
1.3.2 **Defining adequate vitamin D status using 25OHD**

Blood concentrations of serum 25OHD have been used to define vitamin D status. Currently there is no standard definition of optimal vitamin D status. Vitamin D deficiency is defined as serum concentration of 25OHD below which there is an increased risk of osteomalacia and rickets. Vitamin D insufficiency is not associated with clinical rickets or osteomalacia, but is a lesser form of vitamin D deficiency associated with increased risk of disease such as osteoporosis, multiple sclerosis and certain cancers [6, 31]. While Vitamin D deficiency is well-defined, considerable controversy exists over what concentrations of serum 25OHD define vitamin D insufficiency. Most studies examining the relationship between clinical end-points in health and vitamin D have been descriptive case control and cohort studies, with relatively few randomized control trials (RCT).

1.3.3 **Vitamin D deficiency**

In adults, serum concentrations of 25OHD below 12.5 nmol/L have been associated with well-defined osteomalacia [36]. Similar research has found the same or similar cut-offs to be associated with rickets in children, in confirmed cases of rickets the 25OHD serum concentration is almost always below 25 nmol/L [37]. Though the exact serum concentration is somewhat arbitrary, it has been found that over 80% of children diagnosed with rickets have a 25OHD concentration less than 20 nmol/L [38]. Generally, a cut-off of 25 nmol/L is used to indicate vitamin D deficiency.

1.3.4 **Vitamin D insufficiency and sufficiency**

Although there is no universally accepted definition of insufficiency expert groups have favoured cut-offs between 50-80 nmol/L of circulating 25OHD, with higher concentrations for defining optimal status of 75 to over 100 nmol/L 25OHD [32, 39].
Estimates of optimal 25OHD cut-off concentrations have been based on clinical and functional end-points regarding health status [40]. These functional end-points are typically related to bone health and have included calcium absorption, bone mineral density, PTH concentrations and fractures and falls. Multiple issues surrounding these cut-offs exist, including different measurement assays for 25OHD and different functional endpoints used to define status [3]. Further, most of these studies to date have focused on the elderly and are associated with bone and skeletal outcomes [41]. There is little data about sufficient and optimal 25OHD status at other life-stages, particularly pregnant and lactating women.

Currently, the Canadian Pediatric Society recommends a 25OHD concentration greater than 75 nmol/L in pregnancy [42]. The Institute of Medicine (IOM), which advises both Canadian and American governments on dietary reference intakes calls for a serum vitamin D concentration > 50 nmol/L for people at all life stages, including pregnancy and lactation [18]. The current vitamin D recommendations will be further discussed in section 2.5. Due to the uncertainty surrounding optimal 25OHD concentrations I will define optimal 25OHD status as > 50 nmol/L throughout pregnancy and lactation based on the recommendations of the IOM, although other cut-offs are considered in this research.

1.4 Vitamin D metabolism in pregnancy

Alterations of mineral metabolism and hormones occur throughout pregnancy. Calcium metabolism is significantly altered in the mother throughout pregnancy, lactation and after weaning. Calcium needs are increased for fetal bone mineral accretion, breast milk synthesis and restoring the maternal skeleton after birth [43]. Over the course of pregnancy, between 25 and 30 g of calcium is transferred to the fetus; equivalent to approximately 2-3 mg/day during the first and second trimesters, reaching to 250 mg/day in the third trimester.
[44]. Multiple adaptations occur to maintain maternal blood calcium concentrations within normal range during this time. The primary strategies used by the mother to accumulate fetal calcium in gestation include an increase in maternal efficiency of intestinal absorption of calcium, decreases in renal calcium excretion and resorption of maternal skeletal calcium [43].

Serum concentrations of ionized calcium, or albumin-adjusted calcium remain relatively stable throughout pregnancy, while circulating 1,25OH₂D concentrations increase. Serum active 1,25OH₂D concentrations increase 50-100% during the second trimester and by up to 100% during the third trimester of gestation, at term there is an increase in free 1,25OH₂D (ie. not bound to vitamin D-binding protein) [45]. The mechanism for the increased 1,25OH₂D at term gestation is not clear; in non-pregnant adults it is associated with increases in PTH, however PTH concentrations do not appear to be elevated throughout pregnancy, though inconsistent results regarding this have been found [45]. These inconsistencies may be due to small sample sizes, not controlling for calcium intake, or ethnic differences [46].

A modest decline or no change in maternal 25OHD status during pregnancy has been reported in observational studies [46]. It is believed that 25OHD can easily cross the placenta, whereas 1,25OH₂D does not. Cord blood concentrations of 25OHD are similar or up to 20% lower than maternal blood 25OHD concentrations. In order for an infant to be born with adequate concentrations of 25OHD the mother must not be vitamin D deficient [46]. Currently it is unknown whether the ideal concentration of 25OHD in pregnancy is different from that of non-pregnant adults [46].
Vitamin D metabolites can pass into breast milk to varying degrees; vitamin D passes readily, whereas 25OHD passes minimally and 1,25OH₂D does not pass into breast milk at all [47]. Maternal blood concentrations of 1,25OH₂D fall quickly after pregnancy and are “normal” throughout lactation. Quantitatively, breast milk accounts for only small amounts of maternal 25OHD loss; other factors such as seasonal variations or maternal supplementation are greater determinants of variation in serum 25OHD concentrations [46, 48].

1.5 Consequences of inadequate vitamin D in pregnancy

Vitamin D inadequacy in pregnancy can result in adverse outcomes for both mother and infant. Low vitamin D in pregnancy is associated with decreased maternal bone health and rickets in infants. It is well established that vitamin D’s roles extend beyond bone health and there may be additional roles of vitamin D throughout pregnancy and lactation that have not yet been elucidated [49]. Inadequate vitamin D status at any life stage can be detrimental to one’s health; however the consequences of low vitamin D during pregnancy, lactation and infancy may be more serious and further research during these critical time periods should be conducted. Most of the evidence on the consequences of inadequate vitamin D during pregnancy beyond severe deficiency are derived from observational studies linking low serum 25OHD during pregnancy to adverse outcomes. As with all observational research cause-and-effect cannot be established. Indeed, there is very little clinical trial evidence to demonstrate that improving vitamin D status will improve pregnancy outcomes [46].
1.5.1 Maternal consequences of low vitamin D

Maternal vitamin D deficiency is thought to have both skeletal and non-skeletal consequences for maternal health. Non-skeletal consequences include increased risk of pre-eclampsia and gestational diabetes mellitus [50].

1.5.1.1 Musculo-skeletal health

The occurrence of increased bone turnover in pregnancy is controversial, and its relationship to serum 25OHD concentrations is not clear [50]. Studies examining bone turnover in pregnancy have shown no change in bone mineral density (BMD) or total bone mineral content (BMC) [51]. More et al. reported that bone formation biomarkers increased significantly in 20 pregnant women, more specifically osteocalcin increased by 96% and bone-specific alkaline phosphatase (BSAP) by 290% [52]. It is important to note however, that increased bone turnover may occur with equilibrium with no net changes to BMD or BMC; studies conducted on bone formation markers assume they are in balance. Few researchers examining bone turnover during pregnancy have also measured 25OHD; those that have measured 25OHD concentrations have found no association between BMD and vitamin D status [50, 51, 53]. Thus, it remains unclear to what extent that either bone mineral may be mobilized in pregnancy, or what the impact of vitamin D status on maternal bone health might be [50].

Vitamin D deficiency results in secondary hyperparathyroidism and can lead to modest hypocalcemia and osteomalacia but there is little evidence that these conditions worsen throughout pregnancy compared to non-pregnant adults [46]. There are rare case reports of osteoporosis in pregnancy in which patients typically present with back pain or vertebral fractures in the third trimester [54]. It has been suggested that pregnancy-associated
osteoporosis could be due to a genetic disposition to low peak bone mass [54]. It is unlikely that vitamin D deficiency during pregnancy will result in chronic bone disease; however, more research is needed [55].

1.5.1.2  Preeclampsia

Preeclampsia is a pregnancy-specific hypertensive disorder characterized by edema, sudden weight gain and proteinuria which affects approximately 3-7% of first pregnancies [14]. It is the leading cause of fetal and maternal morbidity and mortality; once preeclampsia is diagnosed it is only resolved with the delivery of the placenta and infant [50]. There is a racial disparity in preeclampsia, with black women being more likely to develop preeclampsia as well as more likely to have low vitamin D status compared to women of European ancestry with lighter skin, which led some researchers to propose a role for vitamin D in preeclampsia [14].

A recent nested case-control study found that women who developed preeclampsia had lower 25OHD concentrations of 45.4 nmol/L (95% Confidence Interval (95% CI 38.6, 53.4 nmol/L; n=55) compared to 53.1 nmol/L (95% CI 47.1, 59.9 nmol/L; n=219) in control women [14]. They also found that after adjustment for confounders a 50 nmol/L decrease in 25OHD doubled the risk of preeclampsia [adjusted odds ratio 2.4, (95% CI 1.1-5.4)]. Infants born to mothers with preeclampsia were also twice as likely to have 25OHD < 37.5 nmol/L than control infants (adjusted odds ratio 2.2, 95% CI 1.2-4.1) [14]. In the Norwegian Mother and Child Cohort vitamin D was found to have a protective role against the development of preeclampsia. The authors of the study reported the odds ratio for preeclampsia was 0.76 (95% CI 0.60, 0.90, n=23423) among women with a total vitamin D intake of 15-20 µg/day compared with women consuming < 5 µg/day [56]. A study from Vancouver, BC examined
the 25OHD status of women attending a specialist antenatal clinic due to clinical or biochemical risk factors for preeclampsia. Clinical risk factors included past obstetric history of early onset or severe preeclampsia and biochemical markers such as excess protein in the urine. The latter study reported that 78% of women had 25OHD concentrations < 75 nmol/L, however maternal serum 25OHD concentration was not associated with the increased risk of preeclampsia or gestational hypertension [57]. Discrepancies among studies may result from different time points in gestation of 25OHD sample measurements or the populations surveyed. Women in the Vancouver study were selected because they were at high risk for preeclampsia, whereas the other studies involved women with apparently healthy pregnancies.

Other research has shown that attaining adequate vitamin D during infancy is associated with a reduced risk of preeclampsia in pregnancy in adulthood. In the 1966 Northern Finland Birth Cohort, 68 of 2969 women developed preeclampsia (2.3%). The risk of preeclampsia was halved (odds ratio 0.49, 95% CI 0.26,0.92) in women who received vitamin D supplementation in their first year of life [58].

1.5.1.3 Gestational diabetes mellitus

Contradictory findings have been reported in relation to vitamin D and gestational diabetes mellitus (GDM). Vitamin D receptors are present in the pancreas and although the mechanism is not well defined, vitamin D deficiency has been related to impaired insulin secretion and sensitivity [59]. In a nested case-control study, women who developed GDM had plasma 25OHD concentrations at 16 weeks gestation that were significantly lower than controls (60.4 nmol/L and 75.1 nmol/L, respectively, n=953) [60]. An Australian study with 307 women found that the maternal serum 25OHD concentration was negatively correlated
with maternal fasting insulin and fasting plasma glucose (r=0.20, 95% CI 0.08, 0.31) [61]. In contrast, 66% of South Asian women were reported to be vitamin D insufficient (25OHD < 50 nmol/L), but no association was found between serum 25OHD and GDM [n=559] [62]. These differences among studies may be attributed to the different ethnicities of study populations and sample size differences. Whether supplementing pregnant women with vitamin D would improve glycemic control and reduce gestational diabetes is unknown.

1.5.2 Infant consequences of low maternal vitamin D

Low maternal vitamin D status has been associated with negative health outcomes for the infant, including poor skeletal bone mineralization and increased incidence of asthma and type 1 diabetes.

1.5.2.1 Musculo-skeletal health

Maternal vitamin D deficiency can result in rickets in infants and children. Rickets causes weakened and softened bones in children due to poor bone mineralization and can cause the classic symptoms of bowed legs and rachitic rosary. Rickets can potentially cause morbidity and mortality from hypocalcaemic seizures, increased susceptibility to serious infections, failure to thrive and chronic problems with growth and skeletal deformities [63]. Rickets is unlikely to occur in newborns rather the incidence peaks between 3 to 18 months of age [64]. Recent evidence suggests that rickets may be re-emerging in developed nations such as the US, Australia and Canada where it was previously thought to have been eradicated [11, 63, 65]. In Canada there were 104 confirmed cases of rickets, with an incidence rate of 2.9 per 100,000 between July 1, 2002 to June 30, 2004. The majority of cases were in intermediate or dark skinned infants who were exclusively breastfed and not supplemented with vitamin D [11]. In almost all radiologically confirmed cases of rickets the
infant’s serum 25OHD was below 10 nmol/L [66]. Rickets can easily be treated if discovered at an early stage, usually with a regimen of supplemental calcium and vitamin D [66].

Whether suboptimal vitamin D status during pregnancy contributes to poorer bone health in the child is not clear. A longitudinal Nordic study found that bone mineral content was 0.047 g/cm higher (95% CI 0.011, 0.082) \( p=0.01 \) and cross-sectional area was 12.3 mm\(^2\) (95% CI 2.0, 22.6, \( p=0.02 \)) in 125 infants born to mothers with serum 25OHD concentrations greater than 42.6 nmol/L compared to below 42.6 nmol/L [67]. Javaid and colleagues conducted a longitudinal study in the UK looking at body build, nutrition and vitamin D status of mothers and followed up with their children at 9 y. They found 49 (31%) mothers had insufficient 25OHD levels (27.5 - 50 nmol/L) and 28 (18%) mothers were deficient in 25OHD (< 27.5 nmol/L). They established that low maternal 25OHD was associated with reduced whole-body (\( r=0.21, p=0.0008 \)) and lumbar spine (\( r=0.17, p=0.03 \)) bone mineral content at follow-up at 9 y [9]. In these observational studies low maternal 25OHD was associated with poor offspring bone outcomes that appeared to persist until at least 9 years of age.

1.5.2.2 Asthma

Evidence exists for a relationship between vitamin D and immune function. A study by Chi et al found that cord blood 25OHD concentrations were inversely associated with the proportion of immune cells (\( r=0.21, p<0.005 \)) suggesting a role for vitamin D in immune system regulation early in life [68]. Epidemiological evidence has suggested a role for vitamin D in the development of asthma and allergies as low maternal vitamin D deficiency is associated with greater risk of asthma and respiratory infections in offspring [69]. A cohort of 1724 Spanish children showed that those born to mothers in the highest quartile of
maternal 25OHD status during pregnancy decreased the odds of respiratory tract infection compared to those in the lowest quartile (odds ratio 0.67, 95% CI 0.50, 0.90, \( p=0.016 \)), however, they reported no association between maternal 25OHD status and child asthma at 4-6 y [70]. In the United States, Camargo and colleagues examined the relationship between cord blood serum 25OHD and found they were inversely associated to risk of wheeze at 15 months, 3 years and 5 years of age (all \( p<0.05 \)) [13]. In an American cohort from Massachusetts of 1194 mother-infant pairs looked at maternal dietary intake of vitamin D and recurrent wheeze in offspring at 3-4 y follow-up. They found mothers in the highest quartile of vitamin D intake (724 IU) had a lower risk of having children with recurrent wheeze [odds ratio = 0.39, 95% CI (0.25, 0.62), \( p<0.001 \)] [71]. However, dietary vitamin D intake does not always reflect vitamin D status as measured by 25OHD.

1.5.2.3 **Type 1 diabetes mellitus**

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which the insulin producing \( \beta \)-cells of the pancreas are destroyed and the body’s insulin needs are unable to be met. Hypotheses for vitamin D’s role in T1DM include its role in immune function and that both vitamin D deficiency and T1DM are more prevalent in the winter and at higher latitudes. An Indian case-control study demonstrated that in 50 children with T1DM the mean serum 25OHD concentration was significantly lower compared to controls (50 ± 27 mmol/L vs. 65 ± 31 mmol/L, \( p=0.009 \)) [12]. Supplementation has been studied to see if it reduces the risk of T1DM incidence. Bener and colleagues found that vitamin D supplementation in conjunction with breast feeding was lower in children with T1DM (37%) versus control children (48%) [72]. A Finnish birth-cohort study of 12,055 mother-infant pairs found that children who complied with 2000 IU of supplemental vitamin D had a decreased risk of
T1DM compared to those who did not supplement regularly [relative risk = 0.22, 95% CI (0.05, 0.89)] [73]. However, these findings should be regarded with caution as very few women did not supplement their infants with vitamin D (n=32) and in this group only two children developed T1DM. The women who did not supplement their infants with vitamin D were found to be less educated compared to those who did supplement [73].

Maternal vitamin D intake during pregnancy has been assessed for its association with offspring T1DM incidence in longitudinal studies again with conflicting results. A study of 3,723 Finnish women and their infants who were at genetic risk for T1DM revealed that vitamin D intake from either food or supplements was not associated with β-cell autoimmunity biomarkers or type 1 diabetes [74]. The Diabetes Autoimmunity Study In Youth (DAISY) from Denver, in the United States, found that increased maternal dietary vitamin D intake was significantly associated with decreased autoimmunity biomarkers appearing in offspring [hazard ratio = 0.37, 95% CI (0.17, 0.78)] [75]. Conflicting results could result from different biomarkers measured in studies, different population demographics and the Finnish study looked at vitamin D intake from supplements and diet, whereas DAISY only examined maternal dietary intake.

1.6 Current recommendations for vitamin D

The recommended intake of vitamin D during pregnancy varies considerably between different governments and organizations. Health Canada recommends intake reference values for vitamin D in the form of Dietary Reference Intakes (DRIs). These DRIs are generated by expert panels of Canadian and American scientists who are overseen by the US Food and Nutrition Board of the Institute of Medicine (IOM) of the National Academy of Sciences.
The DRIs are nutrient reference values for life stage and gender that can be used to plan and assess nutrient intakes in healthy populations [76].

Health Canada first published DRIs for vitamin D in 1998, at this time it was thought that there was insufficient evidence to establish an Estimated Average Requirement (EAR), which is the intake that is estimated to meet the requirement of half of the population in a certain gender and age group [76]. The EAR is then used to calculate the Recommended Dietary Allowance (RDA), which is the amount of a nutrient thought to meet or exceed the requirement for 97.5% of the population [77]. In 1998 an Adequate Intake (AI) was established for vitamin D. The AI is used when there is insufficient evidence to establish an EAR and set an RDA; the AI is a recommended average daily nutrient intake level based on experimentally determined approximations or estimates of nutrient intake in a group of apparently healthy individuals [76]. The AI for vitamin D was set at 200 IU/day for all gender and life stages less than 50 y, including pregnant and lactating women. Due to emerging evidence based on bone health outcomes, the IOM updated the DRI’s in 2010; however, they determined that there is insufficient evidence regarding the role of vitamin D in other health outcomes such as cancer, diabetes, multiple sclerosis etc. [18]. The current RDA is 600 IU/day for all genders and life stages up to 50 y, including pregnant and lactating women. The IOM concluded that pregnant and lactating women do not need more vitamin D than their non-pregnant counterparts [18].

Other organizations both within and outside of Canada and the US recommend different intakes of vitamin D. The Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommended in 2002 that pregnant and lactating women consume 200 IU/day of vitamin D; however, in 2011 they were updating their
recommendations which may be subject to change in the near future [78]. The Canadian Pediatric Society recommends that pregnant women should receive 2000 IU/day for vitamin D, which is 10 times the recommendation from the FAO/WHO and over 3 times higher than the most recent IOM recommendations [42]. Within the European Union (EU), the United Kingdom, Ireland, France, Nordic countries and others recommend 400 IU/day of vitamin D in pregnancy and lactation [79].

1.6.1 Adverse outcomes of high vitamin D

Vitamin D toxicity does occur but there is considerable controversy about the dose and duration of vitamin D required to cause it. Hypervitaminosis D is rare, but can be dangerous resulting in hypercalciuria and potentially fatal hypercalcemia. Hypercalciuria is defined as a urinary calcium excretion over 6.2 mmol/L over 24 h and hypercalcemia is a serum calcium concentration > 2.5 mmol/L. Hypercalciuria and hypercalcemia can result in dementia, weakness, muscle twitches and if untreated, ultimately, death. The current DRI includes a Tolerable Upper Level of Intake (UL), which poses an extremely low risk of adverse effects. The current UL of vitamin D for Canadian adults is 4000 IU/day, which also applies to pregnant and lactating women [77]. Some experts have suggested that much higher intakes of vitamin D pose no significant health risk. The greatest risk of hypervitaminosis D comes from nutrition supplements, as diet and sunlight are unlikely to result in toxicity. Synthesis of vitamin D in the skin via UVB light can generate large amounts of previtamin D₃, however much of this is degraded into inert photoproducts via physiological feedback mechanisms [80]. Dietary intake of vitamin D is also unlikely to result in toxicity; average dietary intake of vitamin D for women ages 19-50 y (n=5,018) is approximately 200 IU/d of vitamin D [81].
Clinical trials conducted with oral or supplemental dosages of 10,000 IU of vitamin D have shown no significant effects or increases in serum calcium levels, suggesting this dosage is safe [29, 82]. In conclusion, doses of up to 10,000 IU/day vitamin D do not appear to cause hypercalcemia. Nonetheless, there is little safety data from studies of pregnant women as it would be unethical and there may be other long-term adverse effects of high doses of vitamin D that have not yet been assessed.

1.7 Vitamin D status of pregnant and lactating women

A few researchers have reported on the vitamin D status of pregnant women in Canada most of whom have used convenience samples. Studies have included women of European background, Aboriginals, as well as Asian women and data has been collected from various regions in Canada including the Northwest Territories, Edmonton, Vancouver, Manitoba and Newfoundland and Labrador (Table 1.1).

Researchers from Newfoundland and Labrador measured 25OHD concentrations of pregnant women, primarily of European background. A cross-sectional study measured 25OHD concentrations in 25 pregnant women in each of the winter and summer months, and reported that 2% of women had vitamin D concentrations classified as deficient (< 25 nmol/L) and 78% were vitamin D insufficient (25-75 nmol/L). Circulating 25OHD concentrations were not significantly lower in the winter versus summer months (51.9 nmol/L and 61.1 nmol/L, respectively) [83]. In a second study, researchers randomly sampled pregnant women from 79 census-consolidated subdivisions across Newfoundland and Labrador in winter and summer months. They sampled 304 pregnant women in the summer and 289 in the winter and found that the 25OHD concentrations of mothers were significantly lower in the winter (52.1 nmol/L) versus the summer (68.6 nmol/L, p<0.001).
They found that 6.6% of women were vitamin D deficient in the winter (25OHD < 25 nmol/L) and only 1.7% were deficient in the summer [84]. Vitamin D insufficiency (25-75 nmol/L) was also more prevalent in the winter months (83%) than summer months (62%) [84].

Researchers from the Northwest Territories measured vitamin D concentrations in 121 pregnant women (33 Caucasians, 51 Inuit and 37 Aboriginal) and their newborns. At delivery the 25OHD concentrations were lower in Aboriginal mothers (50.1 ± 19.3 nmol/L) and their offspring (34.2 ± 13.1 nmol/L) compared to their Inuit and Caucasian counterparts (59.8 ± 29.4 nmol/L and 41.4 ± 23.5 nmol/L, respectively) despite many women taking a prenatal vitamin D supplement of 400 IU/day [85]. In Edmonton, a study looking at 25OHD status in clinical practice populations found that 19 of 83 pregnant women (23%) had 25OHD concentrations below 40 nmol/L [86]. In a cross-sectional study in Vancouver, 336 pregnant women between 20-36 weeks of gestation had a mean serum 25OHD concentration of 67 nmol/L [95% CI (64, 69)] [15]. Over 80% of the women reported that they took a prenatal supplement containing vitamin D, however within the study the Caucasian women had a 25OHD status 9-13 nmol/L greater than other ethnic groups [15].

Data from the 2007-2009 Canadian Health Measures Survey (CHMS) provides the most recent information on the 25OHD status of Canadians. This study did not sample pregnant women, however as there is little information on pregnant women it is reasonable to examine the 25OHD status of non-pregnant women of reproductive age. From this CHMS data, females of childbearing age (20-39y) have a mean 25OHD concentration of 70 nmol/L (95% CI 66,73, n=650) [16]. Of these women, 10% had serum concentrations below 38
24 nmol/L (95% CI 6, 15) and 64% had 25OHD concentrations below 75 nmol/L (95% CI 56, 71) [16].

Overall, it appears that pregnant women and women of childbearing age in Canada have suboptimal vitamin D status, which is not surprising given the high latitude of Canada and low dietary intake [81].

1.8 Vitamin D supplementation during pregnancy

Vitamin D supplementation and 25OHD dose-response data exists for non-pregnant adults. A study of 67 men followed over 20 weeks concluded that for every 40 IU increase in vitamin D₃ intake the serum 25OHD concentration increased by 0.7 nmol/L [87]. Researchers concluded that healthy men needed 3000-5000 IU/d in the winter to meet >80 % of their 25OHD target blood concentrations (>80 nmol/L) [87]. Another study in 138 healthy Caucasian and African-American men and women suggested that the dose response is 0.66 nmol/L per each 40 IU increase in vitamin D intake [88]. They concluded that in order to reach an optimal 25OHD concentration of >75 nmol/L the optimal daily dose of vitamin D is 4600 IU/d; however, compliance appeared to be low in this study [88]. The previous two studies should not be extrapolated to pregnant adults due to the physiological changes that occur altering the metabolism and uptake of certain nutrients, particularly calcium [89]. A number of studies from 1980 to 2011 have examined the outcomes of vitamin D supplementation during pregnancy on maternal 25OHD and infant outcomes.

1.8.1 Vitamin D supplementation during pregnancy on maternal 25OHD and neonatal outcomes

Researchers have examined the effects of vitamin D supplementation during pregnancy on maternal 25OHD concentrations, and neonatal outcomes, but the studies have
many limitations and the quality of the studies is often low (Table 1.2). Early research by Marya et al provided 120 pregnant Hindu women with vitamin D (either two bolus doses of 600,000 IU or 1200 IU/d) or placebo in their 3rd trimester [90]. Those on the vitamin D supplements gave birth to infants with significantly greater mean birth weights compared to the placebo group. They also found that maternal and cord blood alkaline phosphatase (BSAP) concentrations were significantly lower in the vitamin D supplemented groups. They concluded that the 600,000 IU dose was more efficacious [90]. Marya used bolus dosages of vitamin D, versus daily vitamin supplements and did not assess the baseline or endline 25OHD status of mothers or infants. A study of 200 Indian women found similar results; infants whose mothers received two bolus dosages of 600,000 IU had greater head and arm circumference as well as greater intrauterine growth and greater crown-heel length [91]. These studies did not randomize participants and baseline characteristics such as socioeconomic status, education, season etc could have acted as confounders. Several subsequent researchers have shown very low 25OHD concentrations among Indian women of reproductive age; thus the findings may not be relevant in Canada.

A small French study (n=32) randomized women to either receive supplements or no intervention to serve as a control group. They reported the mean serum 25OHD concentration increased from 25 nmol/L to 65 nmol/L when in mothers supplemented with 1000 IU/d of vitamin D3 from the beginning of the six month of gestation [92]. They also reported significantly increased 25OHD concentrations in cord blood and infants at 4 d after birth in those born to supplemented mothers [p<0.0005] [92]. Serum 25OHD concentrations increased in the control group by 8 nmol/L; the women in this study had much lower 25OHD concentrations than reported for Canadian pregnant women; many women remained
insufficient even after supplementation (<50 nmol/L). Starting supplementation in the third trimester may not provide sufficient time to achieve desirable 25OHD.

Datta et al supplemented 160 non-European minority women living in the UK with 800-1600 IU/d if they were found to be vitamin D deficient at their first antenatal visit for the remainder of pregnancy. Mean serum 25OHD concentrations increased from 15 nmol/L to 28 nmol/L by term [93]. Despite supplementation with 800-1600 IU/d, these mothers were still vitamin D deficient or insufficient at term. A group of immigrant Asian mothers in England (n=113) taking 1000 IU/d of vitamin D$_2$ had significantly greater 25OHD concentrations than taking a placebo (168 nmol/L vs 16 nmol/L) [94]. Mallet et al studied 160 French pregnant women randomized to placebo, 2000 IU/d or a one time dose of 200,000 IU of vitamin D$_2$ in their 7$^{th}$ month of pregnancy [95]. Women who received supplements (either daily or bolus) increased serum concentrations by ~25 nmol/L and gave birth to infants with significantly greater 25OHD cord blood concentrations [95]. These studies suggest that a vitamin D supplement of >1000 IU/d may be necessary to replete vitamin D in vitamin D deficient mothers. One of the confounding variables in these studies is the form of supplement; both vitamin D$_2$ and D$_3$ were used, however vitamin D$_3$ is more effective D$_2$ than at raising serum 25OHD concentrations [21]. Compliance to supplementation was not always assessed.

Researchers examining secondary outcomes have reported increases in cord blood concentrations of 25OHD and decreased hypocalcemia and hypoparathyroidism in vitamin D supplemented mothers. A Scottish study of 1139 women receiving either placebo or 400 IU/d found that serum 25OHD concentrations were significantly greater in maternal, cord and infant samples at day 6 after birth in those receiving vitamin D versus placebo [96]. They also found symptomatic hypocalcemia at 6 days after birth in 6% of infants born to vitamin D
supplemented mothers versus 13% of infants whose mothers took the placebo ($x^2=8.82$, $p<0.005$) [96]. Brooke et al’s data also support these results as they found mothers taking placebo versus vitamin D were twice as likely to give birth to infants that were small for gestational age (29% vs. 15%) and 5 of 67 infants developed symptomatic hypocalcemia [94]. However, these mothers were profoundly deficient at randomization, with mean 25OHD concentrations of 20 nmol/L [94]. An English study that randomized 180 pregnant women to placebo, 800 IU/d vitamin D$_3$ or a one-time dose of 200,000 IU vitamin D$_2$ in 27th week of gestation, found that the mean cord blood concentrations were significantly higher in those who had been supplemented with vitamin D versus placebo [97]. They also reported decreased cases of secondary hypoparathyroidism in women who were taking vitamin D supplements versus placebo [97].

Most recently, Hollis et al conducted a that study randomized 350 American women of varied ethnicities (Hispanic, African-American and Caucasian) to 400 IU/d, 2000 IU/d or 4000 IU/d of vitamin D$_3$ beginning at 12-16 weeks of gestation (Table 1.3). They found decreased levels of PTH in African Americans and increased serum 25OHD concentrations among all groups, with 4000 IU/d resulting in a mean 25OHD serum concentration of 111±40 nmol/L ($p<0.0001$) in mothers at term [98]. They also reported no adverse events when supplementing women throughout pregnancy with 4000 IU/d, which is the current UL for vitamin D [98]. Hollis et al conducted their study at a Southern latitude (33°N), therefore the vitamin D requirements of women living in more Northern regions, including Canada may be even greater. The population is also not representative of Canadian demographics, as they had a larger proportion of African-American and Hispanic pregnant women, which would mean their requirements would be higher.
The majority of recent research conducted examining maternal and infant responses to maternal supplemental vitamin D is of low quality [92]. Issues include; varying duration of supplementation (2nd versus 3rd trimester) [95, 98], varying seasonal affects, lack of randomization or placebo [93], different populations some of them profoundly vitamin D deficient [94], different supplement regimens (daily versus bolus) [90, 91, 95, 97], different vitamin D supplements (D₂ versus D₃) [97] and different analytical methods used to measure 25OHD concentrations and have produced inconsistent results [94, 98]. No research examining maternal and infant response to vitamin D supplementation has been conducted in Canadian populations to date. Supplementation is the most practical and cost-effective way to increase 25OHD concentrations and it has been reported that most Canadian women take a pre-natal multivitamin during pregnancy, which contains 200-400 IU of vitamin D [15].

In summary, there is a need for rigorous RCT’s examining the effects of physiologically relevant intakes of varying dosages of supplemental vitamin D on maternal and infant outcomes from early pregnancy through lactation. Confounders such as skin colour/ethnicity, latitude, season, BMI and dietary intake of vitamin D and calcium need to be controlled for. Given the importance of vitamin D throughout pregnancy, the primary objective of this research is to determine maternal and neonatal responses to vitamin D₃ supplementation (400 IU/d, 1000 IU/d and 2000 IU/d) in a group of pregnant women, of various ethnicities in Vancouver, Canada (49°N). It is hoped that the results of the proposed study will provide insight to establish a recommended intake for vitamin D during pregnancy and lactation.
**Figure 1.1** Hydroxylation of vitamin D$_3$ [99].

**Figure 1.2** Vitamin D endocrine system [100]
Table 1.1 25OHD status of Canadian pregnant women

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location &amp; Latitude</th>
<th>Population (n)</th>
<th>Time point in pregnancy</th>
<th>Mean 25OHD nmol/L (n)</th>
<th>25OHD nmol/L n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (2) 21 (42) 39 (78) 10 (20)</td>
<td></td>
</tr>
<tr>
<td>Sloka et al</td>
<td>Newfoundland and Labrador 46°N &amp; 53°N</td>
<td>ND [likely Caucasian] (593)</td>
<td>Early 2nd Trimester</td>
<td>Winter 52.1 (304) Summer 68.6 (289)</td>
<td>&lt; 25 25-75 &gt;75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 (4) ND 431 (73) 137 (23)</td>
<td></td>
</tr>
<tr>
<td>Waiters et al</td>
<td>Inuvik Zone, NWT 68°N</td>
<td>Inuit (51), Native Indian (37), Caucasian (33)</td>
<td>25OHD measured at delivery</td>
<td>Inuit 48.8 (51) Native Indian 52.1 (37) Caucasian 59.8 (33)</td>
<td>&lt; 25 25-75 &gt;75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND ND ND ND ND</td>
<td></td>
</tr>
<tr>
<td>Genius et al</td>
<td>Edmonton 53°N</td>
<td>Primarily Caucasian (83)</td>
<td>During ob/gyn visit ND</td>
<td>ND pregnant women All ob/gyn patients 63.02 (507)</td>
<td>&lt;40 40-80 &gt;80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 (5) [40] 15 (18) 44 (53) 20 (24)</td>
<td></td>
</tr>
<tr>
<td>Li et al</td>
<td>Vancouver 49°N</td>
<td>European (155), Chinese (66), South Asian (30), Other (85)</td>
<td>20-35 weeks gestation</td>
<td>66.7 (336)</td>
<td>ND 80 (24) 218 (65) 118 (35)</td>
</tr>
<tr>
<td>Langlois et al</td>
<td>Canada wide</td>
<td>All ethnicities</td>
<td>Childbearing age (not pregnant)</td>
<td>69.5</td>
<td>&lt;27.5 &lt;37.5 ND 36.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.2) (9.7) ND (36.3)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1.2 Controlled trials of vitamin D supplementation throughout pregnancy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location &amp; Latitude</th>
<th>Population (n)</th>
<th>Study Type</th>
<th>Vitamin D Supplement</th>
<th>25OHD (nmol/L) Initial Endpoint*</th>
<th>Other Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marya et al</td>
<td>India, 29°N</td>
<td>Hindu women (120)</td>
<td>RT</td>
<td>Dose 1200 IU/d (25) 600000 IU/d (20) Placebo (75) 600000 IU/d (200) Placebo (100)</td>
<td>D2 ND ND</td>
<td>ALP ↓ &amp; maternal and cord calcium ↑ in D (60000 IU) vs. placebo Birth weight ↑ in both D vs. placebo</td>
</tr>
<tr>
<td>Marya et al</td>
<td>India, 29°N</td>
<td>Asian-Indian (300)</td>
<td>RT</td>
<td>Dose 400 IU/d (506) Placebo (633)</td>
<td>D3 ND ND</td>
<td>↑Birthweight, crown-heel length, head and arm circumference in D group ↑ Maternal &amp; cord calcium and ↓ALP in D ↑ Infant 25OHD at 6th d with D; symptomatic hypocalcemia 0.4% with D, 0.9% placebo</td>
</tr>
<tr>
<td>Cockburn et al</td>
<td>Scotland, 55°N</td>
<td>Scottish (1139)</td>
<td>QRT</td>
<td>Dose 1000 IU/d (39) Placebo (55)</td>
<td>D2 ND 43 33</td>
<td>Cord blood 25OHD ↑ in D; 5 cases symptomatic hypocalcemia in placebo group</td>
</tr>
<tr>
<td>Brooke et al</td>
<td>England, 51°N</td>
<td>Asian (113)</td>
<td>DBRCT</td>
<td>Dose 1000 IU/d (39) Placebo (55)</td>
<td>D2 20 +148 20 -4</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Location &amp; Latitude</td>
<td>Population (n)</td>
<td>Study Type</td>
<td>Vitamin D Supplement</td>
<td>25OHD (nmol/L) Initial Endpoint*</td>
<td>Other Outcomes</td>
</tr>
<tr>
<td>------------</td>
<td>---------------------</td>
<td>----------------</td>
<td>------------</td>
<td>----------------------</td>
<td>----------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Yu et al</td>
<td>England, 51°N</td>
<td>Indian Asian (45), Middle Eastern (45), Black (45) &amp; Caucasian (45)</td>
<td>RT</td>
<td>20000IU/d (60) 800IU/d (60) None (60) 2000IU/d (21) 20000IU/d (27) None (29)</td>
<td>ND 34</td>
<td>↓ Secondary hypoparathyroidism with D Cord 25OHD ↑ with D</td>
</tr>
<tr>
<td>Mallet et al</td>
<td>France, 45-48°N</td>
<td>French (160)</td>
<td>RT</td>
<td>1000IU/d (15) None (17)</td>
<td>ND 9</td>
<td>Cord 25OHD ↑ in D group</td>
</tr>
<tr>
<td>Delvin et al</td>
<td>France, 45°N</td>
<td>French (32)</td>
<td>RT</td>
<td>800 – 1600IU/d (80)</td>
<td>ND 28</td>
<td>Infant (4d) and cord 25OHD ↑ with D</td>
</tr>
<tr>
<td>Datta et al</td>
<td>United Kingdom, 52°N</td>
<td>Non-European minorities (80)</td>
<td>Intervention</td>
<td>From 1st antenatal visit</td>
<td>ND 14.5</td>
<td>PTH hormone levels unchanged after D supplementation</td>
</tr>
</tbody>
</table>

25OHD, 25 hydroxyvitamin D; RT, randomized trial; QRT, quasi randomized trial; DBRCT, double blind randomized controlled trial; ND, not determined

*All measurements at term. Values relative to initial 25OHD levels where initial concentration was measured.

Adapted from Dr. Tim Green and Wangyang Li [101].
Table 1.3 Hollis et al. supplementation during pregnancy: double-blind, randomized, clinical trial of safety and effectiveness [98]

<table>
<thead>
<tr>
<th>Supplement Dose (n)</th>
<th>Ethnicity n (%)</th>
<th>Baseline</th>
<th>25OHD nmol/L ± SD</th>
<th>Change from baseline to delivery</th>
<th>Achieved &lt; 80 nmol/L n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 IU (111)</td>
<td>Black 28 (25.2)</td>
<td>37.3 ± 17.1</td>
<td>48.8 ± 21.1</td>
<td>12.7</td>
<td>43 (50)</td>
</tr>
<tr>
<td></td>
<td>Hispanic 45 (40.5)</td>
<td>59.1 ± 21.6</td>
<td>76.9 ± 21.7</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White 38 (34.2)</td>
<td>81.3 ± 23.8</td>
<td>95.2 ± 20.6</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>2000 IU (122)</td>
<td>Black 37 (30.3)</td>
<td>41.0 ± 19.1</td>
<td>72.2 ± 28.4</td>
<td>49.4</td>
<td>63 (70.8)</td>
</tr>
<tr>
<td></td>
<td>Hispanic 48 (39.3)</td>
<td>59.2 ± 18.9</td>
<td>85.2 ± 16.8</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White 37 (30.3)</td>
<td>71.9 ± 19.0</td>
<td>94.9 ± 18.3</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>4000 IU (117)</td>
<td>Black 33 (28.2)</td>
<td>40.7 ± 20.1</td>
<td>81.0 ± 26.4</td>
<td>57.4</td>
<td>68 (82.0)</td>
</tr>
<tr>
<td></td>
<td>Hispanic 44 (37.6)</td>
<td>63.3 ± 27.6</td>
<td>101.4 ± 28.2</td>
<td>60.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White 40 (34.2)</td>
<td>71.3 ± 17.3</td>
<td>109.8 ± 19.2</td>
<td>50.4</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 2: Research Study

2.1 Purpose

The purpose of the present study is to examine the response to maternal vitamin D supplementation among three different doses 400 IU/d, 1000 IU/d and 2000 IU/d and maternal and neonatal serum 25OHD concentrations. This study will provide critical data to establish a recommended intake for vitamin D during pregnancy and lactation and provide health care workers, policy makers and mothers with critical information on the amount of vitamin D required to promote optimal pregnancy and infant outcomes.

2.2 Objectives and hypothesis

Hypothesis: The current recommended dietary allowance (RDA) of 600 IU/d of vitamin D will be inadequate for Canadian women and their newborns to achieve recommended 25OHD concentrations during pregnancy as defined by the IOM of > 50 nmol/L.

Objectives: The overarching goals of this research project are to determine maternal and neonate responses to maternal vitamin D supplementation throughout pregnancy and lactation. My research and analysis will focus on maternal outcomes, specifically;

1. The effect of three doses of supplemental vitamin D on maternal and neonate serum 25OHD concentrations.

2. The percentage of women taking different supplemental dosages of vitamin D that achieve serum 25OHD concentrations of > 50, 75 and 100 nmol/L.

3. Determine the effect of three doses of supplemental vitamin D on maternal and neonate serum BSAP, a marker of bone formation.
4. Factors that predict vitamin D status among pregnant women at 36 weeks gestation, including the importance of dietary vitamin D intake, season and ethnicity.

2.3 Methods

2.3.1 Overview

This is a randomized, double-blind controlled dose-response trial with three doses of vitamin D: 400, 1000 or 2000 IU/d. A convenience sample of pregnant women (n=110) from Vancouver and surrounding areas were recruited to participate. Participants were randomized to one of three dosages of vitamin D₃ to take during pregnancy, and completed 3 clinic visits at baseline (13-22 weeks) and 36 weeks of gestation, and 8 weeks postpartum. Cord blood was also collected at delivery when possible. Participants completed a demographic and lifestyle questionnaire and a food frequency questionnaire (FFQ), which estimated the intake of vitamin D and calcium consumed in the month prior to each visit. A non-fasting blood sample and spot urine was collected each time. Anthropometric measures such as height, weight and blood pressure were also collected.

2.3.2 Sample size

We estimated that a sample size of 52 subjects per group will be required to estimate the dose most women would require (97.5%) to achieve a serum 25OHD of > 50 nmol/L to within 10% of the true dose with 95% confidence. The primary outcome measure was 25OHD concentration at 36 weeks; 55 women per dose will allow the detection of a minimum difference of 10 nmol/L 25OHD between any two doses assuming a standard deviation of 25 nmol/L [102]. Assuming a 20% attrition rate, we recruited 225 women total; 70 for each dose of vitamin D. This thesis focuses on the first 110 women who enrolled and
completed the study, and therefore the sample size in this thesis is insufficient to ensure adequate power to meet the overall study endpoint.

2.3.3 Recruitment and participant selection

The current research was approved by The University of British Columbia Children’s and Women’s Clinical Research Ethics Board H09-01261. All women gave informed written consent to participate (Appendix A). A convenience sample of 116 pregnant women was recruited from Vancouver and the lower mainland (49°N) between June 2010 and August 2011. Participants were actively recruited (researchers approached patients directly) at BC Women’s Ultrasound and Crossroads Obstetrics and Gynecology Clinic. Participants were recruited passively via brochures and flyers left in waiting areas, flyers at coffee shops, blogs and newspaper advertisements (Appendix B, C & D). Recruitment was also conducted passively in the community at various midwifery groups including: South Community Birth Program, Ravensong, The Midwifery Group, Pomegranate Community, Open Door Midwifery, and various other local community programs and shops which attract pregnant women as a demographic. Participants were also recruited via “word of mouth”.

Pregnant women between 13 and 22 weeks of gestation with a singleton pregnancy and identified as low-risk and between the ages of 18-42 were eligible to participate in the study (Inclusion). Women were not eligible to participate if they had any: comorbid conditions such as gestational diabetes, preeclampsia, cardiac or renal disease, HIV/AIDS or other communicable diseases, hypertension, or autoimmune disease; conditions associated with vitamin D malabsorption (celiac or Crohn’s disease) (Exclusion). Women scheduled individual appointments at BC Women’s and Children’s Hospital and a standard protocol was used for each participant.
2.3.4 Procedures

Women who consented to the study participated in three clinic visits at 13 to 22 weeks and 36 weeks gestation and 8 weeks postpartum, with each visit approximately 30-60 minutes in length. Maternal and infant outcomes from the final visit are included in this thesis. At baseline women were randomized to a supplement dose group. The randomizations were blocked by ethnicity (either Caucasian and non-Caucasian) and then to one of the three dosages within their ethnicity block without bias. Women filled out a socio-demographic questionnaire which asked questions on country of birth, date of birth, ethnicity, infant’s ethnicity, education, occupation, total family income, self-reported pre-pregnancy weight, due date, past pregnancy history (preterm labour, live births, twins or triplets, first pregnancy), smoking status, alcohol consumption, diet and supplement and medication use (Appendix E). In cases where participants indicated they belonged in more than one ethnic group, a single ethnic group was assigned based on the self-reported 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. The researchers made anthropometric measurements; blood pressure was measured using an automated machine, height was measured to the nearest 0.1 cm using a stadiometer and weight was measured to the nearest 0.1 kg in light clothing with no shoes using a standing floor scale. At each visit participants completed a food frequency questionnaire (FFQ) that focused on food sources of vitamin D and calcium intake over the last month (Section 2.3.6). BC Women’s and Children’s Hospital laboratory staff collected a blood sample and a spot urine sample for each subject at each time point.
2.3.5 Supplements

The supplements provided to the participants were a vitamin D₃ supplement with either 400, 1000 or 2000 IU of vitamin D together with a standard prenatal multivitamin that contained no vitamin D (Table 2.1). The vitamin D tablets contained vitamin D₃ at one of three dosages with vegetable grade magnesium stearate as a lubricant and microcrystalline cellulose and dicalcium phosphate dehydrate as fillers. The supplements were all identical in size and colour. Both the investigators and participants were blinded to the supplement dose through coding by Natural Factors Inc., the manufacturer of all the supplements used in the current study. Natural Factors Inc. analyzed the vitamin D supplements at various time points throughout the study for vitamin D₃ content to ensure proper dosage. For external evaluation the samples were sent to Dr. Ronald Horst at Heartland Assays LLC (Table 2.2). No placebo was used in the current study as most prenatal supplements currently available contain at least 200 IU of vitamin D₃ and it would be unethical to randomize women to no additional vitamin D. Supplements were dispensed at clinic visit 1 (~22 weeks) and clinic visit 2 (~36 weeks). Participants were instructed to finish one set of supplements before consuming the next set. Compliance was assessed with pill counts at the final visit (~8 weeks postpartum) and through a questionnaire at their second and final visit asking women how many times they consumed their vitamin D supplement over the last week and over the last two months (Appendix F). Compliant subjects are defined as those who consumed over 80% of their supplements in the two months prior to their study visit.

2.3.6 Dietary assessment

Maternal intake of vitamin D and calcium from food sources, including fortified foods and supplements in a typical month during pregnancy were estimated using a semi-
quantitative FFQ validated for use in a variety of ethnic groups (Appendix G). The FFQ was developed and validated in a group of Canadian healthy young adults of diverse ethnicities in Southern Ontario \((n=107)\) for rapid assessment of vitamin D intake during late winter 2007. The FFQ was highly correlated with 7-day food diaries and serum 25OHD concentrations \((r=0.529, p < 0.001; r=0.481, p < 0.001, \text{respectively})\) [103]. The FFQ allows participants to choose their frequency of consumption for each food item (never or less than once per month, 1 per month etc…up to 2+ times per day) and specify a serving size of either small, medium or large based upon the serving size listed. Participants also provided information on nutritional supplements used in the previous month at the first visit. At the second and third clinic visit they only provided nutritional supplement information if they were consuming supplements in addition to the supplied prenatal and vitamin D supplements.

The FFQ’s were scanned and analyzed by the University of Saskatchewan College of Pharmacy and Nutrition, which developed the questionnaire. EHSA Food Processor (Version 8.0, EHSA Research, Ore), which included 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 [103]. Fortification amounts for foods that were recently approved in Canada were updated (soy beverage, orange juice) [103]. Dietary intake was exported to excel spreadsheets.

### 2.3.7 Blood and urine samples

Blood samples were taken at the outpatient blood collection laboratory at BC Children’s Hospital, Vancouver. For each patient at each clinic visit three tubes of blood were collected by venipuncture into vacutainers; one sample for the preparation of serum in a 10 mL plastic serum tube with Increased Silica Act Clot Activator and two samples for the
preparation of plasma in a 2 mL and 6 mL plastic tube with potassium ethylene diamine acetic acid (K<sub>2</sub> EDTA) as an anticoagulant. The 2 mL plasma tube was used for a complete blood cell count (CBC) in the Hematopathology lab labs at BC Women’s and Children’s Hospital. At birth, nurses from BC Women’s Hospital collected a 6 mL blood sample from the umbilical cord, which was then picked up by myself or another research assistant that day or the next morning, if delivery occurred at night. Blood samples for the preparation of plasma were centrifuged within 30 minutes (3700g, 15 minutes) and blood for the preparation of serum were centrifuged within 1 hour (3000 g, 10 minutes). Plasma and serum were removed from the pellet and stored in -80°C at the Child and Family Research Institute, Vancouver for subsequent analyses. Samples did not undergo any freeze-thaw cycles and were stored up to 20 months before being analyzed for 25OHD in November 2011 and April 2012. Serum samples were shipped to Dr. Hope Weiler at McGill, Montreal Quebec on dry ice for analyses. 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 (25OHD<sub>2</sub> and 25OHD<sub>3</sub>) in human serum. The measurement range of the assay was 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); the accuracy using the mid-range of manufacturer specifications was 88% for low control and 90% for high control. The assay has high specificity (25OHD<sub>2</sub> 104%; and 25OHD<sub>3</sub> 100%). Supplementation trials have shown that serum levels of 25OHD > 300 nmol/L don’t cause hypercalciuria, the first indicator of hypervitaminosis D. We used a cut-off of 25OHD > 225
nmol/L to define hypervitaminosis D, which is also the cut-off of the United States Food and Drug Administration (FDA) [87, 98].

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

Spot urine samples were collected at the end of each clinic visit in a 200 mL urine cup. Urine was aliquoted and stored at -80°C for subsequent analyses. Maternal serum and urine creatinine, phosphate and calcium were measured on laboratory equipment from Johnson & Johnson, Ortho-Clinical Diagnostics VITROS© 5600 System according to standardized methodology and laboratory normative data at BC Women’s and Children’s Hospital. The cut-offs used were 2.22 – 2.67 mmol/L for serum calcium, 0.87 – 1.52 mmol/L for serum phosphate, 39-106 umol/L for serum creatinine and < 0.7 mg/dL for urine calcium to creatinine ratio as reported by BC Women’s and Children’s Hospital [104].

2.3.8 Data analyses

Statistical analyses were performed using SPSS Statistics 20.00 for Macintosh (SPSS Inc., Chicago, IL 2012). Data were checked for normality using a histogram and Shapiro-Wilk's test; it was found to be normally distributed. A cut-off value of 25OHD < 25 nmol/L was used to indicate deficiency [36]. Three commonly used cut-offs, 50 nmol/L, 75 nmol/L
and 100 nmol/L 25OHD were used to define insufficiency and sufficiency [32, 39]. Data analysis was conducted as both intention-to-treat analysis (ITT) and as-treated (AT). As-treated models analyze data only from participants who completed the study and adhered to protocols. AT provides an advantage as it only analyzes those with complete data sets, allowing researchers to examine study outcomes under the most ideal conditions. AT analysis is prone to numerous, potentially important biases. The ITT model was also used to reduce bias. ITT is often used in clinical trials and compares outcomes between supplement randomizations as assigned without making any assumptions regarding adherence to dosing or compliance with supplement regimen [98]. ITT carries the last data value forward if a participant withdraws or is lost to follow-up as these deviations from protocol might not be random; therefore reducing bias. This approach can be applied in a public-health context as the level of adherence in the study could be similar to that observed in the community, informing community-based decisions regarding the effectiveness of the experimental intervention [105]. Both models were used to observe any differences in the data that may arise from those who did not adhere to study protocols versus those who did.

One-way analysis of variance (ANOVA) and chi-square tests for categorical variables was used to determine differences in baseline participant characteristics across the supplement groups. Estimates were considered statistically significant if \( p < 0.05 \). Chi-square tests were used to determine the prevalence of vitamin D insufficiency and deficiency among treatment groups. One-way ANOVA and paired sample t-tests were used to compare 25OHD, BSAP, creatinine, calcium and phosphate concentrations across treatments groups at the two time points during pregnancy. Dietary calcium and vitamin D intake across supplement groups were analyzed with one-way ANOVA and paired samples t-tests. A
general linear model was used to determine the effect of season, ethnicity and supplement dose on 25OHD concentrations at 36 weeks of pregnancy and to determine if there were any interactions between season, ethnicity and dosage. Compliance was assessed using chi-square tests.

2.3.9 Miscellaneous

Each participant was provided with a $25.00 voucher to a local Vancouver retailer of their choice such as Safeway, London Drugs, Chapters etc. at each study visit. They were also provided with a parking pass or bus fare to cover their transportation costs to and from BC Women’s and Children’s Hospital. Upon completion of the study, women will receive a letter summarizing the study findings, their individual 25OHD concentrations at each time point and their infant’s and breastmilk 25OHD concentrations.
Table 2.1 Natural Factors prenatal vitamin product monograph

**Vitamins**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta carotene (provitamin A)</td>
<td>1500 IU</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>1500 IU</td>
</tr>
<tr>
<td>Vitamin B1 (thiamine mononitrate)</td>
<td>3 mg</td>
</tr>
<tr>
<td>Vitamin B2 (riboflavin)</td>
<td>3.4 mg</td>
</tr>
<tr>
<td>Niacinamide</td>
<td>20 mg</td>
</tr>
<tr>
<td>Vitamin B6 (pyrodoxine hydrochloride)</td>
<td>10 mg</td>
</tr>
<tr>
<td>Vitamin B12 (cyanocobalamin)</td>
<td>12 mcg</td>
</tr>
<tr>
<td>D'Pantothenic acid (calcium pantothenate)</td>
<td>10 mg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>1 mg</td>
</tr>
<tr>
<td>Biotin</td>
<td>30 mcg</td>
</tr>
<tr>
<td>Vitamin C (sodium ascorbate/ascorbic acid)</td>
<td>100 mg</td>
</tr>
<tr>
<td>Vitamin E (d-alpha tocopheryl acid succinate)</td>
<td>30 IU</td>
</tr>
</tbody>
</table>

**Minerals**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (citrate)</td>
<td>250 mg</td>
</tr>
<tr>
<td>Magnesium (oxide)</td>
<td>50 mg</td>
</tr>
<tr>
<td>Iron (ferrous fumarate)</td>
<td>27 mg</td>
</tr>
<tr>
<td>Potassium (citrate)</td>
<td>5 mg</td>
</tr>
<tr>
<td>Zinc (citrate)</td>
<td>25 mg</td>
</tr>
<tr>
<td>Manganese (gluconate)</td>
<td>1 mg</td>
</tr>
<tr>
<td>Iodine (potassium iodide)</td>
<td>0.15 mg</td>
</tr>
<tr>
<td>Copper (copper gluconate)</td>
<td>2 mg</td>
</tr>
<tr>
<td>Chromium (chromium HVP chelate)</td>
<td>25 mcg</td>
</tr>
<tr>
<td>Manganese (manganese citrate)</td>
<td>5 mg</td>
</tr>
<tr>
<td>Molybdenum (molybdenum)</td>
<td>25 mcg</td>
</tr>
<tr>
<td>Selenium (selenium HVP chelate)</td>
<td>25 mcg</td>
</tr>
</tbody>
</table>

HVP hydrolyzed vegetable protein

Non-medicinal ingredients: Microcrystalline cellulose, croscarmellose sodium, coating (carbohydrate gum, polyethylene glycol), vegetable grade magnesium stearate (lubricant).
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Date</th>
<th>400 IU Tablet</th>
<th>1000 IU Tablet</th>
<th>2000 IU Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Factors Inc.</td>
<td>January 17, 2011</td>
<td>473</td>
<td>1266</td>
<td>2523</td>
</tr>
<tr>
<td></td>
<td>August 3, 2011</td>
<td>463</td>
<td>1211</td>
<td>2209</td>
</tr>
<tr>
<td>Heartland Assays LLC</td>
<td>March 27, 2012</td>
<td>484</td>
<td>1144</td>
<td>2379</td>
</tr>
</tbody>
</table>
Chapter 3: Results

3.1.1 Recruitment and randomization

Figure 3.1 shows the participant enrollment, randomization and follow-up throughout the trial. Between July 2010 and August 2011, 114 pregnant women who met all the eligibility criteria were enrolled. Between enrolment and randomization, 4 women left the study; 2 participants had miscarriages, 1 terminated her pregnancy and 1 declined to participate. The remaining 110 participants attended a baseline clinic and were randomized to vitamin D treatment; 36 women in the 400 IU/d group, 38 women in the 1000 IU/d group and 36 women in the 2000 IU/d group. Twenty-one subjects did not attend their second clinic visit at 36 weeks gestation. The retention rate at 36 weeks was 81.8%. Reasons for withdrawal included not tolerating study vitamins \( n=3 \), illness \( n=5 \), personal issues \( n=3 \), too busy to continue participating \( n=2 \) or no reason cited \( n=7 \). Several participants \( n=6 \) missed their second study visit due to early delivery, preterm delivery, incorrect estimated delivery date calculations, or were on vacation; these women were shipped their vitamins and remained in the study, attending their third and final visit postpartum [which will not be covered in this thesis]. Twenty-five women in the 400 IU group, 28 women in the 1000 IU group and 28 women in the 2000 IU group completed the study, of these 18, 18 and 15, respectively, had their cord blood collected at delivery. The retention rate at the end of the study was 73.6% \( n=81 \).

3.1.2 Participant characteristics at baseline

Participant characteristics across supplement allocation groups are shown in Table 3.1. After allocation into treatment groups, there were no significant differences in the
participants’ characteristics across treatment groups for any measures. For example, there were no differences in maternal age, stage of gestation at enrollment, gravidity or parity. At baseline the mean ± standard deviation (SD) serum 25OHD was 70.5 ± 24.4 nmol/L, 66.0 ± 22.3 nmol/L, and 67.6 ± 15.6 nmol/L in the 400 IU, 1000 IU, and 2000 IU groups, respectively. Of the participants, 25%, 23.7%, and 5.6% in the 400 IU, 1000 IU, and 2000 IU groups, respectively, had a serum 25OHD less than 50 nmol/L, and overall 18.2% of participants were vitamin D insufficient at baseline.

Overall, 76% had a normal pre-pregnancy body mass index (BMI) (between 18.5-24.9 kg/m²). Participants were generally well-educated, with most having completed an undergraduate degree at minimum, and many having completed a post-graduate program or more. The women were primarily Caucasian (white/European) ethnicity (77%) and 23% of the women were of non-Caucasian ethnicities comprised of First Nations (0.9%), Chinese (10%), East Indian (4.5%) and ‘other’, which included Arab, Iranian, South-East Asian and Japanese. Most women had a household income of greater than $50,000 per year. More women entered the study in the summer months (April- September) versus the winter months (October-March). Dietary and supplemental vitamin D and calcium intake did not differ among treatment groups at baseline.

3.1.3 Compliance

Of the women who attended the clinic visit at 36 weeks (n=90) 85% of them were considered compliant, taking more than 80% of their vitamin D supplements in the two months prior to their visit. In the 400 IU, 1000 IU and 2000 IU allocations 83.3%, 72.4% and 96.8% of women were over 80% compliant over the last two months. There was a statistically significant difference in compliance between treatment groups at 36 weeks.
In the ITT analysis, the mean ± SD 25OHD concentrations at baseline were not significantly different by treatment ($p=0.653$); 70.5 ± 24.4 nmol/L in the 400 IU, 66 ± 22.3 nmol/L in the 1000 IU and 67.6 ± 15.6 nmol/L in the 2000 IU group. There were also no significant differences in 25OHD concentrations at baseline between supplement allocations in the AT analyses ($p=0.136$). At baseline, in those ≥ 80% compliant serum 25OHD greatest in the 400 IU group ($n=25$) 74.1 ± 25.7 nmol/L compared to the 1000 IU group ($n=24$) 64.6 ± 23.5 nmol/L, was not different ($p=0.307$) (Table 3.8).

In the ITT analysis there was a trend toward increasing serum concentrations of 25OHD in the 1000 IU and 2000 IU treatment groups at 36 weeks gestation; mean 25OHD in nmol/L was 70.4 ± 19.4 in the 400 IU group, 76.5 ± 25.7 in the 1000 IU group and 80.1 ± 20.3 in the 2000 IU group, however the differences were not statistically significant ($p=0.178$). Similar results were found in the AT analysis and ≥ 80% compliant participants ($p=0.165$ and $p=0.243$, respectively). The change in serum 25OHD (nmol/L) from baseline to 36 weeks of gestation however was significantly different between women in the 400 IU group and 1000 IU and between women in the 400 IU and 2000 IU groups in all analyses (ITT, as-treated, and participants over 80% compliant) ($p=0.005$, $p=0.003$ and $p=0.137$, respectively).
Mean newborn umbilical cord 25OHD concentrations were highest in infants born to mothers assigned to the 2000 IU vitamin D supplement, however the concentrations were not significantly different from newborns born to mothers in the 400 IU and 1000 IU allocations ($p=0.11$ (ITT), $p=0.08$ (as-treated) and $p=0.137$ (80% compliance). We reported one cord blood sample with serum 25OHD $> 225$ nmol/L; at 281 nmol/L. This appears to be an error in 25OHD measurement as the mother’s 25OHD at 36 weeks and the infant’s 25OHD measurement at 8 weeks postpartum were both within the normal range. This outlier was removed from the data set.

3.1.5 Vitamin D insufficiency and sufficiency

The second objective of this research was to determine the number of women by treatment groups achieving sufficient 25OHD concentrations ($> 50$ nmol/L) at 36 weeks gestation. A number of other commonly used cut-offs were also evaluated (Table 3.3 and Table 3.4). Using ITT, overall, at 36 weeks only 11% (11/110) of women had a serum 25OHD less than 50 nmol/L. Including only those women who attended the 36 week visit (as-treated) 7% of these women had a serum 25OHD less than 50 nmol/L. More women in the 1000 IU allocation were diagnosed as vitamin D insufficient (25OHD $< 50$ nmol/L) in both the ITT analysis ($n=7$, 18.4%) and AT analysis ($n=5$, 17.2%) compared to the 400 IU and 2000 IU supplement groups; differences were not significant ($p=0.227$, $p=0.069$ respectively). In the ITT analysis 22 women (61.6%) in the 400IU group and 19 women (50.0%) in the 1000 IU group had serum 25OHD concentrations below 75 nmol/L, compared to 14 women (38.9%) in the 2000 IU group ($p=0.169$). Similar results were found in the AT analysis ($p=0.247$). More women in the 1000 IU and 2000 IU supplement allocations reached 25OHD concentrations greater than 100 nmol/L compared to the 400 IU group.
(p=0.354 (ITT), p=0.504 (as-treated). For women who were over 80% compliant to supplement dosing significantly more women were classified as insufficient (25OHD < 50 nmol/L) in the 1000 IU group, with 0 women in the 400 IU group, 4 in the 1000 IU group and 1 in the 2000 IU group classified as vitamin D deficient at 36 weeks of gestation [p=0.026] (Table 3.5).

3.1.6 Factors affecting 25OHD concentrations at 36 weeks

Factors affecting serum 25OHD concentrations at 36 weeks of gestation are shown in Table 3.9 (ITT analysis) and Table 3.10 (AT analysis). Variables included were treatment group (400 IU, 1000 IU and 2000 IU), season at 36 weeks (winter or summer) and ethnicity (Caucasian or non-Caucasian). No interactions were found when the analysis was adjusted for the participant’s baseline 25OHD concentration, the number of weeks on supplement and dietary intake of vitamin D (IU) at 36 weeks. Supplement dose was a predictor of 25OHD concentrations, such that those in the 400 IU group had vitamin D concentrations a mean of 12.34 nmol/L lower than those in the 2000 IU group in ITT analysis (p=0.004) and 12.26 nmol/L lower in AT analysis (p=0.004). Season was a statistically significant predictor of 25OHD status such that those women who were 36 weeks pregnant in the summer months had 25OHD concentrations a mean of 14.1 nmol/L higher than those who were 36 weeks gestation in the winter months (p<0.000 for both ITT and as-treated analysis). Ethnicity was not a significant predictor of 25OHD concentrations, though a trend was seen that 25OHD concentrations were higher in Caucasian participants (p=0.33 ITT and p=0.35 AT).

3.1.7 Dietary vitamin D and calcium

Dietary vitamin D and calcium intake at baseline, 36 weeks and the change between baseline and 36 weeks are shown as ITT analysis in Table 3.11 and AT analysis in Table
3.12. The mean ± SD dietary vitamin D intake at baseline was 184.6 ± 115.4 IU/day and
200.1 ± 118.5 at 36 weeks gestation in the 400 IU group, 170.7 ± 114.6 and 193.7 ± 114.9
IU/day in the 1000 IU group and 198.6 ± 127.3 versus 221.2 ± 119.1 IU/day in the 2000 IU
group in ITT analysis. In the ITT analysis there was no difference among treatment groups in
vitamin D intake at baseline ($p=0.603$) or at 36 weeks gestation ($p=0.579$). Similar results
were seen in AT analyses and no significant differences were found. Although there were no
significant differences between groups in vitamin D intake at baseline and 36 weeks
($p=0.977$) vitamin D intake in IU/day did increase significantly from baseline to 36 weeks
within all groups [$p=0.047$].

3.1.8 Bone specific alkaline phosphatase

Maternal and newborn cord blood bone-specific alkaline phosphatase (BSAP)
concentrations are presented as means ± SD and the range in Table 3.13 and 3.14. There
were no significant differences in serum BSAP concentrations between treatment groups at
baseline or 36 weeks in either ITT or AT analyses. At 36 weeks gestation the mean serum
BSAP was 11.3 ± 5.3 ug/L in the 400 IU group, 11.2 ± 4.4 ug/L in the 1000 IU group and
11.8 ± 4.4 ug/L in the 2000 IU group in the ITT analysis ($p=0.85$). In the AT analyses BSAP
concentrations were 12.2 ± 5.3 ug/L in the 400 IU group, 11.9 ± 4.5 ug/L in the 1000 IU
group and 12.1 ± 4.6 ug/L in the 2000 IU group ($p=0.973$). BSAP increased significantly in
all groups from baseline to 36 weeks gestation ($p < 0.000$ for both ITT and AT). The mean
increase in BSAP from baseline to 36 weeks gestation for ITT analysis was 2.7 ± 3.4 ug/L
($p=0.958$) and for AT analysis was 3.3 ± 3.4 ug/L ($p=0.96$).
Newborn BSAP concentrations as measured from cord blood were significantly higher than maternal BSAP concentrations. There were no significant differences in cord blood BSAP concentrations across treatment groups ($p=0.968$ ITT and $p=0.949$ AT).

### 3.1.9 Safety measures

Serum and urine creatinine, calcium and phosphate in the treatment groups at baseline and 36 weeks gestation are reported as means ± SD. The ITT analysis is reported in Table 3.15 and AT analysis in Table 3.16. Urine calcium to creatinine ratios and any changes from baseline to 36 weeks are also reported. There were no significant differences in any measures at 36 weeks, for example in ITT analysis the serum calcium was $2.06 \pm 0.22$ mmol/L in the 400IU group, $2.10 \pm 0.21$ mmol/L in the 1000 IU group and $2.06 \pm 0.22$ mmol/L in the 2000 IU group ($p=0.618$). There were also no significant differences in the measures in the AT analysis. No differences were found among the groups in the change in the calcium to creatinine ratio (mg/dL) from baseline to 36 weeks ($p=0.439$ ITT and $p=0.464$ AT).

At 36 weeks gestation no participants had any measures that exceeded the normal laboratory cut-offs for serum creatinine or calcium (> 104 umol/L and 2.7 mmol/L, respectively). There were 5 women who had low serum creatinine (< 39 umol/L) at 36 weeks, and 40 women who had low serum calcium at 36 weeks (< 2.2 mmol/L). There was one woman with low serum phosphate (< 0.87 mmol/L) and 4 women whose serum phosphate values exceeded the recommended range (> 1.52 mmol/L). At 36 weeks gestation 2 women had a urinary calcium to creatinine ratio that exceeded the recommended range (> 0.7 mmol/L).
Figure 3.1 Flow diagram of pregnancy study

Assessed for eligibility and enrolled (n=114)

Excluded (n=4)
- miscarriage (n=2)
- declined participation (n=1)
- pregnancy termination (n=1)

Allocate group (n=36)
- received allocated intervention (n=36)

Attended second visit, 36 weeks (n=30)
- did not attend 2nd visit (n=6)
- missed 2nd visit, attended 3rd (n=1)

Complete dy (n=25)
- cord blood collected (n=18)
- lost to follow up (n=8)
- withdrew (n=3)

Allocate group (n=38)
- received allocated intervention (n=38)

Attended second visit, 36 weeks (n=29)
- did not attend 2nd visit (n=9)
- missed 2nd visit, attended 3rd (n=1)

Complete dy (n=28)
- cord blood collected (n=18)
- lost to follow up (n=7)
- withdrew (n=3)

Allocate group (n=36)
- received allocated intervention (n=36)

Attended second visit, 36 weeks (n=31)
- did not attend 2nd visit (n=5)
- missed 2nd visit, attended 3rd (n=2)

Complete dy (n=28)
- cord blood collected (n=15)
- lost to follow up (n=5)
- withdrew (n=3)

Co-mplet ed study (n=25)
- cord blood collected (n=18)
- lost to follow up (n=8)
- withdrew (n=3)

Excluded (n=4)
- miscarriage (n=2)
- declined participation (n=1)
- pregnancy termination (n=1)
Table 3.1 Baseline participant characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>400 IU group (n=36)</th>
<th>1000 IU group (n=38)</th>
<th>2000 IU group (n=36)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>33.2 ± 3.7</td>
<td>32.4 ± 4.9</td>
<td>33.2 ± 4.3</td>
<td>0.655</td>
</tr>
<tr>
<td>Gestation at enrollment (weeks), mean ± SD</td>
<td>20.9 ± 2.0</td>
<td>20.3 ± 2.1</td>
<td>20.2 ± 3.1</td>
<td>0.374</td>
</tr>
<tr>
<td>Maternal gravidity, median (range)</td>
<td>1 (0-6)</td>
<td>1 (0-7)</td>
<td>1.5 (0-7)</td>
<td>0.416</td>
</tr>
<tr>
<td>Maternal parity, median (range)</td>
<td>0 (0-2)</td>
<td>0 (0-4)</td>
<td>0 (0-3)</td>
<td>0.567</td>
</tr>
<tr>
<td>Pre-pregnancy BMI, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.183</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>24 (68.6)</td>
<td>23 (65.7)</td>
<td>28 (84.8)</td>
<td></td>
</tr>
<tr>
<td>24.9-29.9</td>
<td>2 (11.4)</td>
<td>8 (22.9)</td>
<td>3 (9.1)</td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>7 (20.0)</td>
<td>4 (11.4)</td>
<td>2 (4.2)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.985</td>
</tr>
<tr>
<td>White</td>
<td>28 (77.8)</td>
<td>29 (76.3)</td>
<td>28 (77.8)</td>
<td></td>
</tr>
<tr>
<td>Non-white$^1$</td>
<td>8 (22.2)</td>
<td>9 (23.7)</td>
<td>8 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.238</td>
</tr>
<tr>
<td>High school</td>
<td>0 (0)</td>
<td>1 (2.7)</td>
<td>3 (8.8)</td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>8 (22.2)</td>
<td>9 (24.3)</td>
<td>4 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Undergraduate or more</td>
<td>28 (77.8)</td>
<td>27 (73.0)</td>
<td>27 (79.4)</td>
<td></td>
</tr>
<tr>
<td>Household income per year, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.919</td>
</tr>
<tr>
<td>&lt; $50,000</td>
<td>4 (11)</td>
<td>5 (13.5)</td>
<td>7 (20.6)</td>
<td></td>
</tr>
<tr>
<td>$ 50,000 - $ 100,000</td>
<td>13 (36.1)</td>
<td>15 (40.5)</td>
<td>10 (29.4)</td>
<td></td>
</tr>
<tr>
<td>≥ $ 100,000</td>
<td>19 (52.8)</td>
<td>17 (45.9)</td>
<td>17 (16.8)</td>
<td></td>
</tr>
<tr>
<td>Season at study entry, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.873</td>
</tr>
<tr>
<td>April - September</td>
<td>26 (72.2)</td>
<td>26 (68.4)</td>
<td>24 (66.7)</td>
<td></td>
</tr>
<tr>
<td>October - March</td>
<td>10 (27.8)</td>
<td>12 (31.6)</td>
<td>12 (33.3)</td>
<td></td>
</tr>
</tbody>
</table>
Dietary vitamin D intake (IU), mean ± SD 185 ± 115 170 ± 114 198 ± 127 0.603  
Supplement vitamin D intake (IU), mean ± SD 439 ± 311 367 ± 148 460 ± 276 0.272  
Dietary calcium intake (mg), mean ± SD 777 ± 358 753 ± 478 837 ± 427 0.691  
Supplement calcium intake (mg), mean ± SD 219 ± 93 259 ± 157 229 ± 121 0.337  
25OHD (nmol/L) at baseline, mean ± SD 70.5 ± 24.4 66 ± 22.3 67.6 ± 15.6 0.653  
Baseline serum 25OHD (nmol/L), n (%)  
| < 50 nmol/L   | 9 (25%)  | 9 (23.7%) | 2 (5.6%) | 0.056  
| < 75 nmol/L   | 21 (58.3%) | 26 (68.4%) | 24 (66.7%) | 0.629  
| > 100 nmol/L  | 3 (8.3%)  | 3 (7.9%)  | 1 (2.8%)  | 0.56  

BMI, Body Mass Index  
1First Nations (0.9%), Chinese (10%), East Indian (4.5%) and other

**Table 3.2 Compliance by supplement dosage**

<table>
<thead>
<tr>
<th>Compliance</th>
<th>400 IU (n=30)</th>
<th>1000 IU (n=29)</th>
<th>2000 IU (n=32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 80% compliant1, n (%)</td>
<td>5 (16.7)</td>
<td>8 (27.6)</td>
<td>1 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Over 80% compliant, n (%)</td>
<td>25 (83.3)</td>
<td>21 (72.4)*</td>
<td>30 (96.8)*</td>
<td>0.033</td>
</tr>
</tbody>
</table>

1 Compliance calculated as taking 80% or more of 60 supplement tablets two months prior to 36 weeks gestation  
* Results are significantly different (p < 0.05), Bonferroni post-hoc analyses
### Table 3.3 Serum 25OHD concentrations (nmol/L) during pregnancy and in cord blood (ITT)

<table>
<thead>
<tr>
<th>Serum 25OHD (nmol/L)</th>
<th>400 IU (n=36)</th>
<th>1000 IU (n=38)</th>
<th>2000 IU (n=36)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline, mean ± SD</td>
<td>70.5 ± 24.4</td>
<td>66 ± 22.3</td>
<td>67.6 ± 15.6</td>
<td>0.653</td>
</tr>
<tr>
<td>Range</td>
<td>(40-149.0)</td>
<td>(16-124)</td>
<td>(30-102)</td>
<td></td>
</tr>
<tr>
<td>36 weeks gestation, mean ± SD</td>
<td>70.4 ± 19.5</td>
<td>76.5 ± 25.7</td>
<td>80.1 ± 20.3</td>
<td>0.178</td>
</tr>
<tr>
<td>Range</td>
<td>(34.1-106)</td>
<td>(34.9-151)</td>
<td>(29.6-140)</td>
<td></td>
</tr>
<tr>
<td>Change from baseline to 36 weeks, mean ± SD</td>
<td>(-0.08) ± 15.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.5 ± 20.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.4 ± 13.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.005</td>
</tr>
<tr>
<td>Cord blood, mean ± SD</td>
<td>72.2 ± 21.4</td>
<td>65.5 ± 22.6</td>
<td>91.5 ± 19.8</td>
<td>0.111</td>
</tr>
<tr>
<td>Range</td>
<td>(24-108)</td>
<td>(30.3-124)</td>
<td>(51.2-126)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>ab</sup>Results are statistically significant, p<0.05 (Bonferroni), results with the same letter are significantly different from one another

ITT, Intention to Treat model used

### Table 3.4 Serum 25OHD concentrations (nmol/L) during pregnancy and in cord blood (AT)

<table>
<thead>
<tr>
<th>Serum 25OHD (nmol/L)</th>
<th>400 IU (n=30)</th>
<th>1000 IU (n=29)</th>
<th>2000 IU (n=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline, mean ± SD</td>
<td>72.6 ± 24.4</td>
<td>62.2 ± 19.7</td>
<td>68.4 ± 14.2</td>
<td>0.136</td>
</tr>
<tr>
<td>Range</td>
<td>(43-149)</td>
<td>(16-100)</td>
<td>(35-102)</td>
<td></td>
</tr>
<tr>
<td>36 weeks gestation, mean ± SD</td>
<td>72.5 ± 18.5</td>
<td>75.9 ± 25.9</td>
<td>82.7 ± 18.5</td>
<td>0.165</td>
</tr>
<tr>
<td>Range</td>
<td>(34.1-106)</td>
<td>(43.1-151)</td>
<td>(49.0-140)</td>
<td></td>
</tr>
<tr>
<td>Change from baseline to 36 weeks, mean ± SD</td>
<td>(-0.14) ± 17.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13.7 ± 22.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 13.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>Cord blood, mean ± SD</td>
<td>79.2 ± 18.1</td>
<td>67.5 ± 21.5</td>
<td>91.5 ± 19.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Range</td>
<td>(49-108)</td>
<td>(48-124)</td>
<td>(51.2-126)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>ab</sup>Results are statistically significant, p<0.05 (Bonferroni), results with the same letter are significantly different from one another
Table 3.5  Serum 25OHD concentrations (nmol/L) during pregnancy and in cord blood for those over 80% compliant

<table>
<thead>
<tr>
<th></th>
<th>Serum 25OHD (nmol/L)</th>
<th></th>
<th></th>
<th></th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400 IU (n=25)</td>
<td>1000 IU (n=24)</td>
<td>2000 IU (n=30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mean ± SD</td>
<td>74.1 ± 25.7</td>
<td>64.6 ± 23.5</td>
<td>68.6 ± 14.3</td>
<td>0.307</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(43.0-149.0)</td>
<td>(16.0-124.0)</td>
<td>(34.8-102.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 weeks gestation, mean ± SD</td>
<td>73.4 ± 17.8</td>
<td>77.9 ± 27.6</td>
<td>83.2 ± 18.6</td>
<td>0.243</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(50.0-106.0)</td>
<td>(43.0-151.0)</td>
<td>(49.0-140.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change from baseline to 36 weeks, mean ± SD</td>
<td>-0.61 ± 14.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.9 ± 24.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.7 ± 13.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Cord blood, mean ± SD</td>
<td>77.4 ± 16.2</td>
<td>65.6 ± 18.2</td>
<td>90.8 ± 20.3</td>
<td>0.137</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(49.0-102.0)</td>
<td>(48.0-108.0)</td>
<td>(51.2-126.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>ab</sup>Results are statistically significant, p<0.05 (Bonferroni), results with the same letter are significantly different from one another.

Compliancy assessed as those consuming 80% or more of 60 supplement tablets two months prior to 36 weeks gestation.
### Table 3.6 The percentage of women achieving different cut-offs of serum 25OHD at 36 weeks gestation (ITT)

<table>
<thead>
<tr>
<th>Supplement Dose</th>
<th>&lt; 50</th>
<th>&lt; 75</th>
<th>≥ 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 IU (n=36)</td>
<td>4 (11.)</td>
<td>22 (61.1)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>1000 IU (n=38)</td>
<td>7 (18.4)</td>
<td>19 (50.0)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>2000 IU (n=36)</td>
<td>2 (5.6)</td>
<td>14 (38.9)</td>
<td>5 (13.9)</td>
</tr>
<tr>
<td>p value</td>
<td>0.227</td>
<td>0.169</td>
<td>0.354</td>
</tr>
</tbody>
</table>

ITT, Intention to Treat model used

### Table 3.7 The percentage of women achieving different cut-offs of serum 25OHD at 36 weeks gestation (AT)

<table>
<thead>
<tr>
<th>Supplement Dose</th>
<th>&lt; 50</th>
<th>&lt; 75</th>
<th>≥ 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 IU (n=30)</td>
<td>1 (3.3)</td>
<td>17 (56.7)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>1000 IU (n=29)</td>
<td>5 (17.2)</td>
<td>14 (48.3)</td>
<td>4 (13.8)</td>
</tr>
<tr>
<td>2000 IU (n=31)</td>
<td>1 (3.2)</td>
<td>11 (35.5)</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td>p value</td>
<td>0.069</td>
<td>0.247</td>
<td>0.504</td>
</tr>
</tbody>
</table>

### Table 3.8 The percentage of women achieving different cut-offs of serum 25OHD at 36 weeks gestation (>80% compliant)

<table>
<thead>
<tr>
<th>Supplement Dose</th>
<th>&lt; 50</th>
<th>&lt; 75</th>
<th>≥ 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 IU (n=30)</td>
<td>0 (0)</td>
<td>14 (38.9)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>1000 IU (n=29)</td>
<td>4 (10.5)</td>
<td>10 (26.3)</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>2000 IU (n=31)</td>
<td>1 (2.8)</td>
<td>10 (27.8)</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>p value</td>
<td>0.026</td>
<td>0.297</td>
<td>0.792</td>
</tr>
</tbody>
</table>
### Table 3.9 Estimated difference in 25OHD concentrations (nmol/L) according to selected variables (ITT)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated difference in 25OHD (nmol/L)</th>
<th>Marginal Mean</th>
<th>(95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 IU</td>
<td>-12.34</td>
<td>70.79</td>
<td>(64.5, 77.1)</td>
<td>0.004</td>
</tr>
<tr>
<td>1000 IU</td>
<td>-3.53</td>
<td>79.6</td>
<td>(72.9, 86.3)</td>
<td>0.386</td>
</tr>
<tr>
<td>2000 IU</td>
<td>0</td>
<td>83.13</td>
<td>(76.7, 89.6)</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Winter</td>
<td>0</td>
<td>84.9</td>
<td>(78.5, 91.4)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>14.14</td>
<td>70.8</td>
<td>(66.2, 75.4)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>White</td>
<td>4.08</td>
<td>79.9</td>
<td>(75.9, 83.8)</td>
<td></td>
</tr>
<tr>
<td>Non-white</td>
<td>0</td>
<td>75.8</td>
<td>(68.3, 83.2)</td>
<td></td>
</tr>
</tbody>
</table>

Estimates adjusted for baseline 25OHD, Dietary vitamin D intake at 36 weeks and time (weeks) on supplement ITT, Intention to Treat model used

### Table 3.10 Estimated difference in 25OHD concentrations (nmol/L) according to selected variables, as-treated

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated difference in 25OHD (nmol/L)</th>
<th>Marginal Mean</th>
<th>(95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 IU</td>
<td>-12.26</td>
<td>68.5</td>
<td>(61.41, 75.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>1000 IU</td>
<td>-4.17</td>
<td>76.8</td>
<td>(69.5, 84.2)</td>
<td>0.343</td>
</tr>
<tr>
<td>2000 IU</td>
<td>0</td>
<td>81.2</td>
<td>(74.1, 88.3)</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Winter</td>
<td>0</td>
<td>74.2</td>
<td>(69.3, 79.1)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>14.01</td>
<td>76.8</td>
<td>(69.4, 84.2)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>0.352</td>
</tr>
<tr>
<td>White</td>
<td>4</td>
<td>78.4</td>
<td>(74.0, 82.8)</td>
<td></td>
</tr>
<tr>
<td>Non-white</td>
<td>0</td>
<td>72.6</td>
<td>(64.4, 80.9)</td>
<td></td>
</tr>
</tbody>
</table>

Estimates adjusted for baseline 25OHD, Dietary vitamin D intake at 36 weeks and time (weeks) on supplement
### Table 3.11 Dietary vitamin D and calcium intake at baseline and 36 weeks gestation (ITT)

<table>
<thead>
<tr>
<th>Vitamin D intake (IU)/day</th>
<th>Baseline, mean ± SD</th>
<th>36 weeks, mean ± SD</th>
<th>Change in dietary vitamin D intake (IU), mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (IU) or Calcium (mg)/day</td>
<td>400 IU (n=36)</td>
<td>1000 IU (n=38)</td>
<td>2000 IU (n=36)</td>
</tr>
<tr>
<td>Vitamin D intake (IU)/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mean ± SD</td>
<td>184.6 ± 115.4</td>
<td>170.7 ± 114.6</td>
<td>198.6 ± 127.3</td>
</tr>
<tr>
<td>36 weeks, mean ± SD</td>
<td>200.1 ± 118.5</td>
<td>193.7 ± 114.9</td>
<td>221.2 ± 119.1</td>
</tr>
<tr>
<td>Change in dietary vitamin D intake (IU), mean (95% CI)</td>
<td>15.5 (-18.3, 49.3)</td>
<td>23 (-2.7, 48.8)</td>
<td>22.6 (-10.6, 55.7)</td>
</tr>
<tr>
<td>Calcium intake (mg)/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mean ± SD</td>
<td>777.7 ± 358.7</td>
<td>753.5 ± 478.3</td>
<td>836.6 ± 427.6</td>
</tr>
<tr>
<td>36 weeks, mean ± SD</td>
<td>820.6 ± 345.8</td>
<td>814.4 ± 469.7</td>
<td>885.2 ± 437.1</td>
</tr>
<tr>
<td>Change in dietary calcium intake (mg), mean (95% CI)</td>
<td>42.9 (-81.6, 167.5)</td>
<td>60.9 (-29.9, 151.7)</td>
<td>48.6 (-103.9, 201.1)</td>
</tr>
</tbody>
</table>

ITT, Intention to Treat model used

### Table 3.12 Dietary vitamin D and calcium intake at baseline and 36 weeks gestation (AT)

<table>
<thead>
<tr>
<th>Vitamin D intake (IU)/day</th>
<th>Baseline, mean ± SD</th>
<th>36 weeks, mean ± SD</th>
<th>Change in dietary vitamin D intake (IU), mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D (IU) or Calcium (mg)/day</td>
<td>400 IU (n=30)</td>
<td>1000 IU (n=29)</td>
<td>2000 IU (n=31)</td>
</tr>
<tr>
<td>Vitamin D intake (IU)/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mean ± SD</td>
<td>181 ± 112.4</td>
<td>157.4 ± 109.7</td>
<td>196.4 ± 125.6</td>
</tr>
<tr>
<td>36 weeks, mean ± SD</td>
<td>199.6 ± 116.6</td>
<td>182.2 ± 115.9</td>
<td>222.6 ± 116</td>
</tr>
<tr>
<td>Change in dietary vitamin D intake (IU), mean (95% CI)</td>
<td>18.6 (-22.3, 59.4)</td>
<td>21.69 (-17.3, 60.7)</td>
<td>26.2 (-12.5, 64.9)</td>
</tr>
<tr>
<td>Calcium intake (mg)/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, mean ± SD</td>
<td>764.3 ± 350.6</td>
<td>742.5 ± 439.6</td>
<td>843.1 ± 429.6</td>
</tr>
<tr>
<td>36 weeks, mean ± SD</td>
<td>815.8 ± 336</td>
<td>829.5 ± 434.4</td>
<td>899.5 ± 439.2</td>
</tr>
<tr>
<td>Change in dietary calcium intake (mg), mean (95% CI)</td>
<td>51.5 (-99.3, 202.3)</td>
<td>59.5 (-82.9, 201.9)</td>
<td>55.7 (-122, 234.8)</td>
</tr>
</tbody>
</table>
### Table 3.13 Serum bone-specific alkaline phosphatase (BSAP) concentrations (ug/L) during pregnancy and cord blood (ITT)

<table>
<thead>
<tr>
<th>Measure</th>
<th>400 IU ($n=36$)</th>
<th>1000 IU ($n=38$)</th>
<th>2000 IU ($n=36$)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSAP (µg/L) at baseline, mean ± SD</td>
<td>8.51 ± 2.30</td>
<td>8.57 ± 2.76</td>
<td>9.03 ± 2.51</td>
<td>0.626</td>
</tr>
<tr>
<td>Range</td>
<td>(5.0-13.0)</td>
<td>(3.9-16.0)</td>
<td>(5.0-17.0)</td>
<td></td>
</tr>
<tr>
<td>BSAP (µg/L) at 36 weeks, mean ± SD</td>
<td>11.33 ± 5.29</td>
<td>11.17 ± 4.38</td>
<td>11.77 ± 4.41</td>
<td>0.85</td>
</tr>
<tr>
<td>Range</td>
<td>(5.0-28.0)</td>
<td>(4.5-20.9)</td>
<td>(6.0-26.0)</td>
<td></td>
</tr>
<tr>
<td>Mean difference in BSAP from baseline to 36 weeks, mean ± SD</td>
<td>2.82 ± 4.09</td>
<td>2.54 ± 3.09</td>
<td>2.73 ± 2.86</td>
<td>0.958</td>
</tr>
<tr>
<td>Cord blood BSAP (µg/L), mean ± SD</td>
<td>25.94 ± 10.15</td>
<td>26.54 ± 12.2</td>
<td>25.63 ± 8.21</td>
<td>0.968</td>
</tr>
<tr>
<td>Range</td>
<td>(15.0-47.0)</td>
<td>(8.2-59.0)</td>
<td>(14.0-45.0)</td>
<td></td>
</tr>
</tbody>
</table>

ITT, Intention to Treat model used

### Table 3.14 Serum bone-specific alkaline phosphatase (BSAP) concentrations (ug/L) during pregnancy and cord blood (AT)

<table>
<thead>
<tr>
<th>Measure</th>
<th>400 IU ($n=30$)</th>
<th>1000 IU ($n=29$)</th>
<th>2000 IU ($n=31$)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSAP (µg/L) at baseline, mean ± SD</td>
<td>8.86 ± 2.26</td>
<td>8.55 ± 2.72</td>
<td>8.89 ± 2.53</td>
<td>0.851</td>
</tr>
<tr>
<td>Range</td>
<td>(5.4-13.0)</td>
<td>(3.9-16.0)</td>
<td>(5.0-17.0)</td>
<td></td>
</tr>
<tr>
<td>BSAP (µg/L) at 36 weeks, mean ± SD</td>
<td>12.24 ± 5.3</td>
<td>11.96 ± 4.46</td>
<td>12.06 ± 4.6</td>
<td>0.973</td>
</tr>
<tr>
<td>Range</td>
<td>(6.28.0)</td>
<td>(4.7-20.9)</td>
<td>(6.0-26.0)</td>
<td></td>
</tr>
<tr>
<td>Mean difference in BSAP from baseline to 36 weeks, mean ± SD</td>
<td>3.38 ± 4.26</td>
<td>3.4 ± 3.12</td>
<td>3.17 ± 2.85</td>
<td>0.96</td>
</tr>
<tr>
<td>Cord blood BSAP (µg/L), mean ± SD</td>
<td>25.92 ± 10.58</td>
<td>26.8 ± 12.53</td>
<td>25.63 ± 8.21</td>
<td>0.949</td>
</tr>
<tr>
<td>Range</td>
<td>(15.0-47.0)</td>
<td>(8.2-59.0)</td>
<td>(14.0-45.0)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3.15 Serum and urine creatinine calcium and phosphate at baseline and 36 weeks gestation (ITT)

<table>
<thead>
<tr>
<th>Measure</th>
<th>400 IU (n=36)</th>
<th>1000 IU (n=38)</th>
<th>2000 IU (n=36)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol/L), mean ± SD</td>
<td>51.1 ± 8.2</td>
<td>49.7 ± 8.7</td>
<td>49.4 ± 8.9</td>
<td>0.693</td>
</tr>
<tr>
<td>36 weeks (µmol/L), mean ± SD</td>
<td>50.8 ± 11.5</td>
<td>48.4 ± 10.6</td>
<td>50.0 ± 9.23</td>
<td>0.6</td>
</tr>
<tr>
<td>Urine Creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol/L), mean ± SD</td>
<td>7931.6 ± 6144.8</td>
<td>7607 ± 7394.7</td>
<td>8000.6 ± 5130.3</td>
<td>0.962</td>
</tr>
<tr>
<td>36 weeks (µmol/L), mean ± SD</td>
<td>6498.9 ± 4585.2</td>
<td>7516.1 ± 5063.2</td>
<td>10572.9 ± 24730.5</td>
<td>0.48</td>
</tr>
<tr>
<td>Serum Calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>2.23 ± 0.17</td>
<td>2.25 ± 0.24</td>
<td>2.21 ± 0.23</td>
<td>0.734</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>2.06 ± 0.22</td>
<td>2.10 ± 0.21</td>
<td>2.06 ± 0.22</td>
<td>0.618</td>
</tr>
<tr>
<td>Urine Calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>4.23 ± 3.18</td>
<td>3.78 ± 2.6</td>
<td>3.87 ± 2.7</td>
<td>0.772</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>4.31 ± 3.02</td>
<td>3.92 ± 2.95</td>
<td>3.75 ± 3.11</td>
<td>0.732</td>
</tr>
<tr>
<td>Serum Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>1.17 ± 0.13</td>
<td>1.19 ± 0.20</td>
<td>1.17 ± 0.17</td>
<td>0.828</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>1.13 ± 0.17</td>
<td>1.14 ± 0.20</td>
<td>1.13 ± 0.22</td>
<td>0.991</td>
</tr>
<tr>
<td>Urine Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>18.24 ± 17.39</td>
<td>14.46 ± 9.12</td>
<td>14.58 ± 9.05</td>
<td>0.352</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>15.55 ± 11.66</td>
<td>17.13 ± 10.67</td>
<td>15.3 ± 10.45</td>
<td>0.741</td>
</tr>
<tr>
<td>Urine Calcium Creatinine Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mg/dL), mean ± SD</td>
<td>0.229 ± 0.121</td>
<td>0.256 ± 0.195</td>
<td>0.218 ± 0.178</td>
<td>0.628</td>
</tr>
<tr>
<td>36 weeks (mg/dL), mean ± SD</td>
<td>0.283 ± 0.204</td>
<td>0.247 ± 0.214</td>
<td>0.250 ± 0.192</td>
<td>0.718</td>
</tr>
<tr>
<td>Change from baseline to 36 weeks (mg/dL), mean ± SD</td>
<td>0.047 ± 0.21</td>
<td>-0.009 ± 0.17</td>
<td>0.038 ± 0.197</td>
<td>0.439</td>
</tr>
</tbody>
</table>

ITT, Intention to Treat model used
<table>
<thead>
<tr>
<th>Measure</th>
<th>400 IU (n=30)</th>
<th>1000 IU (n=29)</th>
<th>2000 IU (n=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum Creatinine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol/L), mean ± SD</td>
<td>52.2 ± 8.1</td>
<td>48.8 ± 8.9</td>
<td>49.6 ± 9.3</td>
<td>0.284</td>
</tr>
<tr>
<td>36 weeks (µmol/L), mean ± SD</td>
<td>53.1 ± 7.9</td>
<td>47.1 ± 11</td>
<td>50.2 ± 9.7</td>
<td>0.059</td>
</tr>
<tr>
<td><strong>Urine Creatinine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (µmol/L), mean ± SD</td>
<td>8067.5 ± 6269.4</td>
<td>7959 ± 8235.3</td>
<td>8401.7 ± 5187.4</td>
<td>0.966</td>
</tr>
<tr>
<td>36 weeks (µmol/L), mean ± SD</td>
<td>6655.6 ± 4425.1</td>
<td>7832.2 ± 5512.9</td>
<td>11197.1 ± 25841.2</td>
<td>0.277</td>
</tr>
<tr>
<td><strong>Serum Calcium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>2.27 ± 0.12</td>
<td>2.22 ± 0.25</td>
<td>2.21 ± 0.24</td>
<td>0.519</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>2.18 ± 0.8</td>
<td>2.12 ± 0.16</td>
<td>2.12 ± 0.23</td>
<td>0.277</td>
</tr>
<tr>
<td><strong>Urine Calcium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>3.99 ± 2.93</td>
<td>3.51 ± 2.22</td>
<td>4.02 ± 2.62</td>
<td>0.697</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>4.10 ± 2.75</td>
<td>3.66 ± 2.81</td>
<td>3.88 ± 31.5</td>
<td>0.846</td>
</tr>
<tr>
<td><strong>Serum Phosphate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>1.17 ± 0.13</td>
<td>1.17 ± 0.22</td>
<td>1.16 ± 0.18</td>
<td>0.974</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>1.22 ± 0.15</td>
<td>1.20 ± 0.21</td>
<td>1.19 ± 0.23</td>
<td>0.828</td>
</tr>
<tr>
<td><strong>Urine Phosphate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mmol/L), mean ± SD</td>
<td>18.85 ± 18.28</td>
<td>13.89 ± 9.26</td>
<td>15.08 ± 9.17</td>
<td>0.304</td>
</tr>
<tr>
<td>36 weeks (mmol/L), mean ± SD</td>
<td>15.46 ± 11.8</td>
<td>17.28 ± 11.24</td>
<td>15.88 ± 10.64</td>
<td>0.808</td>
</tr>
<tr>
<td><strong>Urine Calcium Creatinine Ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (mg/dL), mean ± SD</td>
<td>0.227 ± 0.12</td>
<td>0.238 ± 0.17</td>
<td>0.222 ± 0.18</td>
<td>0.929</td>
</tr>
<tr>
<td>36 weeks (mg/dL), mean ± SD</td>
<td>0.288 ± 0.21</td>
<td>0.227± 0.2</td>
<td>0.258 ± 0.20</td>
<td>0.522</td>
</tr>
<tr>
<td>Change from baseline to 36 weeks (mg/dL), mean ± SD</td>
<td>0.548 ± 0.23</td>
<td>-0.012 ± 0.19</td>
<td>0.042 ± 0.22</td>
<td>0.464</td>
</tr>
</tbody>
</table>
Chapter 4: Discussion

At the outset of this research study, Health Canada recommended pregnant women consume 200 IU/d of vitamin D, which many experts and other public health authorities deemed inadequate [8, 65]. In 2010 the IOM (used by Health Canada) subsequently raised the recommended dietary allowance (RDA) to 600 IU/d of vitamin D to reduce the risk of rickets in children and osteomalacia and osteoporosis in adults for all individuals under 70 years of age, including pregnant and breastfeeding women [76]. Canadian women of childbearing age consume an estimated average of 208 ± 8 IU/d of dietary vitamin D while the median intake is 144 IU/d [81]. Canadian research has shown over 80% of pregnant women consume a prenatal supplement [15]. The remainder of a pregnant women’s vitamin D would usually include an additional 300-400 IU/d of vitamin D from a prenatal supplement; the resulting combined dietary and supplemental vitamin D intake is ~ 600 IU/d of vitamin D, meeting the recommended dietary allowance (RDA) set by Health Canada. Even higher vitamin D intakes have been called for, as vitamin D insufficiency (25OHD < 50 nmol/L) is still common in Canada with 24% of pregnant women in Vancouver reported to be vitamin D insufficient, even though most were taking supplements [15]. The need, safety and effectiveness of vitamin D supplementation remains controversial and in 2010 Health Canada called for Canadians research to determine optimal vitamin D intakes for pregnant and lactating women [19]. To address this call for research, we conducted a study in which we compared serum 25OHD concentrations among pregnant women randomized to 400 IU, 1000 IU or 2000 IU of supplemental vitamin D during pregnancy in Vancouver, British Columbia, Canada (49°N) with measure of their serum 25OHD in their infants umbilical
cord. The overall aim was to determine the percentage of women on different supplemental dosages of vitamin D achieving 25OHD concentrations of > 50, 75 or 100 nmol/L.

4.1 25OHD Outcomes

4.1.1 Serum 25OHD concentrations at 36 weeks

The recent study found that serum 25OHD concentrations at 36 weeks gestation were 70.4 ± 19.5 nmol/L in the 400 IU group, 76.5 ± 25.7 nmol/L in the 1000 IU group and 80.1 ± 20.3 nmol/L in the 2000 IU group \( p=0.178 \) in ITT analysis. Though there was not a significant difference between treatment groups, there was a trend toward increasing serum 25OHD concentrations with increased supplement dosage. There are few other studies that have looked at vitamin D supplementation over various time points during pregnancy; the highest quality study is a recent vitamin D supplementation trial in South Carolina conducted by Hollis et al. that found significant differences between treatment groups \[98\].

Hollis et al. randomized pregnant women \( (n=350) \) to 400 IU, 2000 IU or the UL of 4000 IU and found that 1 month prior to delivery, serum 25OHD concentrations were 81.2 ± 35.9 nmol/L in the 400 IU group, 102.6 ± 36.4 nmol/L in the 2000 IU group and 114.2 ± 35.5 nmol/L in the 4000 IU group \( p<0.0001 \) \[98\]. The Hollis et al. study found significant differences between treatment groups and much greater increases in 25OHD concentrations from baseline to 1 month prior to delivery. Compared to Hollis et al.’s study we didn’t report significant changes in 25OHD concentrations among treatment groups. There are many factors that could account for these differences, including sample size, baseline 25OHD concentrations, time on supplement and ethnicity.

First, women in Hollis et al.’s study were on higher amounts of supplemental vitamin D; our highest vitamin D dosage was 2000 IU/d whereas they supplemented with 4000 IU/d,
which is the current tolerable upper level of intake (UL). The subjects in their study had lower serum 25OHD concentrations at baseline; 62 nmol/L in their 400 IU/d group compared with ours at 70.5 nmol/L, 58.3 nmol/L in their 2000 IU/d group compared to 66 nmol/L in our 1000 IU group and 58.2 nmol/L in their 4000 IU/d group compared to 67.6 nmol/L in our 2000 IU group. There is evidence that baseline 25OHD status is an important factor in how an individual responds to a vitamin D dose; individuals with lower 25OHD concentrations initially will have greater improvements in 25OHD status compared to those with higher initial 25OHD concentrations [106, 107]. Consistent with this finding, women in our study had higher baseline 25OHD concentrations than those in Hollis’s study and did not show the same magnitude of increase in 25OHD concentrations.

Time on supplement can also affect serum 25OHD concentrations [108]. Participants in our trial took the vitamin D supplement for a shorter duration of time (3-4 months) as most entered the study at 20 weeks of gestation in comparison to Hollis et al.’s study in which women entered the study between 12-16 weeks and were on the higher vitamin dosages for approximately 6 months or longer [98].

Though we described no significant differences among groups, it is interesting to note that participants in our trial tended to have higher household income between $50,000-$100,000, and 48% of women had a household income greater than $100,000. Also, only 3.6% of women had only high school education and 74.5% of women had a post-secondary undergraduate degree or greater. Other researchers have reported similar results that low levels of education, low SES and low intakes of vitamin D are associated with vitamin D deficiency [110]. Indeed, in our study women had higher income and level of education and only 20 women (18.2%) had a baseline 25OHD concentration considered insufficient
In this study cohort, only 6 women (5.4%) did not report consuming a prenatal vitamin containing vitamin D compared to the study by Hollis et al. in which only 12.4% consumed a prenatal supplement with vitamin D [98]. This could account for the insignificant increases in 25OHD during pregnancy as women were entering the study already taking a prenatal vitamin resulting in higher baseline 25OHD concentrations and less increases in serum 25OHD during pregnancy.

In our study, we found that there was a significant difference in the change in 25OHD concentrations in nmol/L from baseline to 36 weeks gestation \( p=0.005 \). In the 400 IU group the mean 25OHD change from baseline to 36 weeks was \(-0.08 \pm 15.5\) nmol/L, in the 1000 IU group it was \(10.5 \pm 20.8\) nmol/L and in the 2000 IU group it was \(12.4 \pm 13.7\) nmol/L. Women’s 25OHD concentrations significantly increased in the 1000 IU and 2000 IU groups compared to the 400 IU group, in whom serum 25OHD concentrations did not change. Despite no differences in the mean 25OHD concentrations across treatment groups those on the highest two dosages of vitamin D (1000 and 2000 IU) showed significantly increased 25OHD concentrations from baseline compared to those in the 400 IU group. As most prenatal vitamins only contain 300-400 IU of vitamin D this result suggests that to increase serum 25OHD concentrations from baseline higher vitamin D dosages than in the current supplement are needed. This would be particularly true if a woman was vitamin D deficient going into pregnancy. Research by Datta et al. reported that when vitamin D deficient pregnant women (serum 25OHD 14.5 nmol/L) were supplemented with 800-1600 IU/d from their first antenatal visit onwards they were still vitamin D deficient at term (28 nmol/L) [93]. Mallet et al showed that at least 1000 IU/d of vitamin D was needed to increase 25OHD concentrations in deficient mothers [95]. Though 25OHD concentrations increase more
quickly in individuals with lower baseline 25OHD concentrations, women with low 25OHD concentrations may still need more vitamin D to achieve serum 25OHD > 50 nmol/L. As 24% of Vancouver pregnant women were found to be vitamin D insufficient a supplement of 400 IU/d may not raise their 25OHD concentrations. This may result in in their offspring being vitamin D insufficient or deficient as in order for an infant to be born with adequate adult concentrations of 25OHD the mother must not be deficient [15, 46].

4.1.2 Neonate 25OHD concentrations

We found no significant differences in the mean newborn serum 25OHD concentrations among groups as measured from an umbilical cord blood sample at delivery. In the 400 IU/d group 25OHD concentrations were 72.2 nmol/L, with lower values in the 1000 IU group at 65.5 nmol/L in contrast to the 2000 IU/d group where cord serum concentrations were 91.5 nmol/L. Reasons for these findings are not clear, but since there were only 43 cord blood samples collected this may have occurred through chance.

4.1.3 Vitamin D insufficiency

This study has shown that at 36 weeks of gestation there were no significant differences between treatment groups in the number of women with serum 25OHD concentrations below 50 nmol/L or 75 nmol/L or greater than 100 nmol/L in ITT analysis. Interestingly, though not statistically significant, more women in the 1000 IU/d group (n=7) had serum concentrations below 50 nmol/L than in the 400 IU/d group (n=4) or the 2000 IU/d group (n=2). This unexpected finding maybe likely due to chance, given the small sample size. An observational study by Li et al. also conducted in Vancouver reported that 24% of pregnant women on a vitamin D supplement of 400 IU/d or greater had 25OHD concentrations below 50 nmol/L between 20-36 weeks [15]. In comparison, the present study
found 18% of participants had insufficient 25OHD concentrations at baseline, and these women were also taking a vitamin D supplement providing an average of 421 IU of vitamin D per day.

The results of this thesis research also shows that at baseline there was a statistically significant difference between groups in the number of women with insufficient 25OHD concentrations. Significantly fewer women in the 2000 IU/d group had insufficient vitamin D concentrations at baseline, only 2 women compared to 9 women in each of the 400 IU and 1000 IU groups, which is likely due to chance and small sample size. We also found a statistically significant difference between treatment groups in the number of women who had insufficient 25OHD concentrations at 36 weeks when looking at only those who consumed greater than 80% of their supplements in the previous two months. The statistically significant differences in compliance are likely due to the small sample size as the 1000 IU group had 4 women who were vitamin D deficient at baseline compared to the 400 IU group with 0 women and the 2000 IU group with 1 woman.

4.1.4 Effects of ethnicity and season on 25OHD concentrations

We found no interactions between season or ethnicity and treatment on serum 25OHD concentrations at 36 weeks gestation, suggesting that these were not determinants of response to vitamin D supplementation. Again, sample size may be an issue as numbers were small when stratified by season and ethnicity. It is well established that vitamin D concentrations vary by season, especially at higher latitudes, being higher in the summer months and lower in the winter months due to decreased sun exposure [31]. Our results, however, were consistent with the literature in that 25OHD concentrations were around 14 nmol/L higher (p<0.000) in the summer months than winter.
We also found that non-white ethnicity was associated with a 4.1 nmol/L increase in 25OHD status at 36 weeks but was not a significant predictor of 25OHD concentrations. Many studies have shown that ethnicity is indeed a predictor of vitamin D status; individuals with darker skin tones such as Hispanics, aboriginal populations and blacks have lower 25OHD concentrations [111-113]. Hollis et al found significant differences in 25OHD concentrations analyzed by race; black women lagged at all time points and doses in regards to serum 25OHD concentrations [98]. In our study, only 25 of 110 women were of non-Caucasian ethnicity, likely too small a sample size to show significant differences in 25OHD concentrations. Also, our study population contained no women of black ethnicity, Vancouver does not have a large population of blacks or Hispanics, rather a larger Asian population, particularly Chinese [114].

4.2 Dietary vitamin D intake

Our findings regarding dietary calcium and vitamin D intake are consistent with other Canadian literature from Calvo et al. and Vatanparast et al. [5, 81]. We found that at baseline mean intakes of vitamin D for all participants was 184.4 IU/d, and did not differ among treatment groups and at 36 weeks the mean dietary vitamin D intake at the same time was 201.9 IU/d. Vatanparast et al analyzed data from the 2004 Canadian Health Measures Survey and reported that mean vitamin D intakes for 19-50 year old Caucasian women was 208 IU/d and in non-Caucasians was 180 IU/d [81]. In the present study, dietary vitamin D intake increased significantly in all participants from baseline to 36 weeks (184.4 IU/d vs 202 IU/d, p=0.047) although levels were not significantly different across treatment groups. This is likely a result of the increased energy intake during late pregnancy due to increased caloric needs. Some of which may come from fortified dairy products as Health Canada
recommends adding an additional snack each day, specifically mentioning a piece of fruit and a slice of cheese or yogurt which contain vitamin D [115, 116].

Our data has also provided insight into whether or not Canadian women are meeting the current recommended dietary allowance for vitamin D. At baseline women were consuming a mean vitamin D intake of 421.3 IU/d from a prenatal supplement and 184.4 IU/d from their diets. This confirms that on average women are meeting the RDA of 600 IU/d of vitamin D throughout their pregnancies. The average 25OHD at baseline was > 50 nmol/L and 20 women (18.2%) were still classified as vitamin D insufficient (25OHD < 50 nmol/L), they were likely those either not taking a prenatal supplement at study entry, receive little UVB light exposure or not consuming sufficient dietary vitamin D.

4.3 Bone specific alkaline phosphatase

Bone specific alkaline phosphatase is an isoenzyme associated with osteoblast cells and thought to play a role in bone mineralization; it is used clinically as a bone formation biomarker. The normal reference range is 6.5–20.1 ug/L for adult females and this signals normal bone mineralization. At baseline, 12 women had BSAP concentrations < 6.5 ug/L, however by completion of the study all had increased their BSAP concentrations and all were within the normal range. At 36 weeks of gestation, 4 women had BSAP concentrations > 20.1 ug/L, however this is physiologically normal as the placenta produces alkaline phosphatase during pregnancy, increasing serum concentrations of BSAP. Indeed, in all participants we saw a significant increase in BSAP from baseline to 36 weeks [p<0.000 for both ITT and as-treated analyses] but no differences were seen between vitamin D dose allocations.
In the newborn, BSAP concentrations were 26.1 ± 10.23 ug/L for all cord blood samples and we found no differences in BSAP concentrations among groups, and therefore indicating no difference in bone formation between treatment groups.

4.4 Safety

Serum 25OHD, calcium, phosphate and creatinine concentrations as well as urine calcium to creatinine ratios were assessed as safety measures. There were no serum calcium concentrations that exceeded 2.67 mmol/L at 36 weeks gestation. Two women had calcium to creatinine ratios greater than 0.7 mg/dL (0.94 mg/dL and 0.87 mg/dL). However calcium to creatinine ratios less than 1.0 mg/dL during pregnancy can be physiologically normal due to increased glomerular filtration rate and not indicative of hypervitaminosis D or hypercalciuria [118]. Other biomarker concentrations were consistent with safety guidelines and physiologically normal cut-offs for pregnancy and there were no significant differences among treatment groups in any biochemical outcome measures.

We found no indication of hypervitaminosis D in our study, which is consistent with Health Canada’s UL of 4000 IU/d and the study by Hollis et al. whose highest vitamin D dosage was 4000 IU/d that was deemed to be safe during pregnancy and lactation.

4.5 Limitations

This research had several limitations. The sample size in this study was small resulting in the study not being adequately powered, as the research is still ongoing at the time of publication of this thesis. At the completion of the study the sample size will have sufficient power and more likely will find greater or significant treatment effects. We had a small number of participants of non-Caucasian ethnicity; therefore the study findings cannot be extrapolated to Canadian pregnant women of different ethnicities; although we could find no
evidence that dose response differed by ethnicity. Women were also all from Vancouver and surrounding areas in the lower mainland, therefore our study results cannot make conclusions for all Canadian women due to the varying geography and latitudes throughout Canada. Our study cohort was comprised of women with higher levels of income who were well-educated; these participants are usually more health conscious and likely to follow prenatal recommendations. Women with less education and lower income levels might be at greater risk of vitamin D deficiency and could, therefore, receive greater benefits from prenatal vitamin D supplementation. It is unknown whether or not women in our study may have had higher or lower baseline 25OHD concentrations than women of lower socioeconomic status in Vancouver.

Our participants were also recruited as a convenience sample; many likely participated in the study because they were already interested in vitamin D supplementation and prenatal nutrition, which could have biased our sample. Women were usually recruited at the time of their detailed ultrasound visit that occurs at 18-22 weeks of gestation. It would have been preferable to recruit them around 13-15 weeks or pre-pregnancy so they were on the vitamin D supplement for a longer period of time, perhaps enhancing any effects from treatment. Women self-reported their pre-pregnancy weight; in some cases they guessed their weight or may have used a more socially desirable number. This may have resulted in inaccuracies in self-reported data. Women also self-reported their food intake in the semi-quantitative food frequency questionnaire (FFQ). Self-reported dietary intakes can be inaccurate, however the FFQ was validated and offers the best estimate of dietary vitamin D intake without using more tedious measures such as 7-day food records.
Women were excluded from our study if they had any co-morbidities such as a previous history of preeclampsia, gestational diabetes, renal or cardiac disease. These conditions were self-reported, however a woman could have developed gestational diabetes during the course of the study and not reported her health status to researchers. Women with histories of preeclampsia and diabetes may be at greater risk for vitamin D deficiency and thus could benefit from supplementation.

4.6 Directions for future research

Upon completion of this study, infant birth, weight and head circumference will be collected from the BC Perinatal Registry Database and the relationship between infant birth weight and vitamin D supplement dose will be investigated. We will also determine infant responses to maternal vitamin D supplementation at 8 weeks postpartum and examine the 25OHD concentrations in breastmilk across treatments. When the study is completed, we will have a comprehensive set of data to determine the dose-response relationship for pregnant and lactating women. We will also examine infant dose-response data to maternal supplementation throughout pregnancy. This data set could also be analyzed further by looking at other outcome variables such as maternal and infant PTH concentrations and other bone biomarker concentrations such as osteocalcin, calcitonin and n-telopeptide to get a better picture of bone homeostasis and the resorption and formation occurring during pregnancy and infancy.

Once the dose-response relationship is known studies on functional indices are needed to determine what cut-offs should be used to establish vitamin D requirements. Large randomized controlled trials will be needed to determine if vitamin D supplementation
reduces preeclampsia, gestational diabetes and other adverse pregnancy outcomes that vitamin D deficiency or inadequacy are currently associated with.
Chapter 5: Conclusion

To my knowledge this is the first study looking at maternal responses to three dosages (400, 1000 and 2000 IU) of supplemental vitamin D throughout pregnancy in Canada. At 13-22 weeks of gestation 18.2% \( (n=20) \) of women had insufficient serum 25OHD concentrations \( (25OHD < 50 \text{ nmol/L}) \). At 36 weeks of gestation 10.9% \( (n=12) \) of women had insufficient 25OHD concentrations, which were not significantly different between vitamin D treatment groups. At 36 weeks of gestation there were no significant differences between serum 25OHD concentrations between treatment groups. There was a trend that increased supplemental vitamin D dosage resulted in increased serum 25OHD concentrations. There was a significant change from baseline to 36 weeks gestation in serum 25OHD \( (\text{nmol/L}) \) in the 1000 and 2000 IU groups compared to the 400 IU group, who experienced no change. Newborn 25OHD concentrations did not differ significantly across treatment groups. Season was a significant predictor of 25OHD concentrations at 36 weeks gestation; 25OHD concentrations were 14 nmol/L greater in the summer compared to the winter.

We reported no differences in serum BSAP concentrations between treatment allocations at baseline or 36 weeks gestation, though BSAP concentrations \( (\text{ug/L}) \) increased significantly from baseline to 36 weeks gestation in all participants. There were no differences between treatments in newborn BSAP concentrations. Mean dietary vitamin D intake was 184 IU/d at baseline and 202 IU/d at 36 weeks.

Our data confirm that on average Canadian pregnant women are consuming the RDA for vitamin D of 600 IU/d from diet and prenatal vitamin D supplements combined. Only 18.2% of women were insufficient at baseline, it could be that these women were those not taking a supplement, received little UVB light or not obtaining vitamin D through their diet.
It appears greater increases in supplemental vitamin D are not necessary when using the cut-off for vitamin D sufficiency of 50 nmol/L. With the caveat that our sample was not adequately powered, we fail to reject the null hypothesis that the current RDA of 600 IU/d will be insufficient for pregnant women to achieve circulating 25OHD concentrations > 50 nmol/L as recommended by the IOM. This research has added to the current literature on the safety and efficacy of vitamin D supplementation throughout pregnancy. Current dietary patterns and supplement usage appear sufficient to allow most women to achieve vitamin D sufficiency (25OHD > 50 nmol/L). We found no evidence of hypercalcemia or hypervitaminosis D at any supplement dosage; women could consume higher supplemental dosages of 1000 or 2000 IU/d of vitamin D with no ill effect. Those who are vitamin D deficient might need to consume greater dosages of supplemental vitamin D to attain sufficiency.

Pregnant women or those planning on becoming pregnant in Vancouver should take a prenatal supplement containing at least 400 IU/d of vitamin D and focus on increasing consumption of milk products, eggs or fatty fish in their diet to ensure they are receiving dietary vitamin D. It is difficult to draw firm conclusions regarding the adequacy of the current RDA for achieving sufficient 25OHD concentrations in pregnancy; final conclusions upon completion of the study should provide greater insight.
References


104. BC Women and Children's Hospital RB: *CW Reference Ranges*. (Hospital BWC) Department of Pathology and Laboratory Medicine; 2010.


Appendices

Appendix A

Vitamin D in Pregnancy and Lactation

Informed Consent Form

Principal Investigator
Dr. Tim Green, PhD
Food, Nutrition, and Health
University of British Columbia
**Telephone:** 604 802 8438

Co-investigators
Dr. Sheila Innis, PhD
Paediatrics
University of British Columbia
Dr. Antonia Shand, MD
Obstetrics and Gynaecology
BC Women’s Hospital

Dr. Peter von Dadelszen, PhD, MD
Associate Professor of Obstetrics and Gynaecology
University of British Columbia
Dr. Michael Lyon, MD
Adjunct Professor
Food, Nutrition, and Health
University of British Columbia

Emergency Phone Number
(604) 875-XXXX;
*available 24 hours per day and seven days per week*

Sponsor
Canadian Institutes of Health Research

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.

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 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 mothers 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 at 604-875-2345 (Ext 7156).

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 at 604-822-8598.

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 at 604-875-XXXX (Ext XXXX).
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 in the University of British Columbia Office of Research Services at 604-822-8598.

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

___________________________________________                ___________________,
Signed          Date

_______________________             Tim J Green
Investigator Signature   Printed Name                   Date

Appendix B
Appendix

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
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,
Kaitlin 778-989-XXXX or
Andrea 604-928-XXXX

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 prenatal 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

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 breastfeeding.

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

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

☐ High School  ☐ Completed
☐ College  ☐ Completed
☐ Undergraduate  ☐ Completed
☐ Graduate  ☐ 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 F

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?

___________________________________________________________________________
Appendix G

FOOD FREQUENCY QUESTIONNAIRE

Please use HB pencil making sure response bubble is filled in completely.
Please list nutritional supplements used in the past month, using as much detail as you can remember.

<table>
<thead>
<tr>
<th>BRAND NAME OF SUPPLEMENT OR TYPE</th>
<th>AMOUNT TAKEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>(e.g., Exact calcium 500mg &amp; vitamin D)</td>
<td>(e.g., 1 tablet every other day)</td>
</tr>
</tbody>
</table>

1. We want to know how often you eat or drink certain foods each month.
2. Think about a typical month, not just what you ate this week which might be different.
3. Medium portion sizes are given to help you determine the usual size of the food or drink, and to compare to small and large.
4. If you drink or eat much less (approximately half) than the medium portion size described, then check small. If you drink a large glass of milk every day (approximately 1.5 times the size of medium), then check large.
5. Fill out the form similar to this example:
   - If you drink a carton of chocolate milk (500ml) Monday through Friday, then choose M (medium) and show it as 5 - 6 times per week.

<table>
<thead>
<tr>
<th>TYPE of FOOD or DRINK</th>
<th>Never or less than 1 per month</th>
<th>1 per month</th>
<th>2-3 per month</th>
<th>1 per week</th>
<th>2 per week</th>
<th>3-4 per week</th>
<th>5-6 per week</th>
<th>1 per day</th>
<th>2+ per day</th>
<th>Medium serving</th>
<th>Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td>X</td>
</tr>
<tr>
<td>Type of Food or Drink</td>
<td>Never or less than 1 per month</td>
<td>1 per month</td>
<td>2 - 3 per month</td>
<td>1 per week</td>
<td>2 per week</td>
<td>3-4 per week</td>
<td>5-6 per week</td>
<td>1 per day</td>
<td>2+ per day</td>
<td>Medium Serving Size</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------</td>
<td>-----------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Milk: whole, 2%, 1% or skim</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td></td>
</tr>
<tr>
<td>Chocolate Milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td></td>
</tr>
<tr>
<td>Soy Milk Beverage: Fortified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td></td>
</tr>
<tr>
<td>Soy Drink: Plain (not fortified)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td></td>
</tr>
<tr>
<td>Other plant milks (rice, potato, etc)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td></td>
</tr>
<tr>
<td>Milk or coffee or tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 tablespoon</td>
<td></td>
</tr>
<tr>
<td>Milk on cereal (if not included above)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 cup</td>
<td></td>
</tr>
<tr>
<td>Milk shake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (8 oz or 250 ml)</td>
<td></td>
</tr>
<tr>
<td>Milk dessert (ice cream, pudding)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 cup (one scoop, 1 container)</td>
<td></td>
</tr>
<tr>
<td>Yogurt (milk or soy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 cup (125 g, 1 container)</td>
<td></td>
</tr>
<tr>
<td>Soft Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 tablespoon</td>
<td></td>
</tr>
<tr>
<td>Hard Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cube 2&quot; (2 slices)</td>
<td></td>
</tr>
<tr>
<td>White bread, roll, bun, biscuit, bagel, nan, tortilla</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 slice, 1 small roll, 1/2 bagel</td>
<td></td>
</tr>
<tr>
<td>Dark bread, roll, bagel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 slice, 1 small roll, 1/2 bagel</td>
<td></td>
</tr>
<tr>
<td>Taco chips, nacho chips</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup (28g)</td>
<td></td>
</tr>
<tr>
<td>Waffle, pancake, French toast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 piece (4&quot; round)</td>
<td></td>
</tr>
<tr>
<td>Butter (in any foods eaten)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pat (1 teaspoon)</td>
<td></td>
</tr>
<tr>
<td>Margarine (in any foods eaten)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 pat (1 teaspoon)</td>
<td></td>
</tr>
<tr>
<td>TYPE of Food or Drink</td>
<td>Never or less than 1 per month</td>
<td>1 per week</td>
<td>2-3 per month</td>
<td>1 per month</td>
<td>1 per day</td>
<td>Serving Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------</td>
<td>------------</td>
<td>---------------</td>
<td>------------</td>
<td>----------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tofu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cube (2&quot;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaroni with cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned Salmon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 tablespoons or 1 cup of casserole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 tablespoons or 1 cup of casserole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned Sardines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmon Steak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1/2 can)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other fish: white</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90 g (3 oz)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other fish: oily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90 g (3 oz)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream soups made with milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taco or burrito made with cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(8 oz or 250 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pizza made with cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 regular taco: 1/2 burrito</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentils, beans, peas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 cup cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs: eaten alone or in other foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 large egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes: mashed with milk &amp; margarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 cup (100g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange Juice: not fortified with calcium, vitamin D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange Juice: with calcium, vitamin D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(8 oz or 250 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli, kale, greens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup raw or 1/2 cup cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seafood: e.g. shrimp, crab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 cup meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shellfish: e.g. Mussells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>