ASSOCIATION OF NEWBORN VITAMIN D STATUS WITH PREGNANCY OUTCOME AND INFANT HEALTH

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ABSTRACT

There is little information available about the relationship of newborn vitamin D status with pregnancy outcome and infant health. The purpose of this cross-sectional study was to estimate the prevalence of vitamin D deficiency and insufficiency in newborns in the Saskatoon Health Region, identify risk factors for low neonatal levels of vitamin D, and determine whether any association exists between low levels of vitamin D and adverse pregnancy and neonatal outcomes.

The Newborn Vitamin D Study was conducted between December, 2011 and February, 2012. Sixty-five maternal-fetal dyads delivering in the Saskatoon Health Region were included in the study. Mean cord blood vitamin D level was 64.1 nmol/L (standard deviation = 19.8 nmol/L), which is in the insufficient range. Cord blood vitamin D level was deficient (<50 nmol/L) in 22% and insufficient (50-75 nmol/L) in 48% of the 65 newborns studied. Simple linear regression indicated that low weight gain during pregnancy is significantly associated with low vitamin D levels ($p = 0.04$). However, younger maternal age ($p < 0.01$) and urban area of residence ($p = 0.09$) were the strongest predictors of low cord blood vitamin D levels in a multiple linear regression model (R^2 of 0.519, $p = 0.003$). Cord blood vitamin D levels were not significantly associated with any pregnancy or neonatal outcomes.

Despite 85% of mothers reporting having taken a daily prenatal supplement, 70% of newborns in our study population had either an insufficient or deficient cord blood vitamin D status. This suggests that prenatal supplements, which typically contain 400 IU of vitamin D, contain an inadequate dose of vitamin D to produce sufficient cord blood vitamin D levels in most newborns. Further research is necessary to inform maternal vitamin D supplementation guidelines and to investigate the role of vitamin D in pregnancy outcomes and infant health.

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LIST OF ABBREVIATIONS

ALRI: acute lower respiratory infection

BMI: body mass index

COPD: chronic obstructive pulmonary disease

GDM: gestational diabetes mellitus

HIV: human immunodeficiency virus

IU: international units

LGA: large for gestational age

PTH: parathyroid hormone

RCT: randomized controlled trial

RSV: respiratory syncytial virus

RXR: retinoid X receptor

SGA: small for gestational age

SNP: single nucleotide polymorphism

VDBP: vitamin D binding protein

VDR: vitamin D receptor

$1,25(OH)_2D$: calcitriol, the active form of vitamin D

25(OH)D: calcidiol, the inactive form of vitamin D

CHAPTER 1: INTRODUCTION

1.1 Introduction

In addition to its well-established role in bone health and calcium homeostasis, vitamin D is also a regulator of immune function and is implicated in predisposing to infection, autoimmune and cardiovascular diseases, mental illness, and cancer (McGrath, Saari et al. 2004; Nagpal, Na et al. 2005; Wang, Pencina et al. 2008). In fact, it has been estimated that by optimizing vitamin D status in Canadians mortality could be reduced by 37,000 annually (a 16.1% reduction) and reduce the annual economic burden by $14.4 billion (6.9%) (Grant, Schwalfenberg et al. 2010). Vitamin D's role in preventing adverse health outcomes may begin as early as the first trimester of intra-uterine development; low maternal vitamin D intake during pregnancy has been linked with increased risk of asthma and diabetes later in the offspring's life (Camargo, Rifas-Shiman et al. 2007; Devereux, Litonjua et al. 2007; Erkkola, Kaila et al. 2009; Miyake, Sasaki et al. 2010; Krishnaveni, Veena et al. 2011). However, few studies have assessed the relationship of newborn vitamin D levels with pregnancy outcome and neonatal health.

The prevalence of maternal vitamin D insufficiency and deficiency in Canada is between 46-80% (Weiler, Fitzpatrick-Wong et al. 2005; Newhook, Sloka et al. 2009). In Saskatchewan, the prevalence and health and economic burdens of maternal vitamin D deficiency are likely to be particularly high due to the population's risk factors for low vitamin D status, including northern latitude (49 - 60°N), lack of sun exposure during the winter months, high prevalence of obesity, and high proportion of Aboriginal ethnicity (Camargo, Ingham et al. 2010; Dror, King et al. 2011). However, no studies have investigated the vitamin D status of Saskatchewan mothers, and few studies anywhere have investigated maternal risk factors for having newborns with low

levels of vitamin D.

The lack of consensus on guidelines for vitamin D supplementation during pregnancy may be contributing to the lack of adequate maternal vitamin D supplementation in Canada and abroad (Hypponen and Boucher 2010). Different recommendations by the Canadian Pediatric Society, the Endocrine Society and Health Canada may cause confusion among mothers and health care practitioners alike (Canadian Pediatric Society 2007; Health Canada 2010; Holick, Binkley et al. 2011). Research that examines the pregnancy and neonatal outcomes associated with newborn vitamin D status may aid in determining how much supplementation is necessary for healthy maternal and fetal development and thus help guide development of consensus guidelines for maternal vitamin D supplementation.

The prevalence of newborn vitamin D deficiency ranges from 11 - 93%, depending on the definition of deficiency used and the population studied (Maghbooli 2007; Bowyer, Catling-Paull et al. 2009). Because vitamin D crosses the placenta, the vitamin D level of the newborn is entirely dependent on the maternal vitamin D level (Hillman and Haddad 1974). Therefore, a high prevalence of vitamin D deficiency or insufficiency in pregnant women correlates with a correspondingly high prevalence of vitamin D deficiency or insufficiency in newborns. However, the clinical significance of low newborn vitamin D levels has not been established. Although some studies indicate low neonatal levels of vitamin D may be associated with adverse neonatal outcomes (Belderbos 2011; Camargo, Ingham et al. 2011), it is unknown if low levels of vitamin D may harm infants or whether they are simply reflective of a lower vitamin D requirement.

This research aimed to estimate the prevalence of low neonatal levels of vitamin D in the Saskatoon Health Region, identify maternal risk factors for low neonatal vitamin D levels, and

examine the relationship between low neonatal levels of vitamin D and adverse pregnancy and neonatal outcomes. Information generated by this research will help identify socio-demographic, obstetric and personal health factors that combine to influence vitamin D levels in the newborn, and will help empower pregnant women to optimize their vitamin D status and thereby contribute to improving the long term health of their offspring. These findings will help to inform future studies in order to prevent adverse effects of low levels of vitamin D *in utero* and in childhood.

1.2 Objectives

1) To estimate the prevalence of vitamin D sufficiency, insufficiency and deficiency in newborns delivered in the Saskatoon Health Region.

2) To identify associations between neonatal levels of vitamin D based on cord blood samples and socio-demographic, obstetric, and personal health factors in this population.

3) To identify associations between neonatal levels of vitamin D based on cord blood samples and obstetric and neonatal outcomes such as method of delivery, delivery complications, infant weight, infant length, infant head circumference, Apgar scores, and illness at birth.

1.3 Hypotheses

Hypothesis 1: The majority of newborns in the Saskatoon Health Region will have deficient or insufficient cord blood levels of vitamin D.

Hypothesis 2: Risk factors for low levels of D in newborns will include dark skin pigmentation, high pre-pregnancy BMI, and a lack of vitamin D supplementation.

Hypothesis 3: Low levels of cord blood vitamin D will be associated with Cesarean delivery,

increased risk of delivery complications, low birth weight, low birth length, smaller head circumference, low Apgar scores, and increased risk of illness at birth.

2. LITERATURE REVIEW

2.1 Vitamin D Physiology

2.1.1 Biochemistry of Vitamin D

The term "vitamin D" is in fact a misnomer. Whereas true vitamins are ingested only through exogenous supplementation, vitamin D may be produced endogenously and moves to other areas in the body to exert its action. Vitamin D is therefore a hormone, not a vitamin, but it is referred to as a vitamin due to current convention.

There are two forms of vitamin D. Vitamin D_2 (ergocalciferol) is derived from the ultraviolet irradiation of plant ergosterol, and vitamin D_3 (cholecalciferol) is found in fish oils and is made in the skin. Vitamin D_3 is produced from 7-dehydrocholesterol in skin exposed to ultraviolet B (UVB) radiation (Holick 2006), and both vitamins D_2 and D_3 may be ingested through the diet or through supplementation (Holick 2007). In this thesis, vitamin D will refer to both vitamin D_2 and D_3.

Vitamin D is hydroxylated in the liver and becomes 25(OH)D, or calcidiol, the primary circulating form of vitamin D. 25(OH)D may be converted to $1,25(OH)_2D$ (calcitriol), the active form of vitamin D, by 1-alpha hydroxylase (CYP27B1) in the kidneys and other organs. The production of $1,25(OH)_2D$ in the kidney is regulated by plasma parathyroid hormone levels as well as serum calcium and phosphorus levels (Holick 2007). $1,25(OH)_2D$ is broken down into its inactive metabolite by 24-hydroxylase (CYP24). Negative feedback aids in regulating $1,25(OH)_2D$ levels, as $1,25(OH)_2D$ inhibits renal 1-alpha hydroxylase and stimulates 24-hydroxylase, and this maintains circulating levels within a limited range (Aranow 2011). As well, excess vitamin D in the skin is broken down by sunlight, inhibiting the production of excessive vitamin D after prolonged sun exposure.

More than 80% of the vitamin D requirement is derived from cutaneous synthesis (Holick 2007). Vitamin D can also be ingested through supplementation or through vitamin D-rich foods, including oily fish such as salmon, mackerel and sardines, some fish oils such as cod liver oil, egg yolk, and vitamin D-enriched foods (milk, infant formula, cereal, orange juice, yogurt and margarine) (Holick 2006). However, dietary intake alone, including fortified foods, is unlikely to provide the daily recommended quantity of vitamin D to prevent insufficiency. Supplements are usually required to reach the minimum recommended daily intake of vitamin D, especially during the winter months (Holick 2006).

2.1.2 Vitamin D in Bone Health

2.1.2.1 Normal Vitamin D Physiology

Calcitriol increases levels of ionized calcium in blood by binding to the vitamin D receptor (VDR). In the intestine, the calcitriol-VDR complex acts as a transcription factor for the expression of transport proteins involved in calcium absorption (Bouillon 2003), and an increase in intestinal absorption of calcium results. In the kidney, calcitriol increases renal tubular reabsorption of calcium, and in bone, calcitriol indirectly stimulates osteoclast action to increase calcium release into blood. Calcitriol also acts directly on the parathyroid gland to decrease parathyroid hormone (PTH) production and indirectly decreases PTH by increasing serum calcium concentration (Holick 2006).

Vitamin D is primarily responsible for regulating absorption of calcium and phosphorus from the intestine. Low levels of vitamin D result in only 10% to 15% of dietary calcium and a low level of dietary phosphorus being absorbed. However, when vitamin D levels are sufficient, 30% to 40% of dietary calcium and a higher level of phosphorus are absorbed (Heaney, Dowell

et al. 2003; Holick 2007). Low levels of vitamin D result in insufficient calcium absorption to maintain calcium homeostasis, and low serum calcium stimulates PTH release. PTH increases serum calcium by acting on bone to increase calcium release, acting on the kidney to increase renal tubular calcium reabsorption, and increasing formation of calcitriol (Holick 2006).

2.1.2.2 Effects of Low Vitamin D on Bone Health

Chronic, severe vitamin D deficiency results in impaired calcium absorption. Low calcium stimulates the parathyroid gland resulting in secondary hyperparathyroidism, which allows the body to avoid hypocalcemia by mobilizing bone stores of calcium. In infants and adolescents, these bone stores of calcium occasionally cannot be released quickly enough to meet the demand during periods of rapid growth, and this may result in hypocalcemic seizures or tetany (Narchi, El Jamil et al. 2001; Ladhani, Srinivasan et al. 2004). In school-aged children, chronic mobilization of calcium from bone is usually sufficient to maintain normal serum calcium, but demineralization and subsequent deformity of the bone can result in rickets (Ladhani, Srinivasan et al. 2004). Bone breakdown is also the reason that low vitamin D in pregnancy and in childhood may impair the attainment of peak bone mass in children (Cooper, Javaid et al. 2005). The same process in adults may result in bone pain, proximal muscle weakness, osteomalacia, osteoporosis, and increased risk of fractures (Exton-Smith, Hodkinson et al. 1966; Passeri, Pini et al. 2003).

2.1.3 Nonskeletal Roles of Vitamin D

The presence of the vitamin D receptor in a wide variety of nonskeletal organs and tissues suggests that vitamin D likely plays a role in a many physiological processes. A role for

vitamin D has been implicated in cardiovascular health (Wang, Pencina et al. 2008), autoimmune disease (Munger, Zhang et al. 2004), cancer (Chang, Smedby et al. 2005), neurological development (Eyles, Burne et al. 2011) and immune function (Aranow 2011). As well, vitamin D levels have been associated with preeclampsia (Halhali, Tovar et al. 2000), dermatologic diseases (Kragballe, Barnes et al. 1998), obesity (Gilbert-Diamond 2010), and mental health, specifically schizophrenia (McGrath, Saari et al. 2004) and seasonal affective disorder (Gloth, Alam et al. 1999). Vitamin D receptor ligands have been implicated in; "inflammation (rheumatoid arthritis, psoriatic arthritis), dermatological indications (psoriasis, actinic keratosis, seborrheic dermatitis, photoaging), osteoporosis (postmenopausal and steroid-induced osteoporosis), cancers (prostate, colon, breast, myelodysplasia, leukemia, head and neck squamous cell carcinoma, and basal cell carcinoma), secondary hyperparathyroidism, and autoimmune diseases (systemic lupus erythematosus, type I diabetes, multiple sclerosis, and organ transplantation)." (Nagpal, Na et al. 2005)

The mechanisms by which vitamin D exerts its nonskeletal effects are manifold. Calcitriol directly or indirectly controls more than 200 genes. It is involved in the regulation of cellular differentiation, apoptosis, proliferation and angiogenesis (Nagpal, Na et al. 2005). It increases myocardial contractility (Zittermann 2006), inhibits renin synthesis (Li 2003) and is involved in insulin production (Chiu, Chu et al. 2004). It also acts as an immunomodulator (Penna, Roncari et al. 2005), as it dampens systemic inflammatory responses through functional vitamin D receptors present on all major immune cells. In short, researchers are only beginning to elucidate the role of vitamin D in physiology, and additional research is necessary before the importance of vitamin D supplementation is fully understood.

2.1.4 The Vitamin D Receptor

The vitamin D receptor (VDR) is a steroid hormone receptor that binds calcitriol and acts as a transcription factor during gene expression (Holick 2006). After the VDR is bound to calcitriol, the complex dimerizes with the retinoid X receptor (RXR). The calcitriol-VDR-RXR heterodimer translocates into the nucleus and binds to vitamin D responsive elements in the promoter regions of vitamin D responsive genes, inducing their expression (Aranow 2011).

The VDR is expressed in many organs including those not typically involved in calcium homeostasis or bone metabolism, such as the placenta, brain, gonads, heart, lung, prostate, skin, breast, pancreas, small intestine, colon, immune system and vascular wall (Holick 2007). The placenta, brain, lymph nodes and skin also express alpha-1 hydroxylase, and they are therefore capable of producing calcitriol as an autocrine hormone (Hewison 2000; Eyles, Smith et al. 2005). The presence of the VDR in this wide variety of tissue types suggests that vitamin D's role in physiology is more multifaceted than previously recognized. Further research is necessary to confirm the nature of vitamin D's interaction with these tissue types.

Mutations in the VDR have been associated with many clinical outcomes. VDR genetic mutations can confer susceptibility to bone demineralization, particularly in the context of other risk factors such as age, suboptimal calcium intake, and physical inactivity (Viitanen AM 1996; Gong, Stern et al. 1999; Ralston 2003). Genetic mutations in the VDR have also been associated with increased risk of developing cancers (Slattery, Neuhausen et al. 2004; Uitterlinden, Fang et al. 2004; John, Schwartz et al. 2005) and type 1 diabetes mellitus (McDermott, Ramachandran et al. 1997; Slattery, Neuhausen et al. 2004). Vitamin D and its receptor may both play a role in disease prevention and the maintenance of skeletal and extraskeletal health.

2.1.5 Vitamin D Genetics

Mutations in the proteins involved in vitamin D absorption, metabolism, and excretion may affect serum vitamin D levels and therefore susceptibility to vitamin D-related disease outcomes. The best-studied mutations effecting serum 25(OH)D levels are single nucleotide polymorphisms in the vitamin D receptor (VDR), the group-specific component of the vitamin D binding protein (VDBP), and the cytochrome P450s involved in vitamin D metabolism. The single nucleotide polymorphisms (SNPs) in the VDBP most strongly associated with serum vitamin D levels include rs4588 and rs7041 (McGrath, Saha et al. 2010). The former SNP is associated with increased 25(OH)D levels while the latter is associated with decreased vitamin D levels. The SNP rs2228570 (formerly rs10735810) in the VDR is associated with increased vitamin D concentrations. While the SNP rs10877012 in CYP27B1 (also known as 1-alpha hydroxylase) was found to be significantly associated with vitamin D levels in two studies, the direction of the association differed between the studies (McGrath, Saha et al. 2010).

The mechanism of action for SNP influence on vitamin D levels is poorly understood (McGrath, Saha et al. 2010). However, it is possible that individuals with different vitamin D-related genotypes require varying levels of vitamin D to optimize their vitamin D status. It is also likely that genotype plays a role in susceptibility to vitamin D-related disease outcomes. More studies are required to investigate the relationship between SNPs in the VDR and VDBP, vitamin D levels, and disease pathogenesis.

Evidence that SNPs in vitamin D-related proteins are related to clinical outcomes is mounting. SNPs in the VDR have been linked with multiple primary melanoma (Mandelcorn-Monson, Marrett et al. 2011), prostate cancer (John, Schwartz et al. 2005), colorectal cancer (Slattery, Neuhausen et al. 2004), Alzheimer's disease (Lehmann, Refsum et al. 2011), type 1

diabetes mellitus (McDermott, Ramachandran et al. 1997; Slattery, Neuhausen et al. 2004), multiple sclerosis (Agliardi 2011), and RSV infection in children (Kresfelder, Janssen et al. 2011). VDR genotype has been associated with decreased bone mineral density and increased fracture risk in several studies (Viitanen AM 1996; Feskanich, Hunter et al. 1998; Gong, Stern et al. 1999; Ralston 2003), and maternal VDR genotype has been associated with increased risk of infants being born small for gestational age (Bodnar, Catov et al. 2010). SNPs in the VDBP have been associated with breast cancer (Anderson, Cotterchio et al. 2011), inflammatory bowel disease (Eloranta, Wenger et al. 2011), asthma (Li, Jiang et al. 2011) and COPD (Wood, Bassford et al. 2011). A SNP in CYP2R1 (also called 25-hydroxylase) was associated with increased risk of asthma (Pillai, Iqbal et al. 2011). The existing body of evidence therefore favours the role of vitamin D in the pathophysiology of many diseases. However, further studies must be conducted to clarify the role of genotype in disease susceptibility.

2.1.6 Definition of Vitamin D Deficiency

Although $1,25(OH)_2D$ is the active form of vitamin D, due to a tight regulation of its production as well as a relatively short half-life (4–6 hours), it is not a good indicator of vitamin D status (Papandreou, Malindretos et al. 2010). As well, $1,25(OH)_2D$ may be normal or even elevated in those with vitamin D deficiency due to secondary hyperparathyroidism and does not accurately reflect vitamin D stores (Holick, Binkley et al. 2011). Serum or plasma 25(OH)D has a half-life of two to three weeks and is the major circulating form of vitamin D (Holick, Binkley et al. 2011). 25(OH)D is therefore the most appropriate biochemical marker of vitamin D status (Papandreou, Malindretos et al. 2010).

The optimal level of vitamin D for bone health was determined to be the one at which a

minimal level of PTH was released and a maximal level of calcium was absorbed. PTH levels plateau at their minimum level at a 25(OH)D concentration of 75 nmol/L (Chapuy, Preziosi et al. 1997; McKenna and Freaney 1998; Heaney 2004; Holick, Siris et al. 2005) and serum 25(OH)D levels greater than 75 nmol/L may be required to maximize intestinal calcium absorption (Heaney, Dowell et al. 2003). A 25(OH)D level greater than 75 nmol/L may also be necessary to prevent secondary hyperparathyroidism-induced skeletal conditions (van der Wielen, Lowik et al. 1995; Feskanich, Willett et al. 2003). Based on this and similar evidence, vitamin D sufficiency, insufficiency and deficiency have been defined by the Endocrine Society as 25(OH)D levels greater than 75 nmol/L, 50-75 nmol/L, and less than 50 nmol/L, respectively (Holick, Binkley et al. 2011). Vitamin D intoxication occurs when 25(OH)D levels exceed 374 nmol/L (Holick 2007) (Table 1). Despite these proposed recommendations for categorizing vitamin D status, there is still debate about the correct definition of vitamin D deficiency in both adults and children.

There are no age-specific reference ranges for serum vitamin D status. The Endocrine Society definitions of vitamin D sufficiency, insufficiency and deficiency are the same for children as they are for adults (Holick, Binkley et al. 2011). Some research supports these definitions, as an inverse relationship has been demonstrated between 25(OH)D and PTH levels in children and adolescents (Cheng, Tylavsky et al. 2003; Abrams, Griffin et al. 2005). Furthermore, PTH levels plateau at 25(OH)D levels >80 nmol/L in adolescents (Guillemant, Taupin et al. 1999). However, little is known about the normal 25(OH)D range in neonates, infants, and children. In his review article on the role of vitamin D in child and adolescent health, Daniel Roth argues that because umbilical cord 25(OH)D samples usually contain 50-60% of the maternal 25(OH)D concentration (Waiters, Godel et al. 1999), “it might be inferred

that normal neonatal concentration is >40 nmol/L"(Roth 2007). He notes that additional research on the clinical outcomes of vitamin D deficiency in neonates is necessary to establish age-specific reference ranges (Roth 2007). Until new information is available, the acceptable vitamin D levels for children will remain the same as they are for adults.

Table 1. Vitamin D Status Based on Serum 25(OH)D Level *

Vitamin D Status	Serum 25(OH)D
Deficient	<50 nmol/L (<20 ng/mL)
Insufficient	50-75 nmol/L (20-30 ng/mL)
Sufficient	>75 nmol/L (>30 ng/mL)
Intoxication	>374 nmol/L (150 ng/mL)

*25(OH)D refers to calcidiol. Vitamin D status according to the Endocrine Society (Holick, Binkley et al. 2011)

2.1.7 Risk Factors for Low Levels of Vitamin D

Any factor that limits the cutaneous synthesis of vitamin D is a risk factor for having insufficient or deficient serum levels of vitamin D. Increased clothing coverage, regular sunscreen use, and limited time outdoors are therefore risk factors for vitamin D insufficiency or deficiency (Dror, King et al. 2011). During the winter months, there is insufficient UVB irradiation from sunlight to stimulate cutaneous synthesis of vitamin D, particularly in people living above 42 degrees latitude (Webb 1988). As well, melanin acts as a natural sunscreen in those with darker skin tones, decreasing the cutaneous production of vitamin D by as much as 99%, similar to wearing sunscreen with a sun protection factor of 15 (Clemens 1982 ; Matsuoka 1987; Holick 2006). Consequently, northern latitude, winter season, and darker skin tone are also risk factors for vitamin D insufficiency and deficiency.

Other risk factors for vitamin D deficiency and insufficiency include malabsorptive syndromes (Lo, Paris et al. 1985; Koutkia, Lu et al. 2001), lack of supplementation (Delvin,

Salle et al. 1986), high body mass index (BMI) (Bell, Epstein et al. 1985; Wortsman, Matsuoka et al. 2000; Arunabh, Pollack et al. 2003; Snijder, van Dam et al. 2005), certain medications (Pascussi, Robert et al. 2005), age (MacLaughlin and Holick 1985; Holick, Matsuoka et al. 1989), socioeconomic status (Laitinen, Rasanen et al. 1995; Pehlivan, Hatun et al. 2003; Rasanen, Kronberg-Kippila et al. 2006), and genetic mutations in the vitamin D receptor or the vitamin D binding protein (McGrath, Saha et al. 2010).

When cutaneous synthesis does not produce adequate levels of vitamin D, the body relies on absorption of vitamin D from the intestine. Malabsorptive syndromes, such as inflammatory bowel disease, celiac disease or cystic fibrosis, decrease the ability of the intestine to absorb any ingested vitamin D (Lo, Paris et al. 1985; Koutkia, Lu et al. 2001). A lack of dietary vitamin D makes less vitamin D available for absorption by the intestine, and medication use, particularly anticonvulsants, corticosteroids and rifampin, can interfere with its subsequent metabolism (Bell, Epstein et al. 1985; Pascussi, Robert et al. 2005). Metabolized vitamin D is less physiologically available to those with a high BMI, as the fat-soluble vitamin becomes sequestered in the fat and results in lower serum vitamin D levels (Bell, Epstein et al. 1985; Wortsman, Matsuoka et al. 2000; Arunabh, Pollack et al. 2003; Snijder, van Dam et al. 2005). Increased age results in lower vitamin D levels in both children (Roth, Martz et al. 2005) and the elderly (MacLaughlin and Holick 1985; Holick, Matsuoka et al. 1989), and socioeconomic status, reflected by education level, occupation and household income, is a risk factor for low levels of vitamin D because low socioeconomic status families are less likely to consume sufficient vitamin D in their diets (Laitinen, Rasanen et al. 1995; Rasanen, Kronberg-Kippila et al. 2006). Finally, genetic mutations in the vitamin D receptor and vitamin D binding protein play a role in modulating vitamin D levels (McNally, Leis et al. 2009), though the mechanism of this is poorly understood

(McGrath, Saha et al. 2010).

Ethnicity is also a risk factor for low serum levels of vitamin D (Camargo, Ingham et al. 2010). Although skin pigmentation likely accounts for much of the observed ethnic differences, genetic and environmental differences among ethnic groups may also contribute to lower serum 25(OH)D levels and these warrant further study.

2.1.7.1 Risk Factors for Low Levels of Vitamin D in Newborns

Because vitamin D is transported across the placenta, newborns are entirely dependent on their mothers for vitamin D. Therefore, through contribution to low maternal vitamin D status, the risk factors listed above are also risk factors for low vitamin D status in the newborn. Other established risk factors for vitamin D deficiency in infancy include maternal vitamin D deficiency and breastfeeding without supplementation, as human milk contains only 20 IU/L of vitamin D (Canadian Pediatric Society 1988). Longer gestational age and younger maternal age were associated with low cord blood vitamin D levels in a New Zealand study involving 929 newborns (Camargo, Ingham et al. 2010), and an Australian study involving 901 newborns also found an association between low newborn vitamin D and younger maternal age (Bowyer, Catling-Paull et al. 2009). Longer gestational age provides a longer period for a fetus to draw upon its mother's declining vitamin D levels, and lower maternal age may lead to less sun exposure or less compliance in taking prenatal vitamins (Camargo, Ingham et al. 2010). However, these findings require replication and further investigation before they are accepted as risk factors for low levels of vitamin D in newborns.

2.2 Vitamin D in Pregnancy

2.2.1 Guidelines for Vitamin D Supplementation in Pregnancy

There are no consensus guidelines for vitamin D supplementation during pregnancy. The Canadian Pediatric Society (CPS) recommends vitamin D supplementation of 2000 IU/day during pregnancy and lactation (Canadian Pediatric Society 2007), while Health Canada recommends 600 IU/day for pregnant and breast-feeding women (Health Canada 2010). The Health Canada guidelines are based on the 2010 U.S. Institute of Medicine report, which is primarily intended for food manufacturers, while the CPS guidelines are based a thorough literature review and are intended for clinicians.

Meanwhile, the National Institute for Health and Clinical Excellence in the United Kingdom recommends 400 IU/day of vitamin D during pregnancy (National Institute for Health and Clinical Excellence 2008), the World Health Organization recommends 200 IU/day (World Health Organization 2004), and the Endocrine Society recommends 600 IU/day (Holick, Binkley et al. 2011). However, the Endocrine Society also recognizes that doses of up to 1500 – 2000 IU/day may be required to maintain sufficient serum vitamin D levels (>75 nmol/L) during pregnancy and lactation (Holick, Binkley et al. 2011).

Recent studies have also suggested the need for much higher intakes during pregnancy, with one suggesting that supplementation of 4000 IU/day was safe and most effective in achieving sufficiency in pregnant mothers and their offspring (Hollis, Johnson et al. 2011), and another showing that doses of up to 10,000 IU/day for five months in pregnancy did not elevate levels into the toxic range (Hollis and Wagner 2004). Studies have also indicated that doses of 400 IU/day are inadequate to achieve sufficient serum vitamin D levels in mothers (Dror, King et al. 2011) and newborns (Bodnar, Simhan et al. 2007). The author of a British review article

concluded that, “The lack of unified advice on vitamin D supplementation of pregnant mothers in the UK hinders the implementation of primary prevention strategies and is likely to leave some deficient mothers without supplementation (Hypponen and Boucher 2010).” The same could be said of Canada.

The Endocrine Society Clinical Practice Guidelines suggest that pregnancy and lactation are an indication for 25(OH)D screening (Holick, Binkley et al. 2011), but the British Journal of Nutrition argues that given the prevalence of deficiency and the cost of the assay, it would be much cheaper and more effective to have universal guidelines for Vitamin D supplementation during pregnancy than screening every pregnant woman for deficiency (Hypponen and Boucher 2010).

2.2.2 Prevalence of Vitamin D Insufficiency and Deficiency in Pregnancy

This review will use the definition of vitamin D insufficiency and deficiency espoused by the Endocrine Society, which defines vitamin D sufficiency as a serum vitamin D level of >75 nmol/L, insufficiency as 50-75 nmol/L, and deficiency as <50 nmol/L (Holick, Binkley et al. 2011). Though the definitions of vitamin D deficiency and insufficiency differ among studies, most conclude that low levels of vitamin D are prevalent among pregnant mothers (Kazemi A 2009; Camargo, Ingham et al. 2010; Dror, King et al. 2011). In Britain’s Avon Longitudinal Study of Parents and Children, 90% of the 10,000 white pregnant mothers had 25(OH)D concentrations in the deficiency range (<50 nmol/l) during winter and spring while 28% were seriously deficient (<25 nmol/l) (Golding, Pembrey et al. 2001). In Oakland, California, 54% of 275 mothers and 90% of their neonates had insufficient serum 25(OH)D levels (<75 nmol/L) over a one year period (Dror, King et al. 2011). Similar studies in France (Madelenat, Bastian et

al. 2001), India (Sachan 2005), Iran (Kazemi A 2009), China (Specker, Ho et al. 1992) and New Zealand (Camargo, Ingham et al. 2010) have shown that maternal serum levels of vitamin D are frequently deficient, and a review article on serum vitamin D levels in pregnancy found that 35 of 76 studies reviewed had mean or median vitamin D levels below 35 nmol/L (Schroth, Lavelle et al. 2005).

In Canada, several studies have assessed the vitamin D status of pregnant mothers. In Vancouver, 24% and 65% of 336 multiethnic pregnant women had deficient (<50 nmol/L) and insufficient (<75 nmol/L) serum levels of vitamin D, respectively (Li 2011). In Newfoundland and Labrador, a study of 50 pregnant women found that 38% of pregnant women had insufficient (50-75 nmol/L) and 42% had deficient (<50 nmol/L) levels of vitamin D (Newhook, Sloka et al. 2009). In the Northwest Territories, a study of 121 pregnant mothers revealed that the mean vitamin D level for Caucasian and Native Indian mothers was insufficient (< 75nmol/L), while the mean vitamin D level for Inuit mothers was deficient (<50 nmol/L) (Waiters, Godel et al. 1999). Three small studies in Northern Manitoba (n= 32, 35, 37) found that the median serum vitamin D concentrations in pregnant mothers was seriously deficient (<25 nmol/L) (Smith 2000), and in Montreal, a small study (n = 27) of mothers with twin pregnancies found a mean serum vitamin D concentration in the deficiency range (<50 nmol/L) (Reddy, Norman et al. 1983). Finally, a Winnipeg study (n = 50) found that 46% of mothers who had delivered within the past 48 hours were deficient (<37.5 nmol/L) (Weiler, Fitzpatrick-Wong et al. 2005). Therefore, existing research indicates that vitamin D deficiency in pregnant mothers is prevalent in Canada.

2.2.3 Outcomes of Vitamin D Sufficiency, Insufficiency and Deficiency in Pregnancy

While one 2011 review article concluded that there is insufficient evidence to suggest that low vitamin D levels in the first trimester are associated with adverse pregnancy and neonatal outcomes (Nassar, Halligan et al. 2011), a second 2011 review article concluded that, "Recent evidence supports a role of maternal vitamin D status, particularly early in pregnancy, in modulating the risk of pregnancy complications and in sustaining fetal growth, bone development, and immune maturation" (Dror 2011). Though existing evidence on the role of maternal vitamin D in pregnancy, neonatal, and child health outcomes is sparse and sometimes conflicting, it suggests that maternal vitamin D supplementation is required to prevent adverse health outcomes in mothers, infants and children.

2.2.3.1 Maternal Effects of Gestational Vitamin D Deficiency

Low levels of vitamin D throughout pregnancy have been associated adverse health outcomes for mothers, including preeclampsia (Bodnar, Catov et al. 2007), gestational diabetes (GDM) (Soheilykhah, Mojibian et al. 2010), intrahepatic cholestasis of pregnancy (Wikstrom Shemer and Marschall 2010), periodontal disease (Dietrich, Joshipura et al. 2004; Boggess, Espinola et al. 2011), Caesarean section (Merewood, Mehta et al. 2009) and HIV progression and mortality (Mehta, Giovannucci et al. 2010). This thesis will not cover maternal outcomes of vitamin D deficiency in detail as its focus is on neonatal health.

2.2.3.2 Effects of Gestational Vitamin D Deficiency on Neonatal Health

Size

The existing body of evidence indicates that low vitamin D levels during pregnancy are a

significant predictor for newborn size. An American study found that serum 25(OH)D <37.5 nmol/L was a significant risk factor for infants being born SGA (small for gestational age) among white, but not black, women (Bodnar, Catov et al. 2010). A Dutch study of 3730 mothers found that infants born to mothers with serum 25(OH)D <29.9 nmol/L were more likely to be born SGA and had a significantly lower birth weight than those born to mothers with serum 25(OH)D >50 nmol/L (Leffelaar, Vrijkotte et al. 2010). An Iranian study of 449 pregnant women found that mothers who received the daily recommended doses of calcium and vitamin D had infants with higher birth length and were less likely to have infants with low birth weight (Sabour 2006). Similarly, a US study including 2251 pregnant women found that vitamin D intake was a significant predictor for infant birth weight (Scholl and Chen 2009). However, an Australian study found that first trimester vitamin D levels were not associated with birth weight, head circumference, knee-heel length, or crown-heel length (Morley, Carlin et al. 2006).

Randomized controlled trials (RCTs) of vitamin D supplementation have conflicting evidence on the role of vitamin D in infant size. An RCT in 80 British mothers of Asian descent found that the incidence of SGA was higher in the placebo group than in mothers supplemented with 1000 IU of vitamin D per day (Brooke and Wood 1980; Brooke, Brown et al. 1981). Infants of the placebo group mothers subsequently gained less weight and had lower rates of linear growth than infants of the supplemented group (Brooke, Butters et al. 1981). By contrast, a French RCT involving pregnant women in their third trimester gave one dose of 200,000 IU to one group (n = 27), 1000 IU daily to one group (n = 21), and a placebo to a third group (n = 29) and found no differences in birth weight between groups (Mallet, Gugi et al. 1986).

Studies based on dietary recall support the role of vitamin D in infant size. A study performed in Calgary, Alberta showed that mothers who consumed more than one cup (>250

mL, n = 207) of milk per day during pregnancy had infants who weighed more than mothers who consumed less milk (n = 72). Each additional cup of milk per day was associated with a 41g increase in birth weight. Vitamin D was also a significant predictor of birth weight and each additional microgram of vitamin D per day was associated with an 11g increase in birth weight. However, there was no significant difference in infant lengths and head circumferences between groups (Mannion, Gray-Donald et al. 2006).

The Danish National Birth Cohort included data from 50,117 mother-infant pairs and found similar results. Findings were adjusted for potential confounding variables and milk consumption was inversely associated with the risk of SGA birth and directly associated with large for gestational age (LGA) birth and birth weight. Milk consumption was also associated with very small increases in head circumference and birth length (Olsen, Halldorsson et al. 2007). Though this evidence is compelling, it is difficult to control for all confounding factors and larger, randomized-controlled trials are needed to assess the role of vitamin D in birth size.

Apgar scores

Only one study investigated the relationship of maternal vitamin D with Apgar scores. This study assessed the vitamin D intake of 449 pregnant women in Iran and found that adequate maternal calcium and vitamin D intake was associated with higher 1-minute Apgar scores (Sabour 2006). However, it did not measure serum levels of 25(OH)D, the effects of calcium and vitamin D were not assessed separately, and there was no adjustment for potential confounding factors. Further research is required to support this finding.

Skeletal development

Studies have associated maternal vitamin D status during pregnancy with bone development *in utero* and bone size during infancy and childhood. One study involved 424 pregnant women and used a high-resolution 3D ultrasound to measure fetal femur length and distal metaphyseal cross-sectional area. This study found that lower maternal 25(OH)D concentrations were associated with greater femoral metaphyseal cross-sectional area and a higher femoral splaying index at 19 and 34 weeks' gestation, which suggests that maternal vitamin D status can influence fetal bone development early in pregnancy (Mahon, Harvey et al. 2010). In another study, newborn tibial bone mineral content and cross-sectional area, but not bone mineral density, were higher in a group of newborns whose mothers had 25(OH)D concentrations >42.5 nmol/L compared with newborns whose mothers' 25(OH)D concentrations were below this level, further suggesting the maternal vitamin D concentration influences bone size (Viljakainen, Saarnio et al. 2010). When a subset of the participants in this study were followed at age 14 months, tibial cross-section area remained higher in children whose mothers had better vitamin D status during pregnancy (Viljakainen, Korhonen et al. 2011). Finally, a study in the UK of 198 mother-child dyads found that maternal serum 25(OH)D deficiency or insufficiency was associated with reduced whole-body and lumbar spine bone mineral concentration in their 9-year-old children (Javaid, Crozier et al. 2006). The universal conclusion of these studies is that vitamin D supplementation of pregnant women may lead to increased skeletal development and decreased fracture risk in their offspring.

Severe maternal vitamin D deficiency may result in congenital rickets and hypocalcemic seizures (Russell and Hill 1974; Orbak, Karacan et al. 2007), although this is rare.

2.2.3.3 Effects of Gestational Vitamin Deficiency on Infant and Child Health

Neurological development

Low vitamin D levels during pregnancy may have long-lasting effects on the developing fetus. The presence of vitamin D receptors throughout the brain and its role in the development of the nervous system implies that maternal vitamin D levels may have an impact on fetal brain maturation (Garcion, Wion-Barbot et al. 2002). Only one study has assessed the role of vitamin D in the neurological development of the child. A study performed in the UK (n = 178) reported that sufficient maternal serum 25(OH)D status (>75 nmol/L) did not appear to impact the 9-year-old child's psychological health or intelligence (Gale, Robinson et al. 2008). However, no studies have investigated the relationship of maternal or newborn vitamin D status with neurological development in infancy.

Asthma and atopy

Low dietary and supplemental intake of vitamin D during pregnancy has been associated with increased risk of wheezing illness, asthma and allergic rhinitis. In Scotland, a study assessing the vitamin D intake of 1,212 mothers found that 5-year-old children were more likely to ever wheeze, wheeze in the previous year, or have a persistent wheeze if their mothers had a low vitamin D intake during pregnancy (Devereux, Litonjua et al. 2007). A US study similarly found that 3-year-old children were more likely to have a recurrent wheeze if their mothers had a low vitamin D intake during pregnancy (Camargo, Rifas-Shiman et al. 2007). In Finland, a study of 1669 5-year-old children found that the risk of asthma and allergic rhinitis was increased in children whose mothers had low vitamin D intake during pregnancy (Erkkola, Kaila et al. 2009), and in Japan, a study of 763 mother-child pairs found that low vitamin D intake

during pregnancy was a risk factor for eczema and wheeze in infants aged 16-24 months (Miyake, Sasaki et al. 2010).

Diabetes

Maternal vitamin D deficiency has also been related to the risk of Type 1 and Type 2 diabetes. An Indian study of 568 mothers found that 5 and 9.5-year-old children of vitamin D-deficient (<50 nmol/L) mothers had higher insulin resistance than children whose mothers had sufficient vitamin D levels during pregnancy (Krishnaveni, Veena et al. 2011). A Norwegian survey of 85 diabetic patients and 1,071 control patients found that when mothers took cod liver oil during their pregnancy, their offspring had a lower risk of Type 1 diabetes (Stene, Ulriksen et al. 2000).

2.2.4 Conclusion

Evidence suggests that low levels of vitamin D are extremely prevalent during pregnancy and may adversely affect neonatal, infant and child health. In Saskatchewan, the prevalence of low levels of vitamin D is likely to be high due to the northern latitude of the province, the high Aboriginal population, the high prevalence of obesity, and the lack of sun exposure during the winter months. Further studies will need to be conducted in order to estimate the prevalence of low levels of vitamin D in Saskatchewan's pregnant population and appreciate the role of maternal vitamin D supplementation in infant and child health.

2.3 Vitamin D in Newborns

2.3.1 Prevalence of Cord Blood Vitamin D Insufficiency and Deficiency

25(OH)D easily crosses the placenta (Hillman and Haddad 1974), and maternal levels of 25(OH)D are slightly higher than cord blood 25(OH)D levels (Sachan 2005; Bodnar, Simhan et al. 2007). Therefore, given the high prevalence of maternal vitamin D deficiency throughout the world, a similarly high prevalence of newborn vitamin D deficiency is expected. Deficient cord blood vitamin D has been documented in India (n = 207, mean cord blood 21.0 +/- 14.2 nmol/L) (Sachan 2005), Iran (n = 552, 93% <35 nmol/L) (Maghbooli 2007), Australia (n = 901, 11% <25 nmol/L) (Bowyer, Catling-Paull et al. 2009), Greece (n = 123, 8.1% <25 nmol/L) and the United States (n = 40, 65% <30nmol/L) (Lee, Smith et al. 2007), among other countries. In an American study of 200 black and 200 white neonates, vitamin D deficiency (<37.5 nmol/L) and insufficiency (37.5-80 nmol/L) occurred in 45.6% and 46.8% of black neonates, respectively, compared with 9.7% and 56.4% of white neonates (Bodnar, Simhan et al. 2007). Another study of 210 newborns performed in California found that 90% were either insufficient or deficient (<75 nmol/L) in vitamin D (Dror, King et al. 2011). Even in lower latitudes and warm climates, vitamin D deficiency in newborns is extremely prevalent. However, few studies have been performed in Canada.

Three Canadian studies have assessed cord blood vitamin D concentrations and had similar findings. A Winnipeg study (n = 50) found that 36% of infants had deficient cord blood 25(OH)D levels (<27.5 nmol/L). Average cord blood 25(OH)D in the deficient group was 9.0 ± 6.0 nmol/L, while average cord blood in the sufficient group was 39.7 ± 10.6 nmol/L (Weiler, Fitzpatrick-Wong et al. 2005). In the Northwest Territories, a study of 121 newborns found that both native and non-native newborns were on average deficient (34.2+/-13.1 nmol/L and 41.4+/-

23.5 nmol/L, respectively) (Waiters, Godel et al. 1999). In Newfoundland, a study of 51 newborns showed that average cord blood vitamin D was 48.6 nmol/L ± 17.5 nmol/L in the winter and 63.3 nmol/L ± 13.5 nmol/L in the summer. Approximately 49% of infants were insufficient and 35% were deficient (Newhook, Sloka et al. 2009). No studies have assessed cord blood vitamin D status in Saskatchewan.

2.3.2 Outcomes of Cord Blood Vitamin D Sufficiency, Insufficiency and Deficiency

Although many studies have commented on the prevalence of low cord blood vitamin D levels, few have studied its relationship with infant health. The following studies represent the majority of the literature available on the association of cord blood vitamin D status with infant and child health outcomes.

Neonatal heart failure and symptomatic neonatal hypocalcemia

Similar to the case reports of maternal vitamin D deficiency in relation to neonatal rickets and hypocalcemia (Russell and Hill 1974; Orbak, Karacan et al. 2007), case reports have associated low serum 25(OH)D in newborns with neonatal heart failure (Maiya, Sullivan et al. 2008) and symptomatic neonatal hypocalcemia (Shenoy, Swift et al. 2005; Teaema and Al Ansari 2010). In Qatar, 19 cases of neonatal hypocalcemia due to neonatal serum vitamin D deficiency were ultimately attributed to maternal vitamin D deficiency (Teaema and Al Ansari 2010). Similarly, in England, a review of 16 infants with rickets-associated dilated cardiomyopathy found that no infant or mother was receiving the recommended vitamin D supplementation (Maiya, Sullivan et al. 2008), suggesting that these life-threatening presentations of heart failure could have been prevented.

Infection

Two studies have associated low cord blood vitamin D levels with an increased risk of infections. Low cord blood vitamin D has been associated with an increased risk of RSV (respiratory syncytial virus) in the first year of life (Belderbos 2011) and an increased risk of respiratory infections (Camargo, Ingham et al. 2011). The increased infection rate could be explained by the role of vitamin D in immunity, as one study demonstrated that low cord blood vitamin D decreased in vitro monocyte responses (Walker, Zhang et al. 2011), and another found that high cord blood vitamin D levels were associated with lower numbers of T regulatory cells (Chi, Wildfire et al. 2011). (For more information on the role of vitamin D in the immune system, please see Section 2.4.2). These studies suggest that vitamin D status *in utero* may influence immune function in infancy. Additional studies are required to determine the role of vitamin D in neonatal health and particularly in the risk of infection.

Asthma and atopy

Evidence surrounding the role of neonatal vitamin D levels in asthma and allergy is conflicting. One study found that both high and low levels of cord blood 25(OH)D were associated with increased isoallergen sensitization, but that vitamin D levels were not associated with asthma or allergic rhinitis (Rothers, Wright et al. 2011). Similarly, another study found that low cord blood 25(OH)D was associated with increased risk of respiratory infections and wheezing, but not with an increased risk of asthma (Camargo, Ingham et al. 2011). In one study ($n = 649$), cord blood vitamin D deficiency (< 27 nmol/L) was not associated with food sensitivity (Liu, Wang et al. 2011). However, vitamin D deficiency did increase the risk of food sensitivity among children carrying single nucleotide polymorphisms (SNPs) in the genes

involved in regulating IgE and 25(OH)D concentrations (Liu, Wang et al. 2011). This gene-vitamin D interaction is likely not unique to food sensitivity and probably exists with many other conditions. Future studies should search for associations between health outcomes and genetic mutations, as relationships that are not initially apparent may surface once genetic variation is accounted for.

The mechanism underlying these observations may pertain to vitamin D's role as an immunomodulator. One study found that vitamin D supplementation in mothers resulted in the induction of tolerogenic dendritic cells in cord blood, which suggests that vitamin D status *in utero* may influence the development of atopic diseases later in life (Rochat, Ege et al. 2010). Another found that cord blood vitamin D levels were correlated with interleukin-10 (IL-10) levels, and that IL-10 levels were highest in the summer (Zittermann 2004). Because IL-10 is also involved in tolerizing exogenous antigens, it may also predispose infants to increased atopic risk. Research is necessary to further characterize the role of vitamin D in atopy.

Other health outcomes

One small Canadian study (n = 50) reported that low cord blood 25(OH)D concentration was related to lower bone mineral content in newborns (Weiler, Fitzpatrick-Wong et al. 2005). It also found that low vitamin D concentrations were associated with greater infant weight and length. This particular finding contradicts the general consensus of existing research, which has associated lower gestational vitamin D levels with smaller infant size (see Section 2.2.3.2).

2.3.3 Conclusion

Several studies have indicated a high prevalence of low vitamin D levels in newborns globally. In Canada, low levels of cord blood vitamin D were demonstrated in Newfoundland, the Northwest Territories, and Winnipeg. As previously mentioned, Saskatchewan's many risk factors for vitamin D deficiency put its newborns at high risk for having low levels of vitamin D. An estimate of the prevalence of vitamin D deficiency in Saskatchewan's newborns would establish the relevance of this problem to our province, while the identification of risk factors may indicate populations that require screening and primary prevention strategies. Finally, research that identifies the neonatal outcomes of vitamin D deficiency may aid in clarifying the role of vitamin D in physiology and consequently reinforce the importance of vitamin D supplementation.

2.4 Vitamin D in Children

2.4.1 Prevalence of Vitamin D Insufficiency and Deficiency in Children

It is conceivable, though not proven, that those born with a low level of vitamin D may continue to have a low level of vitamin D later in life. As well, it may not be uncommon for a child to develop vitamin D deficiency or insufficiency during childhood. A solid understanding of the risk factors for vitamin D deficiency may improve health behaviours of families during pregnancy, infancy and childhood and prevent vitamin D deficiency at all stages of life. As well, an understanding of the physiologic mechanisms of vitamin D *in utero* and infancy are the first steps to understanding the physiology of vitamin D in later life. Intrauterine vitamin D deficiency may predispose to chronic diseases which effect individuals at all ages. It is imperative to make connections between studies performed during pregnancy, infancy,

childhood, adolescence, adulthood and old age to fully understand how vitamin D levels may impact an individual's future health.

A study analyzing the results of the 2007/2008 Canadian Health Measures survey indicated that for Canadians aged 6–11 years, only 52% have vitamin D levels in the sufficient range (>75 nmol/L), while only 35% of children aged 12-19 years have vitamin D levels in the sufficient range (Langlois, Greene-Finestone et al. 2010). The prevalence of vitamin D deficiency in young children is high in many countries, including England (Callaghan, Moy et al. 2006), Greece (Nicolaidou, Hatzistamatiou et al. 2006), Iran (Neyestani, Hajifaraji et al. 2012), India (Babu and Calvo 2010), Qatar (Bener, Al-Ali et al. 2009) and the United States (Gordon, DePeter et al. 2004; Gordon, Feldman et al. 2008; Cole, Grant et al. 2010; Merewood, Mehta et al. 2010). Several Canadian studies also indicate a high prevalence of low vitamin D levels in children. In Manitoba, a cross-sectional study of 80 mother-infant pairs showed that 43% of infants 3-24 months had a 25(OH)D level below normal (Lebrun, Moffatt et al. 1993). In Quebec, a study of 1753 school-aged children showed that more than 93% had 25(OH)D levels below 75 nmol/L, and mean levels ranged from 40 to 50 nmol/L (Mark, Gray-Donald et al. 2008). Another Quebec study of 159 children aged 8-11 whose parents were obese or had metabolic syndrome showed that over 90% of them had serum 25(OH)D levels in the deficiency range (Mark 2010). In Edmonton, the mean serum 25(OH)D level of 68 pediatric patients presenting to the emergency room was 47 nmol/L, and all but one of the participants had a vitamin D concentration <80 nmol/L (Roth, Martz et al. 2005). Finally, data collected from our research group in Saskatoon indicated a mean vitamin D level of 49 +/- 24 nmol/L among a group of children admitted to the pediatric intensive care unit with acute lower respiratory infection (McNally, Leis et al. 2009).

The high prevalence of low vitamin D levels among Canadian mothers and children can be explained by the reduced skin synthesis of vitamin D during the winter as well as an inadequate intake of vitamin D in the diet. A study which analyzed the results of the 2004 Canadian Community Health Survey Cycle 2.2 showed that the majority of Canadians consume less than the recommended intake of vitamin D from food, suggesting that more vitamin D should be added to fortified foods, and that a wider range of foods should be fortified (Vatanparast, Calvo et al. 2010). It also showed that white Canadians had a higher vitamin D intake than non-white Canadians in most age sex groups (Vatanparast, Calvo et al. 2010), which, along with darker skin tone and genetic diversity, aids in explaining the discrepancy in vitamin D levels observed between white and non-white ethnicities. Given that the same risk factors may create low levels of vitamin D *in utero* as in infancy and childhood, early detection and prevention of low levels of vitamin D in pregnancy may substantially improve long-term child health.

2.4.2 Outcomes of Childhood Vitamin D Insufficiency and Deficiency

Rickets

Severe vitamin D deficiency, which causes rickets, has been well-known and documented since the 1800s (Welch, Bergstrom et al. 2000). However, despite the relatively recent fortification of foods with vitamin D, nutritional rickets appears to be re-emerging in developed countries (Welch, Bergstrom et al. 2000; Allgrove 2004). In Canada, a survey of 2325 pediatricians over two years estimated the incidence of rickets in Canada to be 2.9 per 100,000 (Ward, Gaboury et al. 2007). Of the 104 cases identified by the survey, the mean age at diagnosis was 1.4 years, 89% had darker skin tones, and 94% had been breast fed. None of the

breast-fed infants had been supplemented according to current guidelines (400 IU/day) (Ward, Gaboury et al. 2007), highlighting the importance of supplementing darker-skinned infants during the first year of life. In a Winnipeg hospital between 1972-1984, 48 cases of rickets were identified (Haworth and Dilling 1986) and in a Toronto hospital between 1988 and 1993, 17 cases of rickets were identified (Binet and Kooh 1996). Given the high prevalence of vitamin D insufficiency and deficiency among Canadian children, clinically apparent cases of deficiency represent only a small fraction of vitamin D-related child health outcomes.

Infections and Immunity

Vitamin D is able to act in an autocrine or paracrine manner in regulating immune function (Aranow 2011). The vitamin D receptor is expressed on B cells, T cells, and antigen-presenting cells, and these cells are also capable of producing 1-alpha hydroxylase, the enzyme responsible for converting 25(OH)D to the active form of vitamin D, $1,25(OH)_2D$. The metabolism of vitamin D is also regulated locally by the immune environment, resulting in local levels different from systemic levels. The macrophage 1-alpha hydroxylase is not PTH-dependant (Wu, Ren et al. 2007) and instead responds to circulating levels of 25(OH)D, interferon (IFN) gamma, interleukin (IL) 1, or tumor necrosis factor (TNF) alpha (van Etten, Stoffels et al. 2008). The macrophage 24-hydroxylase is non-functional, so there is no negative feedback of $1,25(OH)_2D$ production (Aranow 2011). The significance of vitamin D as an autocrine hormone continues to be investigated.

Vitamin D is an important factor influencing inflammatory responses (Abu-Amer 1993; Wang, Nestel et al. 2004). Vitamin D dampens systemic inflammatory response by inhibiting antigen-induced T-cell proliferation, antagonizing the pro-inflammatory Th1 response,

suppressing macrophage release of pro-inflammatory cytokines, altering gene expression, and decreasing adherence and chemotaxis of neutrophils (Bhalla, Amento et al. 1984; Rigby, Denome et al. 1987). These roles are pertinent to inflammatory responses common to many diseases.

Vitamin D status has been associated with infection risk. In 1981, Hope-Simpson published a study that recognized the increased incidence of influenza A during the winter in both northern and southern latitudes, while equatorial countries had a steady incidence of influenza A (Hope-Simpson 1981). Since then, many studies have demonstrated a direct association between hypovitaminosis D and increased risk of infection (McNally, Leis et al. 2009; Hewison 2010; Walker, Zhang et al. 2011). One study examined data from 18,883 individuals over age 12 in the US and found an association between low vitamin D levels (<30 ng/mL) and upper respiratory tract infection (Ginde, Mansbach et al. 2009). Low vitamin D levels have also been associated with increased risk of bacterial vaginosis (Bodnar, Krohn et al. 2009), influenza (Cannell, Vieth et al. 2006), and HIV (Villamor 2006). Most convincingly, a randomized, double-blind, placebo-controlled trial involving 167 schoolchildren showed that vitamin D supplementation reduced influenza A incidence by 42% (Urashima, Segawa et al. 2010).

In children, many studies have associated low vitamin D levels with an increased risk of respiratory infections (Muhe 1997; McNally, Leis et al. 2009). In an Ethiopian study of 500 children with pneumonia and 500 controls, children with pneumonia were 13 times more likely to have rickets, suggesting that low levels of vitamin D may predispose children to respiratory infection (Muhe 1997). An Indian study of 80 cases of acute lower respiratory infection (ALRI) and 70 controls aged 2-60 months found that children with vitamin D levels >22.5 nmol/L had a

decreased risk of ALRI (Wayse, Yousafzai et al. 2004). A study conducted by our research group in Saskatoon found no difference in vitamin D levels between children diagnosed with pneumonia or bronchiolitis (n = 105) and children with no respiratory symptoms (n = 92). However, children admitted to the pediatric intensive care unit with ALRI were more likely to be vitamin D deficient than their healthy counterparts (McNally, Leis et al. 2009). However, no studies have investigated the relationship of cord blood vitamin D status with the risk of infection or illness at birth.

Autoimmune disease and diabetes

Low 25(OH)D levels have been associated with increased risk of autoimmune diseases, such as multiple sclerosis (McMichael and Hall 1997; Munger, Zhang et al. 2004; Willer, Dyment et al. 2005), type 1 diabetes mellitus (Staples, Ponsonby et al. 2003; Willer, Dyment et al. 2005; Svoren, Volkening et al. 2009), rheumatoid arthritis (Merlino, Curtis et al. 2004), lupus (Kamen and Aranow 2008), and inflammatory bowel disease (Cantorna, Munsick et al. 2000). Northern latitude is associated with an increased risk of these diseases, further implicating vitamin D in their pathogenesis (Holick 2007). In fact, the risk of multiple sclerosis is decreased by approximately 50% in those living below 35 degrees latitude for the first 10 years of life (Ponsonby, McMichael et al. 2002; Van Amerongen, Dijkstra et al. 2004). However, a Finnish study had an opposite finding; vitamin D supplementation during the first year of life was associated with an increased risk of asthma, atopy and allergic rhinitis at age 31 years (Hypponen, Sovio et al. 2004). Despite the conflicting findings of present research, existing studies suggest that vitamin D supplementation during infancy may have long-lasting impacts on future health.

Several studies have associated childhood vitamin D levels with the risk of developing Type 1 diabetes. A systemic review and meta-analysis of five studies found that the risk of type 1 diabetes was reduced in infants who were supplemented with vitamin D in all studies (Zipitis 2008). The odds ratio for the risk of diabetes in those supplemented compared with those not supplemented was 0.71 (95% CI 0.60 to 0.84), and there was evidence for a dose-response effect with supplementation (Zipitis 2008).

Studies also support the role of vitamin D in the pathogenesis of diabetes. Vitamin D receptors have been found in both beta islet cells and immune cells, and a protective effect of vitamin D in cytokine-induced beta cell dysfunction has been demonstrated (Mathieu, Gysemans et al. 2005). Another study found that vitamin D deficiency was associated with increased insulin resistance and metabolic syndrome (Chiu, Chu et al. 2004). Finally, low vitamin D status has also been associated with increased fasting blood glucose and other indicators of lipid metabolism in Canadian children, which suggests a role for vitamin D in cardiovascular health as well as diabetes (Delvin, Lambert et al. 2010).

Cancer

In adults, serum 25(OH)D levels have been associated with increased risk of many types of cancer, including colorectal (Lappe, Travers-Gustafson et al. 2007), breast (Garland, Garland et al. 1990; Grant 2002), prostate (Bodiwala, Luscombe et al. 2003; Tuohimaa, Tenkanen et al. 2004), and colon cancer (Garland, Comstock et al. 1989; Pritchard, Baron et al. 1996). People living at high latitudes are also at increased risk for these and other cancers (Holick 2007). A randomized controlled trial involving 1179 postmenopausal women found that vitamin D supplementation reduced the risk of cancer by 60 to 77% (Lappe, Travers-Gustafson et al. 2007),

and there is a growing consensus that vitamin D supplementation may reduce the risk of cancer in all age groups.

Fewer studies have been conducted in the pediatric population. In children and young adults, sunlight exposure reduces the risk of non-Hodgkin's lymphoma by 40% (Chang, Smedby et al. 2005), and decreases the risk of death from malignant melanoma once it develops (Berwick, Armstrong et al. 2005). Lab studies show that vitamin D concentrations >75 nmol/L maintain normal cell growth and prevent cells from undergoing malignant transformation (Mawer, Hayes et al. 1994; Cross, Bareis et al. 2001; Tangpricha, Flanagan et al. 2001). Its role in cellular differentiation, apoptosis, proliferation and angiogenesis (Nagpal, Na et al. 2005) makes vitamin D a promising target for cancer research and increases the importance of vitamin D supplementation in protecting future health.

Cardiovascular Disease

Vitamin D's role in cardiovascular health is also being investigated (Wang, Pencina et al. 2008). As previously mentioned, low vitamin D levels have been associated with increased fasting blood glucose and other indicators of lipid metabolism in Canadian children, an observation that could have implications for future cardiovascular disease (Delvin, Lambert et al. 2010). Another study reported that deficient levels of vitamin D may predispose children to obesity (Gilbert-Diamond 2010). Other studies show that higher latitude increases the risk of hypertension and cardiovascular disease (Rostand 1997; Zittermann 2006), and that low vitamin D levels are associated with congestive heart failure in adults (Zittermann 2006). Interestingly, hypertensive patients who were exposed to ultraviolet light were able to normalize their blood pressure (Krause, Buhring et al. 1998), which suggests a role for vitamin D influencing

cardiovascular health. Further studies are warranted to investigate the role of vitamin D in cardiovascular health.

Neurological Development and Mental Health

Many studies have documented the presence of vitamin D, its receptor and its metabolites in the brain and recognized that vitamin D may be an important modulator of brain development (Eyles, Smith et al. 2005; Eyles, Feron et al. 2009; Eyles, Burne et al. 2011; Harms, Burne et al. 2011). Vitamin D may affect the developing brain through its roles in cellular differentiation, neurotransmitter synthesis, neurotrophic factor expression, and the expression of proteins involved in neuronal cell structure and metabolism (Eyles, Burne et al. 2011). VDR-deficient mice exhibit neurobehavioural abnormalities, including increased anxiety, reduced social behavior, abnormal grooming, pup cannibalism, impaired nest building, and neophobia (Kalueff, Keisala et al. 2006). They also exhibit increased susceptibility to epilepsy and impaired vestibular function (Kalueff, Minasyan et al. 2006; Keisala, Minasyan et al. 2009). These findings suggest that vitamin D is necessary for normal neurological development and that low levels of vitamin D may result in neurological impairment. However, as previously mentioned, only one study has investigated the role of maternal vitamin D deficiency on child psychological health, and it found no association between maternal vitamin D level and child psychological health or intelligence at 9 years of age (Gale, Robinson et al. 2008).

Insufficient or deficient Vitamin D status during infancy and childhood may predispose individuals to an increased risk of mental health diseases. Vitamin D levels have been associated with seasonal affective disorder (Gloth, Alam et al. 1999), and sufficient levels of vitamin D during the first year of life have been associated with a decreased risk of schizophrenia in males

(McGrath, Saari et al. 2004). Low vitamin D levels are occasionally responsible for neonatal hypocalcemic seizures (Camadoo, Tibbott et al. 2007), and researchers are investigating a possible role for vitamin D in epilepsy (Janjoppi, Katayama et al. 2008). Low levels of vitamin D are frequently found in patients with minor and major depression (Hoogendijk, Lips et al. 2008; Arvold, Odean et al. 2009), and supplemental vitamin D and light therapy reduce depressive symptoms (Jorde, Sneve et al. 2008; Shirani and St Louis 2009). It is likely that vitamin D status plays a role in the pathogenesis of many types of mental illness and that supplementation during pregnancy and infancy may improve outcomes in depression and other disorders.

2.4.3 Conclusion

Vitamin D deficiency and insufficiency is prevalent in Canadian children, and the myriad outcomes associated with vitamin D deficiency can adversely affect long term health. While many of these studies are observational and only associations have been made, randomized-controlled trials are unlikely to occur due to the health detriment to any group assigned to a placebo arm. Vitamin D deficiency is associated with many adverse outcomes during infancy, childhood, adolescence and adulthood. Further research is necessary to ascertain vitamin D's role in the pathogenesis of these diseases. It is possible that fetal and infant vitamin D levels may have more long-lasting effects on child and adult health than previously imagined.

2.5 Health and Economic Burdens of Vitamin D Deficiency

Given the substantial evidence for an association between vitamin D levels and health status, including potential life-threatening associations, one study estimated that the Canadian

death rate could fall by 37,000 deaths (22,300-52,300 deaths) if the mean serum 25(OH)D level of Canadians was increased to 105 nmol/L. This represents 16.1% of annual deaths and could reduce the annual economic burden by approximately $14.4 billion (6.9%) (Grant, Schwalfenberg et al. 2010). In Saskatchewan, the health and economic burdens of low vitamin D levels are likely to be higher than the national average because of the northern latitude in which much of the province's population resides, the inadequate winter sun exposure and reduced UVB radiation from winter sunlight strength, the high prevalence of obesity, the high Aboriginal population and the divergence of the Aboriginal population from their traditional fish-based diet. Saskatchewan's pregnant mothers, and therefore their newborns, are likely to exhibit a high prevalence of vitamin D insufficiency and deficiency. If low vitamin D levels occur during a critical period in fetal development, it is possible that the fetus may experience long term adverse health consequences that would have been preventable through adequate maternal supplementation.

2.6 Conclusions From the Literature Review and Rationale for the Current Study

The role of vitamin D in the pathogenesis of many non-skeletal infant and child health outcomes is only beginning to be elucidated. Studies are difficult to interpret because of differences in the definitions of vitamin D insufficiency and deficiency. Furthermore, the acceptable level of vitamin D for neonates, infants and children is still debated. Vitamin D studies in newborns are few and far between despite the inherent vulnerability of the fetus *in utero*.

Canada's, and particularly Saskatchewan's, populations are at high risk of being vitamin D deficient due to their high Aboriginal populations, northern latitude, and limited sun intensity

and exposure during the winter. Results of Canadian and non-Canadian studies indicating the prevalence of maternal vitamin D deficiency during pregnancy are alarming. Given that vitamin D travels through the placenta, a similarly prevalent level of newborn vitamin D deficiency would be expected. However, no studies have examined vitamin D levels of Saskatchewan's newborn population.

Both low maternal and cord blood vitamin D levels have been linked with adverse outcomes in infancy and childhood. Maternal vitamin D levels have been linked with infant size and Apgar scores, and low levels of vitamin D during pregnancy may increase a child's risk of wheezing, asthma, and type 1 diabetes. Cord blood vitamin D levels have been associated with neonatal heart failure and hypocalcemia, increased risk of childhood infections, and increased risk of atopic disease. Maternal supplementation of vitamin D may mitigate the risk of several infant and childhood diseases. Therefore, research indicates that the intrauterine environment may have a long-lasting impact on the fetus and eventually the individual. However, clinical associations have not been adequately explored in newborns, and the pathophysiology of the associations identified in children remains unknown.

Although many studies have demonstrated that cord blood vitamin D deficiency and insufficiency is prevalent, it remains uncertain what constitutes "low" levels of vitamin D for newborns because correlations between low vitamin D levels and clinical outcomes are lacking. It is unclear whether socio-demographic, obstetric or personal health factors may influence newborn vitamin D levels, and it is unknown whether low cord blood vitamin D levels are associated with adverse clinical outcomes at birth. Finally, there are few Canadian studies and no Saskatchewan studies that describe cord blood vitamin D levels.

This research aims to estimate the prevalence of low levels of vitamin D among

Saskatoon's newborns, which may aid in establishing the extent of vitamin D deficiency and insufficiency in this population. This research also aims to identify socio-demographic, obstetric and personal health factors that may influence cord blood levels of vitamin D, and identify whether adverse clinical outcomes may be associated with low vitamin D levels.

The association of newborn vitamin D status with neonatal outcomes will aid the understanding of the role of vitamin D's role *in utero* and its impact on a newborn's health. Clinical associations may also aid in the development of age-specific reference ranges for vitamin D levels, as "low" vitamin D levels for adults may be normal in newborns and may not result in any adverse clinical outcomes.

Finally, despite compelling evidence of the importance of vitamin D sufficiency during pregnancy, there is no consensus on guidelines for supplementation. The prevalence of vitamin D deficiency and insufficiency in newborns and any adverse outcomes to the fetus or child may be decreased or prevented with adequate maternal vitamin D supplementation. The final goal of this research is to educate ourselves and our communities about the importance of vitamin D supplementation. This knowledge will empower pregnant mothers, families and communities to make informed decisions about the benefits of vitamin D supplementation. We hope to promote adequate vitamin D supplementation throughout all stages of life.

CHAPTER 3: NEWBORN VITAMIN D STUDY

3.1 MATERIALS AND METHODS

3.1.1 Study design

This study had a cross-sectional design. Participants were recruited between December, 2011 and February, 2012. The study was advertised through posters in Royal University Hospital (RUH), presentations at prenatal classes, and brochures available through midwives and the West Winds Primary Health Centre.

Some participants were recruited at prenatal classes and informed consent was obtained after the class. Most participants were approached on the Assessment, Antepartum and Labour and Delivery wards at RUH. Participants were approached by the researcher when nurses deemed they were appropriate candidates for the study and depending on the researcher's availability. Nurses of potential participants were always approached prior to entering any room to ensure that potential participants were eligible for the study and comfortable enough to give informed consent. The study was explained to potential participants and their families and they were given approximately half an hour to consider participating.

After consent was obtained, participants were asked to fill out a questionnaire containing questions about the mother's health and demographic information. At delivery, nurses collected a cord blood sample for vitamin D measurement and this was stored in a refrigerator until transport to the research laboratory. After delivery, a spectrophotometer was used to measure the skin reflectance of the infant. The medical records pertaining to labour, birth and immediate post-partum history for the mother and the infant were used to collect information pertaining to the pregnancy and delivery as well as anthropometric measurements and health status of the baby at birth. This information was entered into an electronic database. (See Appendix 1 for Consent

Form, Participant Questionnaire and Recruitment Materials.)

3.1.2 Subjects

The study population included 65 maternal-fetal dyads delivering in the Saskatoon Health Region (latitude 52°N). Consenting mothers of singleton pregnancies delivering in the Saskatoon Health Region were included. Any pregnant women with pre-existing parathyroid or calcium conditions, those who took medications that interfere with calcium metabolism (such as diuretics or calcium channel blockers), those with thyroid disease and those without informed consent were excluded.

Of the 89 participants recruited for the study, proper informed consent was not assured for five participants and their information was not used in the analysis. Of the 84 participants enrolled in the study, 19 failed to undergo cord blood collection at delivery and although their demographic information was collected the variable of interest (cord blood vitamin D level) was not available and therefore the information from these participants was excluded from statistical analysis. As well, these participants were not followed post-partum, and therefore no information about the obstetric or neonatal outcomes of these participants is available. For two of these cases, samples were not collected due to precipitous deliveries. For the remaining 17 cases, the reason for failed blood collection was unknown but was likely due to a failure to communicate participatory status with nurses. The remaining 65 samples were included in the statistical analysis.

3.1.3 Data Collection

3.1.3.1 Cord Blood Collection, Storage and Analysis

At the time of delivery, cord blood (3cc) was collected by needle/syringe withdrawal from an umbilical cord vein into a 3cc capacity heparinized vacutainer. Samples were stored in a refrigerator (4^{o}C) until they were transported to the testing laboratory. There, they were centrifuged at 2500 revolutions per minute for 10 minutes, the supernatant was removed, and the supernatant was stored at -80°C until analysis.

Quantitative plasma 25(OH)D was determined by enzyme immunoassay (Immunodiagnostic Systems Ltd.). The assays were performed by the study investigators in the Pediatric Rheumatic Disease Research Laboratory, University of Saskatchewan as we have previously described (McNally, Matheson et al. 2008; McNally, Matheson et al. 2008) and were run in accordance with the manufacturer's specifications. Vitamin **D** **E**xternal **Q**uality **A**ssessment **S**cheme (DEQAS; www.deqas.org) samples were run in parallel with subject samples.

3.1.3.2 Socio-Demographic, Obstetric and Personal Health Information

At enrolment, a study number was assigned to each participant and the following enrolment information documented (See Appendix 1 for Study Questionnaire):

- Date of enrolment
- Maternal age
- Marital status: married, single, divorced, separated, common-law, widowed (Statistics Canada 2006). Due to the small number of participants in other categories, marital status was dichotomized into married and common-law for the purposes of analysis

- Maternal area of residence: determined by forward sortation area (the first three characters of postal code; as an approximate indicator of rural residence a 'zero' as the second character was considered rural and any other number as the second character as urban) (Statistics Canada 2008)
- Maternal education level: elementary, high school, some university, technical training beyond high school, one or more university degrees, other (Statistics Canada 2006). For analysis purposes the education level was dichotomized as elementary or high school education and some post-secondary education
- Household income: <$10,000, $10,000 – $25,000, $25,001 – $50,000, $50,001 – $100,000, >$100,000. Due to the small number of participants in other categories, these five categories were truncated into three categories (<$50,000, $50,001 - $100,000, and >$100,000) for the purposes of analysis
- Parity: number of previous births trichotomized into no previous births, 1 previous birth and greater than 1 previous birth
- Self-reported pre-pregnancy height (cm) and weight (kg)
- Maternal vitamin D supplementation: amount, frequency, duration. Dichotomized as yes/no, where 'yes' indicated some vitamin D supplementation other than a prenatal supplement
- Infant ethnicity: referred to all ethnic origins of the fetus. Non-white ethnicities included East and Southeast Asian, Aboriginal, Chinese, South Asian, American Indian, East Indian, Caribbean or Arab origins (Statistics Canada 2006). For analysis purposes ethnicity was dichotomized into Caucasian origins only and some non-Caucasian ethnicity

- Maternal occupation: Management Occupations, Business, Finance and Administrative Occupations, Natural and Applied Sciences and Related Occupations, Health Occupations, Occupations in Social Science, Education, Government Service and Religion, Occupations in Art, Culture, Recreation and Sport, Sales and Service Occupations, Trades, Transport and Equipment Operators and Related Occupations, Occupations Unique to Primary Industry, Occupations Unique to Processing, Manufacturing and Utilities, Student, Other (list) (Statistics Canada 2006). Mother's occupation was not used during statistical analysis as many participants answered "Other." As well, it was not clear if the participant had previously worked or was currently working outside the home
- Pre-pregnancy medical conditions: listed. Dichotomized as yes/no (has medical diagnosis for which medication is taken and no medical conditions)
- Infections or illnesses during the pregnancy. Dichotomized as yes/no
- Pre-pregnancy weight was confirmed using pre-pregnancy or early pregnancy weight in the medical chart. Pre-pregnancy height and weight were used to calculate pre-pregnancy BMI (kg/m^2)

All information was entered into an electronic database under the study number of the participant.

3.1.3.3 Anthropometric, Pregnancy and Delivery-Related Information

After delivery, health records pertaining to the pregnancy and delivery were consulted to obtain the following information:

- Maternal weight gain during pregnancy: kg (determined by subtracting weight at first prenatal visit from current weight or weight at last prenatal visit)
- Gestational age: weeks
- Method of delivery (vaginal or Cesarean birth)
- Presence of complications with delivery. Dichotomized as no complications or any complications during delivery; any complications were specified as a string variable and included, as examples, fetal decelerations, shoulder dystocia, vacuum or forceps-assisted delivery, and Cesarean section for non-reassuring fetal heart rate, failure to progress, or malpresentation,
- Infant height (cm), weight (g), head circumference (cm)
- Apgar scores: a measurement of neonatal vitality, measured at 1, 5 and 10 minutes. Apgar scores are very infrequently measured at 10 minutes and 91% of this data was missing. Therefore only Apgar scores at 1 and 5 minutes were included in the analysis
- Presence of any illness in the newborn. Dichotomized as yes/no, where 'no' indicated no illness and 'yes' indicated any neonate who was admitted to the neonatal intensive care unit or required any assisted ventilation or medical intervention immediately after birth

This information was entered into an electronic database under the study number of the participant.

3.1.3.4 Skin Tone

Skin pigmentation was estimated by measuring skin reflectance using a portable spectrophotometer (Model cm-600d, Konica Minolta). Skin reflectance readings (L*) were taken at the inner aspect of the middle right forearm as well as on the sternum. L* is an index of reflectance ranging from 0 (perfect black) to 100 (perfect white).

3.1.4 Statistical Analysis

All statistical tests were performed using SPSS Statistics 19 software. The definitions of vitamin D deficiency, insufficiency and sufficiency are as follows: vitamin D sufficiency (> 75 nmol/L), vitamin D insufficiency (50 to 74.99 nmol/L) and vitamin D deficiency (< 50 nmol/L) (Holick, Binkley et al. 2011).

3.1.4.1 Description of Study Population and Vitamin D Status

Descriptive demographic data were used to provide information about the population under investigation. A comparison between the 65 included and 19 excluded participants was performed using chi-square tests for categorical variables with only two categories and t tests were used for continuous variables.

A histogram and tests for skewness and kurtosis were used to determine whether the cord blood vitamin D level had a normal distribution (see Figure 1 in Section 3.2.1). Once the assumption of normality was satisfied, further statistical analysis using cord blood vitamin D level as a continuous dependent variable could be pursued.

3.1.4.2 Risk Factors for Low Cord Blood Vitamin D Levels

Mean levels of vitamin D were reported for each category within each categorical variable. Independent t-tests and one-way ANOVAs were used to determine whether any statistically significant difference existed between the mean vitamin D levels between categories for each categorical variable. The Pearson correlation coefficient was used to evaluate the correlation between continuous variables and vitamin D levels.

Linear regression was used to determine whether any association existed between cord blood vitamin D levels and the independent variables. Multiple linear regression analysis was performed to find the most parsimonious and best fitting model to predict cord blood vitamin D levels. It was performed in three steps:

1) The crude analysis included all available data within a variable (most variables had some missing data) and involved a bivariate analysis between each independent variable and cord blood vitamin D level (the dependent variable).

2) A Full Model was created which included all variables from the Crude Model with a $p < 0.25$ (Hosmer and Lemeshow 2000).

3) A Reduced Model was created using all variables from the Full Model with a $p < 0.10$. Partial F tests were used to examine whether any variable from the Full Model that was excluded from the Reduced Model (i.e because $p > 0.10$) should be included in the Reduced Model. Following this, all variables that were included in the Full Model but excluded from the Reduced Model were added individually to the Reduced Model to test for their role as a confounder. If any added variable caused greater than a twenty percent change in the coefficients of the original variables, confounding was determined to be present and that variable was retained in the reduced model.

3.1.4.3 Outcomes Associated with Low Cord Blood Vitamin D Levels

The obstetrical and neonatal outcomes of the study population were described. Mean cord blood vitamin D level for each category within each categorical variable was described. Independent t-tests were used to compare the mean cord blood vitamin D levels between categories for each categorical variable. Simple linear regression was used to determine whether any association existed between continuous variables and cord blood vitamin D levels. Finally, cord blood vitamin D level was used as an independent variable in the prediction of each obstetric and neonatal outcome.

3.1.5 Confidentiality and Ethics

The protocol Bio 11-185 for the Newborn Vitamin D Study was approved by the Research Ethics Board on November 3, 2011 at the University of Saskatchewan as well as by the Saskatoon Health Region. Informed assent/consent was obtained from participants included in the study. For 5 participants, reliable informed consent was not obtained and therefore these subjects were excluded from analysis. Data and samples were coded by study number only and stored securely.

3.2 RESULTS

3.2.1 Description of Study Population and Vitamin D Status

Of the 89 participants recruited for the study, proper informed consent was not assured for five participants and their information was not used in the analysis. Of the 84 participants enrolled in the study, 19 failed to undergo cord blood collection at delivery and their information was therefore excluded from the statistical analysis. For two of these cases, samples were not collected due to precipitous deliveries. For the remaining 17 cases, the reason for failed blood collection was unknown but was likely due to a failure to communicate participatory status with nurses. The remaining 65 samples were included in the analysis.

Univariate statistics for the sociodemographic variables of all enrolled participants are shown in Tables 2 and 3. Comparisons were made between those who were included in the analysis and those who were not because of failed blood collections. There were no statistically significant differences between the included and excluded populations. Overall, 85% of included mothers reported taking prenatal supplements and 26% reported taking supplemental vitamin D in addition to prenatal supplements.

Table 2. Categorical Demographic and Obstetric Characteristics of Participants

Variable	Included (n = 65) n (%)	Excluded (n = 19) n (%)	p-value*
Residence			
Rural	21 (32%)	6 (32%)	0.95
Urban	44 (68%)	13 (68%)	
Mother's Education Level			
Elementary or High School	8 (12%)	5 (26%)	0.15
Some Post-Secondary	55 (85%)	14 (74%)	
Missing	2 (3%)	0	

Marital Status			
Married	43 (66%)	13 (68%)	0.22
Single	1 (2%)	1 (5%)	
Separated	0	1 (5%)	
Common-law	18 (28%)	4 (21%)	
Missing	3 (5%)	0	
Household Income			
≤ $50,000	13 (20%)	5 (26%)	0.45
$50,001 - $100,000	23 (35%)	4 (21%)	
>$100,000	21 (32%)	8 (42%)	
Missing	8 (12%)	2 (11%)	
Ethnicity of Infant			
Any Non-White Ethnicity	17 (26%)	6 (32%)	0.79
No Non-White Ethnicity	43 (66%)	13 (68%)	
Missing	5 (8%)	0	
Parity			
0	37 (57%)	8 (42%)	0.64
1	19 (29%)	6 (32%)	
>1	7 (11%)	3 (16%)	
Missing	2 (3%)	2 (11%)	
Prenatal Supplement			
Yes	55 (85%)	11 (58%)	0.31
No	7 (11%)	3 (16%)	
Missing	3 (5%)	5 (26%)	
Vitamin D Supplement			
Yes	17 (26%)	1 (5%)	0.12
No	44 (68%)	12 (63%)	
Missing	4 (6%)	6 (32%)	
Maternal Medical Condition			
Yes	16 (25%)	5 (26%)	0.91
No	48 (74%)	14 (74%)	
Missing	1 (2%)	0	
Illness During Pregnancy			
Yes	23 (35%)	7 (37%)	0.94
No	41 (63%)	12 (63%)	
Missing	1 (2%)	0	

* p-value comparing the included to excluded groups

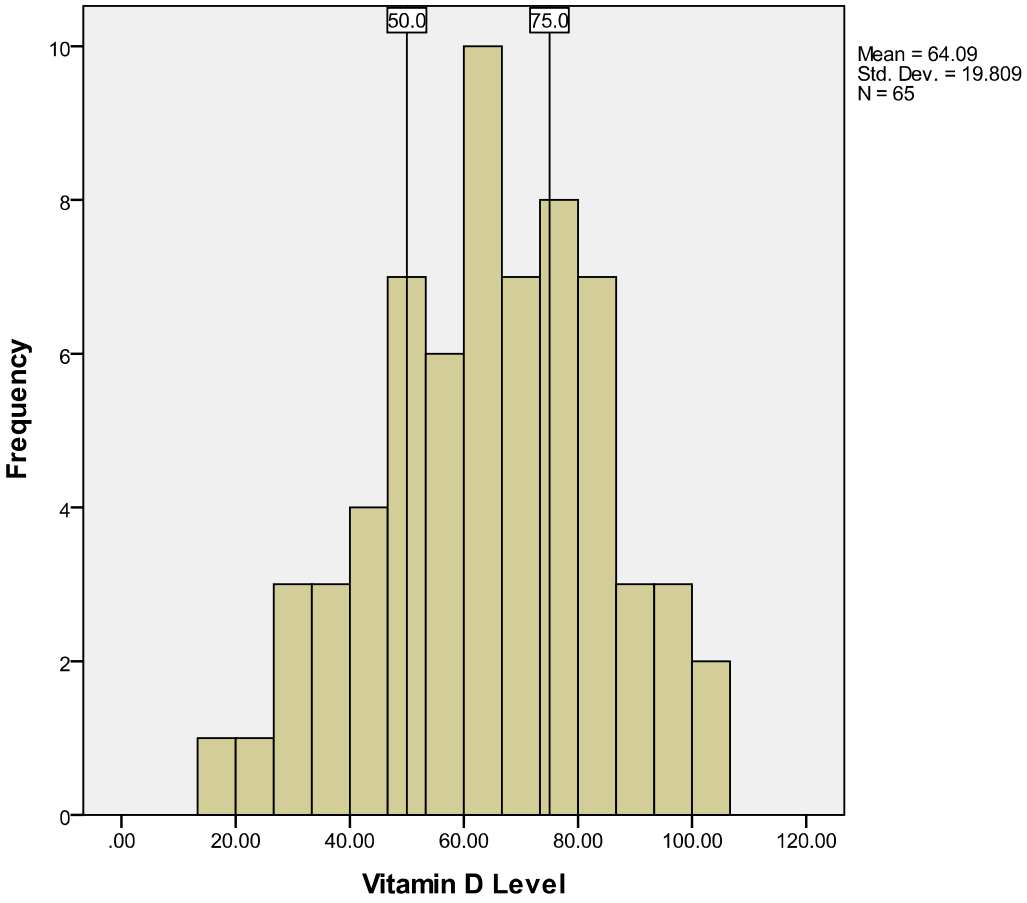
Table 3. Continuous Demographic and Obstetric Characteristics of Participants

Variable	Included (n=65)		Excluded (n=19)		p-value *
	Mean (SD)	n (%)	Mean (SD)	n (%)	
Age (years)	29.6 (4.4)	63 (97%)	30.2 (4.3)	9 (47%)	0.67
Pre-Pregnancy BMI (kg/m^2)	25.2 (5.3)	59 (91%)	26.5 (5.7)	18 (95%)	0.37
Weight Gain During Pregnancy (kg)	13.5 (5.1)	59 (91%)			
Gestational Age (weeks)	39.8 (1.5)	0			
Skin tone forearm (L*)	62.5 (3.5)	52 (80%)			
Skin tone sternum (L*)	63.0 (2.6)	39 (60%)			

* p-value comparing the included to excluded groups

Cord blood vitamin D levels had a relatively normal distribution (Figure 1). Average cord blood vitamin D was 64.1 nmol/L ± 19.8 nmol/L. Samples ranged from 17.5 nmol/L to 106.3 nmol/L. The cord blood vitamin D values were 50.2 nmol/L, 64.2 nmol/L and 78.5 nmol/L for the 25^{th}, 50^{th} and 75^{th} percentiles respectively. When compared with previously established categories of deficient (<50 nmol/L), insufficient (50-75 nmol/L), and sufficient (>75 nmol/L) (Holick, Binkley et al. 2011), cord blood vitamin D level was deficient in 22% and insufficient in 48% of the 65 newborns studied.

Figure 1. Histogram of Cord Blood Vitamin D Levels (n = 65)



3.2.2 Risk Factors for Low Cord Blood Vitamin D Levels

Mean cord blood vitamin D level and standard deviation for each level of the categorical variables is presented in Table 4. There was no statistically significant difference in vitamin D level between categories for any variable. However, there was a trend towards higher vitamin D levels in those with rural residence ($p = 0.09$).

Table 4. Mean (SD) Levels of Cord Blood Vitamin D by Categorical Independent Variables

Variable	n (%)	Mean Cord Blood Vitamin D Level (nmol/L) (SD)	p-value*
Residence			
Rural	21 (32)	70.2 (17.8)	0.09
Urban	44 (68)	61.2 (20.3)	
Education			
Elementary or High School	8 (12)	61.3 (29.7)	0.65
Some Post-Secondary	55 (85)	64.8 (18.3)	
Marital Status			
Married	43 (66)	65.4 (19.3)	0.72
Common-law	18 (28)	63.4 (21.2)	
Income			
≤ $50,000	13 (20)	65.5 (18.6)	0.91
$50,001 - $100,000	23 (35)	64.5 (22.6)	
>100,000	21 (32)	67.1 (18.6)	
Ethnicity of Infant			
Any Non-White Ethnicity	17 (26)	65.0 (21.7)	0.84
No Non-White Ethnicity	43 (66)	63.9 (19.3)	
Parity			
0	37 (57)	61.3 (19.2)	0.20
1	19 (29)	70.6 (18.1)	
>1	7 (11)	63.4 (26.0)	
Prenatal Supplement			
Yes	55 (85)	65.0 (19.6)	0.23
No	7 (11)	55.4 (22.4)	
Vitamin D Supplement			
Yes	17 (26)	65.5 (25.0)	0.59
No	44 (68)	62.4 (17.0)	
Maternal Medical Condition			
Yes	16 (25)	67.8 (19.0)	0.45
No	48 (74)	63.4 (20.0)	
Illness During Pregnancy			
Yes	23 (35)	63.4 (18.5)	0.74
No	41 (63)	65.1 (20.5)	

*p value comparing the vitamin D levels between categories within each variable

The r and p-values for the correlation between each continuous independent variable and cord blood vitamin D level are presented in Table 5. Low weight gain during pregnancy was associated with low cord blood vitamin D levels ($r = 0.27$, $p = 0.04$). Vitamin D level was not significantly correlated with any other maternal demographic or obstetric risk factors. However,

lower maternal age and higher pre-pregnancy BMI showed trends of an association with lower cord blood vitamin D levels ($p = 0.07$ and $p = 0.09$, respectively).

Table 5. Correlation Between Continuous Variables and Cord Blood Vitamin D Level

Variable	n (%)	r value	p-value
Age (years)	63 (97)	0.227	0.07
Pre-pregnancy BMI (kg/m^2)	59 (91)	0.226	0.09
Less Weight Gain During Pregnancy (kg)	59 (91)	0.274	0.04
Gestational Age (weeks)	65 (100)	0.085	0.50
Skin tone forearm (L*)	52 (80)	0.194	0.17
Skin tone sternum (L*)	39 (60)	0.107	0.52

The results from the linear regression analyses are presented in Table 6. This included the examination of the association between each risk factor and cord blood vitamin D level at a crude level and two levels of adjustment. The Full Model contains all variables from the Crude Model with a $p < 0.25$, while the Reduced Model contains all variables from the Full Model with a $p < 0.10$ (Age, Rural). The Full Model has an R^2 of 0.344 ($p = 0.02$) in predicting vitamin D levels ($n = 41$). The Reduced Model has an R^2 of 0.270 ($p < 0.01$) in predicting vitamin D levels ($n = 41$). None of the variables included in the full model that were excluded from the reduced model affected the β estimate of those variables included in the reduced model. As such, these variables were not included as confounders.

Table 6. Crude and Adjusted Results from Linear Regression Analyses Predicting Vitamin D Levels

		Crude		Full (n = 41)		Reduced (n = 41)	
Variable	n (%) of 65	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Age	63 (97%)	1.016 (0.553)	0.07	1.809 (0.568)	<0.01	1.840 (0.557)	<0.01
Rural Address (ref: No)	65 (100%)	9.049 (5.171)	0.09	10.651 (5.727)	0.07	9.296 (5.395)	0.09
Pre-pregnancy BMI	59 (91%)	-0.844 (0.482)	0.09	-0.759 (0.628)	0.24		
Prenatal Supplement (ref: No)	62 (95%)	9.588 (7.983)	0.23	-1.486 (8.037)	0.85		
Skin tone forearm (L*)	39 (60%)	-1.045 (0.746)	0.17	-1.084 (0.729)	0.15		
Weight Gain During Pregnancy (kg)	59 (91%)	1.047 (0.488)	0.04	0.573 (0.583)	0.33		
Post-secondary Education (ref: No)	63 (97%)	3.487 (7.537)	0.65				
Marital Status	62 (95%)	-0.506 (1.393)	0.72				
Household Income	53 (82%)	1.004 (3.523)	0.78				
Non-white Ethnicity	60 (92%)	1.137 (5.735)	0.84				
Parity	63 (97%)	3.534 (3.633)	0.33				
Vitamin D Supplement (ref: No)	61 (94%)	3.051 (5.562)	0.59				
Maternal Medical Condition (ref: No)	64 (98%)	4.330 (5.700)	0.45				
Illness During Pregnancy (ref: No)	64 (98%)	-1.737 (5.163)	0.74				
Gestational Age (weeks)	65 (100%)	-1.151 (1.700)	0.50				
Skin tone sternum (L*)	39 (60%)	-0.810 (1.240)	0.52				

3.2.3 Outcomes Associated with Cord Blood Vitamin D Levels

The obstetric and neonatal outcomes that occurred among participants (n = 65) are presented in Tables 7 and 8. Most participants had vaginal births (82%) and most of their infants (85%) did not have any illness or require any medical intervention at birth. Their infants had a mean length of 52.8 ± 2.9cm, a mean weight of 3616 ± 523g, a mean head circumference of 34.9 ± 1.4cm, and mean Apgar scores of 7.8 ± 1.6 at 1 minute and 8.8 ± 0.6 at 5 minutes. Apgar score at 10 minutes was not considered due to the large number of missing values (Table 8).

Table 7. Frequency (%) of Categorical Obstetric and Neonatal Outcomes (n = 65)

Variable	n (%) of 65
Method of Delivery	
Vaginal	53 (82%)
Cesarean	12 (19%)
Delivery Complication	
Yes	28 (43%)
No	34 (52%)
Missing	3 (5%)
Infant Illness at Birth	
Yes	7 (11%)
No	55 (85%)
Missing	3 (5%)

Table 8. Descriptives of Continuous Obstetric and Neonatal Outcomes (n = 65)

Variable	Mean (SD)	Missing n (%) of 65
Infant Length (cm)	52.8 (2.9)	0
Infant Weight (g)	3616 (523)	0
Infant Head Circumference (cm)	34.9 (1.4)	0
Apgar 1 minute	7.8 (1.6)	0
Apgar 5 minute	8.8 (0.6)	0
Apgar 10 minute	8.2 (1.0)	59 (91%)

The mean cord blood vitamin D level and standard deviation for each categorical variable are presented in Table 9. There was no significant difference between categories within each variable.

Table 9. Mean (SD) Levels of Cord Blood 25(OH)D Stratified by Categorical Variables

Variable	Mean Cord Blood Vitamin D Level (nmol/L) (SD)	p-value*
Method of Delivery		
Vaginal	63.2 (21.2)	0.47
Cesarean	67.8 (12.0)	
Delivery Complication		
Yes	63.8 (17.1)	0.92
No	63.3 (21.4)	
Infant Illness at Birth		
Yes	74.1 (18.3)	0.12
No	62.1 (19.3)	

*p value comparing the vitamin D levels between categories within each variable

The r and p-values for the correlation between each continuous independent variable and cord blood vitamin D level are presented in Table 10. Vitamin D level was not significantly associated with any neonatal outcomes.

Table 10. Correlation Between Continuous Variables and Cord Blood 25(OH)D

Variable	R value	p-value
Infant Length (cm)	0.054	0.67
Infant Weight (g)	0.113	0.37
Infant Head Circumference (cm)	0.029	0.82
Apgar 1 minute	0.159	0.21
Apgar 5 minute	0.128	0.31

Vitamin D level was investigated as a risk factor for various obstetric and neonatal outcomes. Vitamin D level was not significantly associated with any obstetric or neonatal outcomes including Cesarean delivery (OR = 1.002, 95% CI = 0.998 – 1.006, p-value = 0.47), delivery complications (OR = 0.000, 95% CI = 1.006 – 0.994, p-value = 0.92), infant illness at birth (OR = 1.003, 95% CI = 1.007 – 0.999, p-value = 0.12), infant length (β = -0.008, SE = 0.018, p-value = 0.67), infant weight (β = -2.973, SE = 3.305, p = 0.37), infant head

circumference (β = -0.002, SE = 0.009, p = 0.82), Apgar 1 minute (β = -0.013, SE = 0.010, p = 0.21), and Apgar 5 minute (β = -0.004, SE = 0.004, p = 0.31).

3.2.4 Analysis of Statistical Power

This post-hoc analysis estimated the power achieved for all categorical variables with a between-group significance of $p < 0.25$. The categorical variable with the highest power was "Residence," which had an effect size of 0.47, a power of 42% and would have required a sample size of 160 participants to achieve a power of 80%. "Infant Illness at Birth" had an effect size of 0.64, a power of 35% and would have required a sample size of greater than 200 participants to achieve a power of 80%. "Parity" had an effect size of 0.21, a power of less than 30%, and would have required a sample size of 228 to achieve a power of 80%, while "Prenatal Supplement" had an effect size of 0.45, a power of 20% and would have required a sample size of more than 400 to achieve a power of 80%. Because all other categorical variables had lower p values than the four described above, these variables would have required much higher sample sizes to achieve a power of 80%. Therefore, if a statistically significant difference was present in the population, our study was inappropriately powered to detect it most of the time. In the best scenario, for the variable "Residence," a power of 42% means that our probability of committing Type II error was 58% (our study would not detect a significant difference if one was present 58% of the time).

3.3 DISCUSSION

"Readers of medical literature need to consider two types of validity, internal and external. Internal validity means that the study measured what it set out to; external validity is the ability to generalise from the study to the reader's patients."

-David A Grimes and Kenneth F Schulz, The Lancet, 2002

3.3.1 Interpretation of Results

3.3.1.1 Prevalence of Cord Blood Vitamin D Insufficiency and Deficiency

The estimated prevalence of cord blood vitamin D deficiency and insufficiency in the Saskatoon Health Region was lower than the prevalence reported by other Canadian studies. In Winnipeg, 36% of 50 infants had deficient cord blood vitamin D levels (deficiency defined as < 27.5 nmol/L) (Weiler, Fitzpatrick-Wong et al. 2005), in the Northwest Territories, the average newborn cord blood vitamin D level was deficient (n = 121, deficiency defined as < 50 nmol/L) (Waiters, Godel et al. 1999), and in Newfoundland, 35% and 49% of 51 infants had deficient (defined as < 50 nmol/L) and insufficient (defined as 50 – 75 nmol/L) cord blood vitamin D levels, respectively (Newhook, Sloka et al. 2009). Given the Saskatoon population's many risk factors for having low levels of vitamin D, it was expected that the prevalence of vitamin D deficiency and insufficiency in our study population would be similar to or higher than the prevalence found in other Canadian studies. Results indicated that 22% and 48% of our 65 newborns had deficient (< 50 nmol/L) and insufficient (50 – 75 nmol/L) cord blood vitamin D levels, respectively. While the prevalence of cord blood vitamin D deficiency and insufficiency in the Saskatoon Health Region was estimated to be lower than the prevalence reported in the other Canadian studies, it is possible that our study underestimated the prevalence of vitamin D

insufficiency and deficiency (see Sections 3.3.1 and 3.3.2).

Similar to many previous studies, our study found that the 400 IU per day contained in prenatal vitamins was not sufficient to produce sufficient maternal and cord blood levels of vitamin D. For example, in Vancouver, Li *et al*. found that although 80% of 336 pregnant women took a vitamin D-containing supplement containing ≥ 400 IU of vitamin D, 24% and 65% of women had deficient (< 50 nmol/L) and insufficient (< 75 nmol/L) serum levels of vitamin D, respectively (Li *et al.,* 2011). Bodnar *et al*. found that although 90% of women used prenatal vitamins in the last trimester of pregnancy, vitamin D deficiency (cord blood vitamin D levels < 37.5 nmol/L) and insufficiency (cord blood vitamin D levels 37.5 – 80 nmol/L) occurred in 45.6% and 46.8% of black neonates and 9.7% and 56.4% of white neonates (Bodnar, Simhan et al. 2007). Additionally, Dror *et al.* found that of the mothers who took a daily prenatal supplement containing 400 IU of vitamin D, 50% had a serum vitamin D concentration that was either insufficient or deficient (<75 nmol/L) (Dror, King et al. 2011). Similarly, this study showed that despite daily prenatal multivitamin use by 85% of mothers (typically containing 400 IU of vitamin D) and additional vitamin D supplements taken by 26% of mothers, 22% of newborns were deficient and 48% were insufficient in their cord blood vitamin D levels. Maternal vitamin D supplementation of 400 IU per day was not enough to prevent vitamin D insufficiency and deficiency in most newborns. This finding highlights the need for an urgent revision of maternal vitamin D supplementation guidelines in order to produce sufficient cord blood vitamin D levels in newborns.

3.3.1.2 Risk Factors for Cord Blood Vitamin D Insufficiency and Deficiency

Our study aimed to determine whether any socio-demographic, obstetric, or personal

health risk factors existed for low cord blood vitamin D levels in our study population. Each categorical variable was tested to determine whether a significant difference existed between mean cord blood vitamin D levels for each category within each variable (see Table 4). Although there was no significant difference in mean cord blood vitamin D levels between categories for each categorical variable, there was a trend towards higher levels of vitamin D in those with a rural area of residence compared with an urban area of residence ($p = 0.09$).

The finding of a trend towards higher levels of vitamin D in those with a rural area of residence may be due to higher socioeconomic status, better diet and genetic differences between Caucasian and darker-skinned ethnicities. Bedroom communities that are a short distance from Saskatoon likely have populations with a higher socioeconomic status who commute into Saskatoon for employment. Those with higher socioeconomic status are more likely to consume vitamin D-rich foods (Laitinen, Rasanen et al. 1995; Rasanen, Kronberg-Kippila et al. 2006). As well, according to the 2011 Statistics Canada census, rural towns, such as Dundurn and Rosetown, have predominantly Caucasian populations based on their reported mother tongues and languages spoken at home (Statistics Canada, 2011). While the increased absorption of vitamin D from sunlight in Caucasians would normally help to explain differences in vitamin D levels between Caucasian and non-Caucasian populations, our study was conducted during the winter months, and therefore this effect should have been negligible. Instead, genetic differences between Caucasians and darker-skinned ethnicities may aid in explaining differences in vitamin D levels, but little research exists to confirm this hypothesis.

Bivariate analysis revealed that low weight gain during pregnancy was associated with low cord blood vitamin D levels ($R = 0.274$, $p = 0.04$). It also indicated that lower maternal age, higher pre-pregnancy BMI, and urban area of residence were modestly associated with lower

cord blood vitamin D levels ($p = 0.07$, $p = 0.09$, and $p = 0.09$, respectively). The association of low vitamin D levels with urban area of residence is discussed above. High BMI is a well-established risk factor for having low levels of vitamin D (Bell, Epstein et al. 1985; Wortsman, Matsuoka et al. 2000; Arunabh, Pollack et al. 2003), as the fat-soluble vitamin becomes sequestered in the fat and is subsequently physiologically unavailable. As well, high pre-pregnancy BMI has been associated with low pregnancy and neonatal vitamin D levels in a study involving 400 mother-infant dyads (Bodnar, Catov et al. 2007). It is possible that participants with higher BMIs gained less weight during pregnancy and that this could account for the low vitamin D levels among those with low weight gain during pregnancy. This hypothesis is likely, as a bivariate analysis using pre-pregnancy BMI as a predictor for weight gain during pregnancy reveals a strong trend between the two variables given the small sample size ($n = 56$, $p = 0.07$). It is also possible that those with a low weight gain during pregnancy had an inadequate diet to produce both normal weight gain and sufficient vitamin D levels.

Low maternal age has been associated with cord blood vitamin D status in previous literature. Younger maternal age was associated with lower neonatal vitamin D status in a New Zealand study involving 929 newborns (Camargo, Ingham et al. 2010) as well as in an Australian study involving 901 newborns (Bowyer, Catling-Paull et al. 2009). One author speculated that younger mothers may be less compliant with prenatal vitamins or may have less sun exposure (Camargo, Ingham et al. 2010). It is also possible that younger mothers may have a smaller income than older mothers and may consume less expensive, less nutritious foods.

The linear regression model which best predicted cord blood vitamin D levels included only maternal age and rural area of residence. This likely differs from the bivariate analysis due to confounders which affect the relationship between the independent variables and the

dependent variable (cord blood vitamin D level).

3.3.1.3 Vitamin D Level as a Predictor for Obstetric and Neonatal Outcomes

Cord blood vitamin D level did not significantly predict any obstetric and neonatal outcomes. There is little literature that examines the role of cord blood vitamin D levels in the prediction of infant health outcomes, but a substantial body of literature supports the role of maternal vitamin D intake in predicting birth weight and length (Sabour 2006; Scholl and Chen 2009; Leffelaar, Vrijkotte et al. 2010). It is possible that with a larger sample size, an association between cord blood vitamin D levels and obstetric or neonatal outcomes may become apparent. It is also possible that "normal" vitamin D levels for children differ substantially from "normal" vitamin D levels for adults. Although some research conducted in children and adolescents indicates that normal levels of vitamin D for children may be the same as they are for adults (Guillemant, Taupin et al. 1999; Cheng, Tylavsky et al. 2003; Abrams, Griffin et al. 2005), little or no research has been performed in neonates, infants or small children. In fact, one author argues that because cord blood vitamin D samples typically contain 50-60% of maternal vitamin D concentration, "normal" cord blood vitamin D concentration may be >40 nmol/L (Roth 2007). Further research on the clinical implications of cord blood vitamin D deficiency or insufficiency must be conducted before age-specific reference ranges are established.

3.3.2 Assessment of External Validity

The assessment of external validity will compare the study population with the general population and comment on the presence and effect of selection bias.

3.3.2.1 Comparison of Study Population with General Population

The median income of the population under investigation is similar to the median income of Saskatoon's general population. In 2010, the median family income in Saskatoon was $80,570 (Statistics Canada 2009). In this study, 32% of participants reported a household income of greater than $100,000, 35% reported a household income of $50,001 - $100,000, and 20% reported a household income of less than $50,000. Therefore, median income in the study population was in the range of $50,001 - $100,000, which is similar to the median family income of $80,750 reported for the general population.

The study population is also similar to the Saskatoon Health Region population in terms of ethnicity, area of residence, and maternal age at delivery. The most recent census data for Saskatoon indicated that 17.2% of Saskatoon's population had some non-white ethnicity (Statistics Canada 2006), 73% of the Saskatoon Health Region population lived in the city of Saskatoon (Neudorf 2009), and the highest fertility rates for women in the Saskatoon Health Region were in the age groups of 25-29 and 30-34, respectively (Neudorf 2009). In comparison, 26% of our study participants indicated that their newborn had some non-white ethnicity, 68% indicated an urban area of residence, and our study population had a mean maternal age of 29.6 ± 4.4 years. Therefore, for a small sample size, the incomes, ethnicities, area of residence and mean maternal age at delivery of the study population closely matched those of the general population in the Saskatoon Health Region.

Differences between the general population and the study population were most prominent in maternal educational level and marital status. In 2006, 46% of adult women living in Saskatchewan had some form of post-secondary education and 55% had elementary or high school education (Saskatchewan Ministry of Advanced Employment and Labour 2009). In this

study, 85% of women reported some post-secondary education, while only 12% reported elementary or high school education. An analysis of data collected from 5,643 mothers between 1992 and 1994 indicated that 27.3% of pregnant women had never been married, while 70.5% were currently married (Jackson 2003). In our study, 94% of participants indicated their marital status as either "married" or "common-law." These differences in educational and marital status between study and general populations may indicate either a response bias or a genuine difference between populations. In any case, both education level and marital status may be regarded as socioeconomic indicators, and low socioeconomic status is a risk factor for having a low level of vitamin D (Laitinen, Rasanen et al. 1995; Rasanen, Kronberg-Kippila et al. 2006). Our study population indicated that they were more educated and more likely to be married than the general population. If our study population has a higher socioeconomic status than the general population, we may have underestimated the prevalence of vitamin D deficiency and insufficiency in the newborn population of the Saskatoon Health Region.

3.3.2.2 Sampling Bias

Sampling bias is a type of selection bias which causes some members of the population to be less likely to be included than others due to a non-random selection of participants. This type of bias refers to a skewing of the results due to the inclusion of study population that is not reflective of the general population.

The study design may have favoured a healthier population. Potential participants were recruited at prenatal tours as well as on the Labour and Delivery ward at Royal University Hospital. While the population present at prenatal tours (and the population who receives any prenatal care) tends to be a healthier, higher income, and more educated sub-section of the

population, it was hoped that recruitment on Labour and Delivery would allow for the inclusion of more marginalized sections of the population. Although the women who chose to participate in the study were more educated and more likely to be married than the general population, they were similar to the general population in terms of income, ethnicity, area of residence, and maternal age at delivery (see Section 3.3.2 Assessment of External Validity). Women who are more educated may be more likely to consume nutrient-rich foods, and this may skew results in favour of an underestimation of low levels of vitamin D in the general population. However, due to the voluntary nature of participation in this study, this bias could not be controlled for.

The study design specifically excluded individuals with medical conditions or drugs that might affect calcium or vitamin D levels. The exclusion criteria of the study ensured that all mothers with thyroid or parathyroid problems, problems with calcium regulation, or those who took medications that would interact with calcium regulation were excluded. Therefore, in the absence of any undiagnosed conditions that may interfere with calcium or vitamin D regulation, the study population did not have any medical conditions that would have skewed the results. However, the general population has a small percentage of people with calcium regulation problems who were not represented in our study. This bias is likely irrelevant in a population with a small sample size.

3.3.3 Assessment of Internal Validity

"Bias undermines the internal validity of research" (Grimes 2002). Research bias refers to deviation of the research results from the truth. Many types of bias in study design, implementation and interpretation exist. The following type of bias are often present in observational studies: selection bias, information bias, and confounding (Grimes 2002). In this

study, a single group was enrolled, the prevalence of an outcome (low cord blood vitamin D levels) was estimated, and associations between that outcome and several independent variables were examined. The following section will therefore explore selection bias, information bias and confounding in the context of this study.

3.3.3.1 Information Bias

This type of bias results from the incorrect determination of exposure or outcome, or both (Grimes 2002).

Missing maternal demographic data ranged between 0 – 12% for each variable measured by the Participant Questionnaire (see Table 2), and this may have skewed the outcomes measured in the study. A number of participants chose not to answer questions concerning their age, marital status, education level, household income and ethnicity, among other questions. As well, some information, such as weight gain during pregnancy, was not always present in the medical records. The Participant Questionnaire stated clearly that participants did not have to answer any questions that they feel uncomfortable answering, and this may have been one reason that participants chose not to answer certain questions. It is also possible that participants felt a lack of anonymity with their responses, as the questionnaires were collected from them personally by the researcher (despite no names being present on the questionnaire itself). This type of bias could potentially be avoided in the future by providing participants with a postage-paid, addressed envelope so that questionnaires could be mailed in anonymously (identified only by participant number). Although the missing data may have skewed the results towards more socially acceptable responses, this is unlikely given the small amount of missing data for each variable.

The demographic information of the included participants (n = 65) did not differ significantly from the demographic information of the excluded participants (n = 19) (see Tables 2 and 3). Therefore, the exclusion of those participants from the analysis likely did not bias the data. As well, because samples were not collected from 19 participants, these participants were not followed post-partum and no information is available about their obstetric or neonatal outcomes. It is therefore impossible to compare the included to the excluded groups in terms of obstetric and neonatal outcomes. However, given their demographic similarity, the missing data likely did not significantly skew the available data. Future studies could avoid this problem through a study design that improved communication with labour and delivery staff so that samples were not missed.

Information about the mother's occupation was collected but not analyzed. The Statistics Canada categories for occupation did not include "Homemaker" and it could not be discerned whether participants had previously been or were currently working outside the home. As well, many participants chose several categories or indicated "Other," making it very difficult to analyze these results. This could be avoided by asking, "Do you work outside the home?" (Yes/No) prior to asking the participants to choose the single Statistics Canada category that best describes their occupation. Because occupation is used as an indicator of socioeconomic status (and low socioeconomic status is a risk factor for low levels of vitamin D), it is possible that an association would have been present between employment status and cord blood vitamin D levels. However, income and education level, other indicators of socioeconomic status, were analyzed and were not significantly associated with cord blood vitamin D levels.

3.3.3.1.1 Accuracy of Collected Data: The Participant Questionnaire was answered directly by participants and asked participants to answer only questions they felt comfortable

answering. This may have led to some non-response bias. As well, some questions were difficult to answer. For example, "Maximum Education Level" was listed in 6 categories: "elementary," "high school," "some university," "technical training beyond high school," "one or more university degrees," or "other (list)." These categories were loosely based on the Statistics Canada 2006 Census. However, those who chose "other" were left without an analyzable category. Also, the term "technical training beyond high school" makes any non-university training, such as a Basic Life Support course, equivalent to a college degree. In this study, due to the small sample size, Education Level was reduced to "Elementary or high school," and "Some post-secondary education." Future studies would do better to use the categories "elementary," "high school," "training other than college or university," "college degree," and "university degree."

The Questionnaire asked whether or not participants took an "additional vitamin D supplement" but did not ask about prenatal supplements. Due to this lack of clarity, the researcher asked each participant directly about her prenatal supplement use and vitamin D intake in order to quantify the amount of vitamin D taken. This could be avoided through the use of two separate questions stating, "Did you take a daily prenatal supplement?" and "Did you take an additional vitamin D supplement?"

The data collected for ethnicity was difficult to interpret. Participants were instructed to select all ethnicities that applied to their newborns (ethnic groups listed according to the Statistics Canada 2006 Census). Several ethnicities were often selected, and this makes ethnicities present several generations ago equivalent to ethnicities present within one generation. For the purposes of analysis, this data was reduced to "No non-white ethnicity," and "Any non-white ethnicity" based on the ethnicities listed. Non-white ethnicities included East

and Southeast Asian, Aboriginal, Chinese, South Asian, American Indian, East Indian, Caribbean or Arab origins (Statistics Canada 2006). The question could be clarified by asking participants if they or their partners would identify themselves as visible minorities, as the purpose of the question was to search for an association between darker skin tones and lower vitamin D levels.

As previously mentioned, the Statistics Canada categories for maternal occupation were difficult to use. It would have been useful to include the question, "Do you work outside the home?" as there was no option for "Homemaker." The question that asked participants to list any infections or illnesses during pregnancy could have been clarified by asking participants to list only infections or illnesses which required antibiotics or other medical treatment. Finally, the term "household income" was unclear because it did not specify gross or net income.

Although the Participant Questionnaire could have been clearer, the collection of data from participants was standardized. All participants were asked to fill out the Participant Questionnaire and asked directly about their prenatal supplement use and vitamin D intake by the researcher. After delivery, participant and newborn medical charts were consulted to obtain all obstetric and newborn outcome information. Although different health care providers may have completed different charts, this information was likely unbiased as the physicians caring for the participants had no knowledge or consideration of the study.

Finally, the dependent variable, vitamin D, was objectively measured by a lab technician who had no knowledge of participants as samples were identified by participant number only. Levels were determined by enzyme immunoassay (Immunodiagnostic Systems Ltd.) which were run in accordance with the manufacturer's specifications. Vitamin **D** **E**xternal **Q**uality **A**ssessment **S**cheme (DEQAS; www.deqas.org) samples were run in parallel with subject

samples.

In conclusion, although the socio-demographic information could have been collected through clearer questions on the Participant Questionnaire, the questions were identical for all participants. There may have been some non-response bias which could not be controlled for. Vitamin D intake was the only question assessed directly by the researcher. The obstetric and neonatal outcome information derived from medical charts was likely unbiased and the cord blood vitamin D levels were assessed objectively.

3.3.3.2 Confounding

Confounding occurs when a third factor interferes with a relationship between an independent variable and the outcome variable. A confounding factor is associated with the independent variable and effects the outcome variable (Grimes 2002). These relationships should always be assessed when critically appraising research. By completing an adjusted analysis, we attempted to control for potential confounders.

All variables included in the full model but not initially in the reduced model were added individually to the Reduced Model (see Table 6) to test for confounding and because no variables significantly changed the β values of the variables in the Reduced Model, it was concluded that no confounders were present in the data set. However, the small sample size may have masked confounding that would have been significant with a larger data set. As well, variables outside the data set may have been confounders that were not controlled for.

For example, diet and sun exposure were not measured in the study population and may have acted as confounders. Future studies may rectify this by taking detailed diet histories or asking participants to recollect how much time was spent outdoors. Given that the study was

conducted over the winter months, sun exposure was likely minimal for all participants. Diet histories, however, may have been a worthwhile endeavour.

In this study, each categorical variable creates several groups of participants being studied, and these groups cannot be compared to each other without accounting for confounding variables. For example, those with income < $50,000 may be have differences from those with incomes $50,000 - $100,000 and > $100,000 other than income. They may have lower education levels, take fewer prenatal supplements or vitamin D supplements, have an increased risk of illness during pregnancy or have different ethnicities than those with higher incomes. Unless those variables are accounted for, any association between vitamin D level and income is assumed to be directly related to income. Therefore, bivariate analysis may be misleading, as it attributes causation to only a single variable. Multivariate model building controls for all potential confounders included in the model and decreases the risk of selection bias.

In this study, although bivariate analysis was used in the initial testing of variables, a multivariate model was later used which controlled for many confounding variables. However, other differences between groups within each variable, such as better response rates, cannot be measured, as it is impossible to know whether participants with missing data belonged to one group or another. This response bias could not be controlled for.

3.3.3.3 Sample Size and Statistical Power

Statistical power is the probability of rejecting the null hypothesis when it is false (the probability of not making a Type II error). Power is influenced by the sample size, the effect size and the levels of error we are willing to accept (significance criterion). Effect size refers to the detectable difference in the outcome variable between groups (a higher-powered study will

detect smaller differences in the outcome variable). Given that the significance criterion commonly used by the scientific community is 0.05, there is only a 5% chance that any study using this criterion will find a false positive result (Type I, or α, error). Power may be increased (and Type II error decreased) by increasing the significance criterion (for example, to $p = 0.10$), but this increases the risk of Type I error. The accepted balance between Type I and Type II errors is to use a significance criterion of 0.05.

Most researchers consider a power of 80% adequate, as this achieves a 4-to-1 trade-off between Type II (β) and Type I (α) error ($\beta = 0.20$, $\alpha = 0.05$) (Hulley, Cummings et al. 2007). However, the choice of power level and alpha depends on the type of study, as studies reporting adverse events require the highest possible power to avoid Type II error, while studies reporting the success of a treatment should minimize alpha and Type I error. While a significance criterion of $\alpha = 0.05$ was used for this study, the post-hoc analysis of statistical power showed that a power of 80% was not achieved for any categorical variable due to small sample size.

A conservative post-hoc analysis uses categorical rather than continuous variables to estimate power, as categorical variables generally have lower power than continuous variables (Hulley, Cummings et al. 2007). Our power analysis (Section 3.2.4) estimated the power achieved for all categorical variables with a $p < 0.25$ and indicated that our probability of committing Type II error was very high (58% for the highest-powered categorical variable) due to our small sample size. Therefore, a larger sample size may be necessary to rule out the role of chance in this study. Despite this, the effect sizes observed with the aforementioned variables, other than "parity", in this study were all greater than 0.45 indicating a moderate effect size.

Lower power may also indicate that any difference detected by our study may be very likely to be present in the general population (our study only detects large differences).

However, given the number of comparisons made during bivariate analysis, it is equally likely that those findings of significance are due to chance. For example, if 20 independent variables are tested at an $\alpha = 0.05$, there is a very high probability of making a Type 1 error (64%, or $1 - 0.95^{20}$) (Hulley, Cummings et al. 2007). While an adjustment for multiple comparisons would control for this error, the Bonferroni approach, which divides the significance level by the number of hypotheses tested, is usually too stringent. The Bayesian approach asserts that if each individual variable has a reasonable probability of being correct based on previous research, no adjustment for multiple comparisons is necessary (Hulley, Cummings et al. 2007). In this study, each variable was included based on the results of previous research and physiological plausibility. This study was also exploratory in nature. Therefore no adjustment for multiple comparisons was made.

3.3.4 Other Strengths and Limitations

Due to the lack of data about the risk factors and neonatal outcomes associated with cord blood vitamin D levels, this study was exploratory in its intent. This study assessed sixteen potential socio-demographic and personal health risk factors and eight potential obstetric and neonatal outcomes for association with cord blood vitamin D level. In the assessment for risk factors, there was no correction for multiple comparisons because the bivariate analysis was only used as a basis for multivariate model building. All variables with a $p < 0.25$ in bivariate analysis were included in a Full Model, and all variables from the Full Model with a $p < 0.10$ were included in the Reduced Model. Due to the small sample size ($n = 41$ due to missing data) and the small number of variables in the Full and Reduced Models (six and two variables, respectively), no adjustment for multiple comparisons was made. Furthermore, none of the

variables included in the full model that were excluded from the reduced model affected the β estimate of those variables included in the reduced model. Therefore, no confounding was found to exist.

No neonatal outcomes had statistically significant associations with vitamin D levels during the bivariate analysis and therefore no model building was pursued.

The small sample size of this study indicates that the study has little power, or little probability of not making Type II error (falsely accepting the null hypothesis). A study with little power has limited ability to detect associations that are present. However, this also means that any statistical significance detected denotes a stronger association between the independent and dependant variables. Therefore, the association of younger maternal age and urban area of residence with low cord blood vitamin D levels is likely to be strong in this population. A larger sample size may be necessary to confirm or refute this finding as well as ascertain whether further risk factors are associated with low cord blood levels of vitamin D.

The largest strength of this study was that the data is likely to be both reliable (precise and reproducible) and valid (reflective of the general population). Obstetric and neonatal outcomes as well as cord blood vitamin D levels were measured objectively. Although the measurement of socio-demographic factors was subject to non-response bias, the measurement was standardized for all participants. The prevalence of cord blood vitamin D deficiency and insufficiency in the study population is likely to be either reflective or an underestimation of that in the general population. Although a larger sample size would be necessary to help rule out the role of chance to determine whether any risk factors or neonatal outcomes are associated with cord blood vitamin D levels, this small study's negative results are likely to be reproducible.

3.3.5 Conclusions and Future Research Directions

Adequate vitamin D supplementation during pregnancy is likely a factor important to ensuring optimal health outcomes for offspring. Despite a compelling body of research showing that vitamin D deficiency is prevalent among both mothers and infants in Canada and worldwide (see Sections 2.2.2 and 2.3.1), the problem of widespread vitamin D deficiency remains insufficiently addressed. Researchers are only now beginning to appreciate the potential clinical and economic implications of *in utero* vitamin D deficiency; studies have linked maternal vitamin D levels with chronic diseases such as asthma (Devereux, Litonjua et al. 2007; Erkkola, Kaila et al. 2009) and type 1 diabetes (Krishnaveni, Veena et al. 2011) in the offspring. Vitamin D's associations with infection, autoimmune disease, cancer, and bone health are better understood, and low vitamin D status throughout life is associated with adverse health outcomes. In fact, one study estimated that optimizing vitamin D levels in Canadians could reduce mortality by 37,000 annually (a 16.1% reduction) and reduce the annual economic burden by $14.4 billion (6.9%) (Grant, Schwalfenberg et al. 2010). Given the health and economic benefits afforded to the very inexpensive supplement, the prevention of low levels of vitamin D should be a priority for the medical and public health fields as well as the general public. Sufficient vitamin D status should begin *in utero* and be maintained throughout life.

Our research estimates that the prevalence of vitamin D deficiency and insufficiency among newborns in the Saskatoon Health Region is 22% and 48%, respectively. The population's many risk factors for low vitamin D levels, including its northern latitude, lack of sun exposure during the winter months, high Aboriginal population and high prevalence of obesity may aid in explaining these results. However, similar results have been found in warmer climates that lack some or all of these risk factors (Bowyer, Catling-Paull et al. 2009; Dror, King et al. 2011), which presents a stronger case for the role of maternal diet and supplementation in

preventing low newborn levels of vitamin D. The provision of adequate maternal vitamin D supplementation may represent a tremendous opportunity for health and economic gain in Saskatoon and around the world.

A lack of research that demonstrates safe and effective vitamin D dosages during pregnancy has impeded consensus on maternal vitamin D supplementation guidelines. Health Canada, the Endocrine Society, and the Canadian Pediatric Society have different recommendations for vitamin D supplementation during pregnancy (see Section 2.2.1). Further randomized-controlled trials of vitamin D supplementation in pregnancy may be necessary prior to any consensus being reached.

A review of recent literature as well as the results of this study suggest that 400 IU of vitamin D per day is insufficient to prevent vitamin D deficiency and insufficiency in mothers and newborns (Bodnar, Simhan et al. 2007; Ward, Gaboury et al. 2007; Dror 2011; Li 2011). A recent randomized controlled trial demonstrated the safety and efficacy of 4,000 IU of vitamin D per day during pregnancy (Hollis, Johnson et al. 2011), and another study showed that doses of up to 10,000 IU per day did not elevate vitamin D levels into the toxic range (Hollis and Wagner 2004). Although the Endocrine Society recommends a dose of 600 IU of vitamin D per day for pregnant and lactating women, it also recognizes that doses of up to 1500 – 2000 IU per day may be necessary to maintain a serum vitamin D level of greater than 75 nmol/L (Holick, Binkley et al. 2011). The Canadian Pediatric Society's recommendation of 2000 IU per day for pregnant or lactating women (Canadian Pediatric Society 2007) is based on a recent literature review and should perhaps be adopted by other agencies. However, further research is needed to confirm this recommendation or to aid the creation of new guidelines for maternal vitamin D supplementation.

Maternal vitamin D supplementation guidelines may need to be stratified according to risk factors. Skin colour, BMI, sun exposure and a lack of dietary vitamin D consumption are all well-recognized risk factors for having low levels of vitamin D. As well, genotype may play a role in modulating vitamin D levels and requirements. Mothers with darker skin, mutations in vitamin D-specific proteins, and other risk factors for having a low level of vitamin D may require higher levels of supplementation than mothers without risk factors. Our research indicates that young, urban mothers in the Saskatoon Health Region are at the highest risk of having infants with a low cord blood vitamin D level, but further research is necessary to confirm or refute this observation.

The role of vitamin D in fetal and neonatal life has yet to be elucidated. Our research found no association between low neonatal vitamin D levels and obstetric or neonatal outcomes. It is possible that this lack of association is because age-specific reference ranges for vitamin D levels have yet to be determined. "Normal" vitamin D levels for neonates may be a fraction of "normal" levels for adults. It is also possible that low fetal levels of vitamin D do not manifest clinically until later in life, or that a larger study may discover associations between vitamin D status and neonatal outcomes. Research that establishes the clinical relevance of low cord blood vitamin D levels will shed light on both age-specific reference ranges for vitamin D levels and our general understanding of vitamin D in physiology.

In conclusion, this study highlights a growing awareness that vitamin D deficiency and insufficiency is a prevalent problem with a relatively simple solution. Low newborn vitamin D levels are only the proverbial "tip of the iceberg" as vitamin D deficiency and insufficiency are pervasive throughout life. Healthy diets, sun exposure and vitamin D supplementation may be the "wonder-drugs" of the future. Multidisciplinary teams including nutritionists, dieticians,

nurses, physicians, and teachers will be needed to effect change in both supplementation guidelines and public attitudes. Setting up a newborn for a healthy future begins at conception, but we must collaborate to maintain health throughout infancy, childhood and beyond.

6. REFERENCES

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APPENDIX A:

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE
Association of Newborn Vitamin D Status with Pregnancy Outcome and Infant Health

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INTRODUCTION

You are invited to take part in this research study because you will be delivering your baby prior to May 2012.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you will not lose the benefit of any medical care to which you are entitled or are presently receiving.

Please take time to read the following information carefully. You can ask the researcher to explain any words or information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

WHO IS CONDUCTING THE STUDY?

This study is being conducted by researchers at the University of Saskatchewan, Dr. Alan Rosenberg, a pediatrician, Dr. Susan Whiting, a nutritionist, and Dr. Miriam Katzman, a graduate student.

WHY IS THIS STUDY BEING DONE?

We are studying vitamin D levels in newborns to help us understand how common low vitamin D levels are, why some newborns are at higher risk of having low vitamin D levels, and what effect a low vitamin D level has on a newborn's health.

Saskatchewan newborns might be at risk of having low vitamin D levels. During pregnancy, you could have low Vitamin D levels because the ultraviolet B rays from the sunlight during Saskatchewan's winter months are not intense enough to activate Vitamin D in your skin. Diet and genetics can also affect Vitamin D levels in you and your newborn baby. We don't know how many Saskatchewan newborns have low vitamin D levels or what a low level would mean for your baby, but normal vitamin D levels may be important for ensuring the health of your baby. The results of this research may help prevent low vitamin D levels and any health risks associated with them.

In this study we aim to answer the following questions:

1. How common are low vitamin D levels in newborns delivered in the Saskatoon Health Region?
2. Why are some newborns at a higher risk for having a low vitamin D level than others?
3. What effect does a low vitamin D level have on a newborn's health?

WHO CAN PARTICIPATE IN THE STUDY?

You are eligible to participate in this study if:

- You are 18 years of age or older
- You will be delivering your baby at the Royal University Hospital prior to May, 2012
- You have a single pregnancy (not twins or triplets)

You may not participate if:

- You have thyroid or parathyroid problems
- You have problems with calcium regulation
- You take any medications that interfere with calcium regulation (such as diuretics or calcium channel blockers);
- If you are unsure of whether your medication is a diuretic or calcium channel blocker, please ask!

WHAT DOES THE STUDY INVOLVE?

Your participation in this study will take approximately 20 minutes of your time.

If you decide to participate, you will be asked to:

- Fill out a short questionnaire. You do not have to respond to any questions that you are uncomfortable answering.
- Allow the researchers to access your and your newborn's health records to get information about this pregnancy and delivery
- Allow the researchers to measure your baby's skin tone using a device that is placed for several seconds on the baby's arm.
- Allow the researchers to take a sample of your newborn's cord blood*

*Cord blood is blood taken from the leftover umbilical cord after it is cut. Cord blood samples are normally taken by hospital staff. We would like to use a sample of cord blood to determine your newborn's vitamin D level.

Your medical information will be protected: You will assigned a Project ID number and all of the medical information collected from the questionnaire, health records and cord blood sample will be entered into an electronic database under this number. Only members of the research team will have access to information linking your identity with your project ID number. Your identity will be protected in all published material.

The information collected would include:

Information collected prior to delivery:

- Age

- Marital status
- The first three characters of your postal code
- Ethnic group
- Education level
- Occupation
- Household income
- Parity (number of pregnancies you have had)
- Quantity and frequency of vitamin D supplementation you have taken during your pregnancy
- Height and weight before you became pregnant
- Medical conditions you had prior to becoming pregnant

Information collected after delivery:

- Medical problems during pregnancy
- Weight gain during pregnancy
- Gestation (if your baby was born at the expected time)
- Method of delivery
- Complications of delivery
- Infant length, weight and head circumference
- Apgar score (a measure of newborn health)
- Infant medical problems
- Infant skin reflectance (skin tone)
- Infant vitamin D level

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

There may be no direct benefits to you. We expect that the information gained from this study will help provide advice to women and health care providers about vitamin D supplementation during pregnancy. We will also gain a better understanding of whether vitamin D has a role in ensuring the health of newborn babies.

ARE THERE POSSIBLE RISKS AND DISCOMFORTS?

There are no risks to participating in this study.

WHAT HAPPENS IF I DECIDE TO WITHDRAW?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. Your medical care will not be affected. Please contact the researchers via phone to withdraw.

If you choose to enter the study and then decide to withdraw later, all data collected about you during your enrolment will be retained for analysis.

WILL I BE INFORMED OF THE RESULTS OF THE STUDY?

If you would like to be informed of the results of the vitamin D level that is obtained on your baby's cord blood as part of this research study and if you would like us to inform your child's primary care physician (such as your child's family doctor or pediatrician) of the results, you may indicate this in the "Consent to Participate" section at the end of this document.

The results of the study will be available by January, 2013 on www.newbornvitamind.com. As well, the results will be published in a Master's of Health Science thesis paper.

WILL I BE REIMBURSED FOR PARTICIPATING?

You will not be charged for any research-related procedures. You will not be paid for participating in this study. You will not receive any compensation, or financial benefits for being in this study, or as a result of data obtained from research conducted under this study.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

In Saskatchewan, the Health Information Protection Act (HIPA) protects the privacy of your personal health information.

Your 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 and the University of Saskatchewan Research Ethics Board 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. The results of this study may be presented in a scientific meeting or published, but your identity will not be disclosed.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

If you have any questions or desire further information about this study before or during participation, you can refer to www.newbornvitamind.com or contact study personnel at info@newbornvitamind.com or the Vitamin D Study Office at 306-966-7608.

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at

306-966-2975. The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

CONSENT TO PARTICIPATE

Study Title: Association of Newborn Vitamin D Status with Pregnancy Outcome and Infant Health

- I have read (or someone has read to me) the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I understand that I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships.
- I give permission to the use and disclosure of my de-identified information collected for the research purposes described in this form.
- I understand that by signing this document I do not waive any of my legal rights.
- I understand I will be given a signed copy of this consent.

I would like the researchers to contact me with the results of my child's cord blood vitamin D level:

□ Yes

□ No

If you answered "Yes":

Please provide your name and address:

Name:_____

Address: _____

I would like the researchers to contact my child's primary care physician with the results of my child's cord blood vitamin D level:

□ Yes

□ No

If you answered "Yes":
Please provide your child's physician's name and address:

Physician's Name:_____

Physician's Address: _____

I agree to be contacted for possible future studies related to vitamin D in infants and children:

□ Yes

□ No

I agree to participate in this study:

_____

Printed name of participant: Signature Date

_____

Printed name of person obtaining consent: Signature Date

UNIVERSITY OF SASKATCHEWAN

Vitamin D Newborn Vitamin D Study

Study Questionnaire for Participants

Date of Enrolment:

Project ID number:

You do not have to answer any questions that you feel uncomfortable answering

Age:

Marital Status:

- □ Married
- □ Single
- □ Divorced
- □ Separated
- □ Common-law
- □ Widowed

Forward Sortation Area (first 3 characters of postal code):

Maximum Education level:

- □ Elementary
- □ High School
- □ Some university
- □ Technical training beyond high school
- □ One or more university degrees
- □ Other (specify) _____

Household income:

- □ Less than $10,000
- □ Between $10,000 and $25,000
- □ Between $25,001 and $50,000
- □ Between $50,001 and $100,000
- □ More than $100,000

Number of previous births (not including current):

Height (ft/inch):

Pre-pregnancy weight (lb):

Do you take additional vitamin D supplements? If yes:

How much vitamin D do you take?:
How many times per day?:
How long have you been taking vitamin D supplements?:

Ethnic group:

Low levels of vitamin D might be more common in newborns of certain ethnic groups. Please place an 'X' in the box or boxes that will best describe your newborn's origin/descent.

□ British Isles origins	**□ Other North American origins**	**□ Canadian**	**□ European origins**	**□ English**	**□ French origins**
□ French	**□ Scottish**	**□ Western European origins**	**□ Irish**	**□ German**	**□ Eastern European origins**
□ Southern European origins	**□ East and Southeast Asian origins**	**□ Aboriginal origins**	**□ Italian**	**□ Chinese**	**□ South Asian origins**
□ American Indian	**□ Ukrainian**	**□ Northern European origins**	**□ Dutch (Netherlands)**	**□Scandinavian origins**	**□ Polish**
□ East Indian	**□ Caribbean origins**	**□ Russian**	**□ Arab origins**	**□ Welsh**	

Mother's Occupation:

□ Management Occupations
□ Business, Finance and Administrative Occupations
□ Natural and Applied Sciences and Related Occupations
□ Health Occupations
□ Occupations in Social Science, Education, Government Service and Religion
□ Occupations in Art, Culture, Recreation and Sport
□ Sales and Service Occupations
□ Trades, Transport and Equipment Operators and Related Occupations
□ Occupations Unique to Primary Industry
□ Occupations Unique to Processing, Manufacturing and Utilities
□ Student
□ Other _____

Please list any pre-pregnancy medical conditions:

Have you had any infections or illnesses during your pregnancy?

□ Yes
□ No

If yes, please specify and indicate how many months pregnant at the time:

_____

Recruitment Materials

Thank you very much for your interest in the Newborn Vitamin D Study. I am enclosing copies of the information sheet, questionnaire and consent form for your review. These will provide you with detailed information about the study purpose and procedures. Letters for your doctor and delivery nurse have also been included.

You will have time to consider the information and may contact our research staff with any questions or to participate in the study.

Congratulations and best wishes with the new member of your family!

Sincerely,

Miriam Katzman, MD
Newborn Vitamin D Study Research Team
Vitamin D Study Office: (306) 966-7608
Email: info@newbornvitamind.com
www.newbornvitamind.com

The Newborn Vitamin D Study:

Information for pregnant women interested in participating

What is the purpose of this research?

Our study aims to answer three questions:

1. How common are low vitamin D levels in newborns delivered in the Saskatoon Health Region?
2. Why are some newborns at a higher risk for having a low vitamin D level than others?
3. What effect does a low vitamin D level have on a newborn's health?

Why is this study important?

This study will help to determine how frequently low vitamin D levels are found in newborns and if there is an association between vitamin D levels at birth and the health of the baby in the newborn period.

Who is performing this study?

This study is being conducted by researchers at the University of Saskatchewan, Dr. Alan Rosenberg and Dr. Susan Whiting and a graduate student, Dr. Miriam Katzman.

Who can participate in this study?

You are eligible to participate in this study if you are over 18 years old, have a single pregnancy (not twins or triplets) and will be delivering at the Royal University Hospital prior to May, 2012.

What will the people participating in the study be asked to do?

Participating takes about 20 minutes of your time. If you decide to participate in the study, you will be asked to fill out a short questionnaire, allow the researchers to access your and your newborn's health records that relate to your pregnancy, delivery and health of your baby at birth, allow the researchers to measure your baby's skin tone, and allow the researchers to take a sample of your newborn's cord blood.

Who can I contact for more information about this study?

If you have any questions or are interested in participating, please contact:
Dr. Miriam Katzman, Graduate Student, or Shauna Richards, Study Nurse
Email: info@newbornvitamind.com Website: www.newbornvitamind.com Phone: 306-966-7608

Dear Nurse,

Your patient has consented to participate in the **Newborn Vitamin D Study**. We would very much appreciate it if you would collect a 3cc sample of venous cord blood in the 3mL vacutainer tubes intended for this study and store the sample in the study fridge behind the G wing desk in Labour and Delivery. Please contact myself, the study administrator, Miriam Katzman, at 716-1937, once this participant has delivered her newborn. As a thank-you for your participation, you may enter your name into a monthly draw to win a Starbucks card. The draw box is located next to the study fridge.

Thank you very much for your help,

Miriam Katzman, MD
Graduate Student, Newborn Vitamin D Research Team
Email: info@newbornvitamind.com

UNIVERSITY OF SASKATCHEWAN

Dr. Alan M. Rosenberg

Department of Pediatrics

Royal University Hospital

103 Hospital Drive

Date

Dear Dr.

Re: Patient Name

DOB: **PHN:**

Address:

Parent Name:

Phone Number:

Newborn Vitamin D Study Participant (Study ID#)

Your patient, identified above, is a participant in a research study titled**:** ***Association of Newborn Vitamin D Status with Pregnancy Outcome and Infant Health.***
The purpose of this study is to determine vitamin D status in a population of newborns, to identify factors that influence vitamin D levels, and to determine whether a low vitamin D status is associated with any adverse neonatal outcomes. In Saskatchewan, and other northern latitude locations, vitamin D deficiency is an increasing concern.

This letter is to inform you that we have received permission from your patient to participate in the study. If she wishes, the results of the vitamin D test will be mailed to you.

Further information about this study can be found on the study's website (www.newbornvitamind.com*)*.

Please do not hesitate to contact me if you have any questions or suggestions relating to this research.

Yours truly,

Alan M. Rosenberg M.D.

Professor of Pediatrics
Royal University Hospital

APPENDIX B: Newborn Vitamin D Study: Cord Blood 25(OH)D Levels

Table B.1. Cord blood 25(OH)D levels (nmol/L)

17.46	65.28
21.53	66.36
27.63	67.11
30.65	67.29
33.14	67.37
33.73	67.47
37.34	70.36
37.54	71.62
44.63	72.54
44.80	73.38
45.54	74.13
45.92	74.85
47.99	75.05
48.62	76.13
50.16	77.01
50.23	77.42
50.23	79.67
51.68	80.29
51.89	80.42
53.94	80.62
54.23	80.73
55.15	81.03
56.10	81.65
58.55	85.70
58.66	88.97
60.55	89.49
60.56	92.50
61.12	95.12
61.49	95.85
61.76	98.59
62.33	104.01
62.51	106.29
64.21	