Validity of a screening survey tool to identify neonates at high risk for vitamin D deficiency

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Abstract

Many infants in Canada are born with low vitamin D stores, putting them at risk of developing complications such as rickets, if untreated. Newborns are not routinely screened for vitamin D status. Identifying those at high risk may facilitate targeted education to parents regarding supplementation for infants. However, a screening tool is not available. The objective was to develop and test the validity of a screening survey tool to identify term-born neonates at high risk for vitamin D deficiency (25-hydroxyvitamin D (25(OH)D) < 30 nmol/L). Healthy mother-infant pairs (n=1112) were recruited at the Lakeshore General Hospital, Montreal, from March 2016 to March 2019. Parental demographic and lifestyle factors were surveyed. Newborn blood samples, collected < 36 h after birth, were tested for serum 25(OH)D (Liaison, Diasorin Inc.). Content validity was based on 21 known risk factors. Logistic regression models were used to identify key variables associated with risk of low neonatal vitamin D concentrations. Receiver operating characteristic (ROC) curves were used to demonstrate sensitivity and specificity of the screening tool against known vitamin D status. Mothers (age 32.4 ± 4.5 y) were mostly white (58%) and 57% had a pre-pregnancy body mass index (BMI) in the healthy range (18.5-24.9 kg/m^2). Mean neonatal serum 25(OH)D concentration was 44.5 ± 19.9 nmol/L, with 23% (95% CI 0.20, 0.25) below 30 nmol/L. Six out of the 21 existing risk factors assessed were most predictive of neonatal low vitamin D status ($P < 0.05$). These risk factors (maternal age at delivery < 26.0 y, pre-pregnancy BMI < 18.5 or ≥ 25 kg/m^2, non-white skin color, prenatal supplement intake < 2-3 times/wk before or during pregnancy and delivery in October through April) are easily assessable from hospital charts. Regression coefficients for each risk factor were transformed into integer scores. The average of the total scores was taken as the cut-off score for neonates at high risk for vitamin D deficiency. The screening tool had a sensitivity of 68.8% and

specificity of 70.0%. Area under the ROC curve was 0.712 (95% CI 0.679, 0.745; P < 0.0001). This screening survey tool consisting of 6 easily assessable questions was moderately successful in identifying neonates at high risk for vitamin D deficiency; however, further research is needed to refine and validate this screening tool in other populations before it can be used in clinical practice.

Résumé

Au Canada, de nombreux nourrissons naissent avec des réserves de vitamine D faibles, ce qui les expose à un risque de complications telles que le rachitisme, s'ils ne sont pas traités. Il n'y a aucun programme de dépistage des nouveau-nés pour le déficit en vitamine D. Identifier les nourrissons à haut risque peut faciliter une éducation ciblée des parents sur la supplémentation pour les nourrissons. Cependant, aucun outil de dépistage est disponible. L'objectif était de développer et de tester la validité d'un outil d'enquête de dépistage permettant d'identifier les nouveau-nés présentant un risque élevé de carence en vitamine D (25-hydroxyvitamine D (25(OH)D) <30 nmol/L). Des couples mère-enfant en bonne santé (n = 1 112) ont été recrutés à l'Hôpital Général du Lakeshore, à Montréal, de mars 2016 à mars 2019. Une enquête sur les facteurs liés à la démographie et au mode de vie a été réalisée. Des échantillons de sang de nouveau-né, recueillis moins de 36 h après la naissance, ont été soumis à un test de sérum 25(OH)D (Liaison, Diasorin Inc.). La validité du contenu était basée sur 21 facteurs de risque connus. Des modèles de régression logistique ont été utilisés pour identifier les variables clés associées au risque de faibles concentrations néonatales de vitamine D. Les courbes ROC (Receiver Operating Characteristic) ont été utilisées pour démontrer la sensibilité et la spécificité de l'outil de dépistage par rapport au statut connu en vitamine D. Les mères (âgées de 32.4 ± 4.5 ans) étaient en majorité blanches (58%) et 57% avaient un indice de masse corporelle (IMC) avant la grossesse dans la plage des valeurs saines (18.5-24.9 kg/m^2). La concentration sérique moyenne en 25(OH)D néonatal était de 44.5 ± 19.9 nmol/L, avec 23% (IC 95% 0.20, 0.25) inférieure à 30 nmol/L. Six des 21 facteurs de risque existants évalués prédisaient davantage le statut néonatal en vitamine D bas (P <0.05). Ces facteurs de risque (âge maternel à l'accouchement <26.0 ans, IMC <18.5 ou ≥25 kg/m^2 avant la grossesse, couleur de la peau non

blanche, consommation de supplément prénatal <2-3 fois par semaine avant ou pendant la grossesse et accouchement d'octobre à avril) peuvent être facilement évalués à partir des dossiers hospitaliers. Les coefficients de régression pour chaque facteur de risque ont été transformés en scores entiers. La moyenne des scores totaux a été considérée comme le score limite pour les nouveau-nés présentant un risque élevé de carence en vitamine D. L'outil de dépistage avait une sensibilité de 68.8% et une spécificité de 70.0%. L'aire sous la courbe ROC était de 0.712 (IC à 95% 0.679, 0.745; P <0.001). Cet outil d'enquête de dépistage, composé de 6 questions faciles à évaluer, a permis de bien identifier les nouveau-nés présentant un risque élevé de carence en vitamine D. Cependant, des recherches supplémentaires sont nécessaires pour affiner et valider cet outil de dépistage dans d'autres populations avant de pouvoir l'utiliser en pratique clinique.

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Contribution of authors

S. Patel was the primary author of this thesis and the included manuscript. S. Patel was involved in all aspects of the participant recruitment process from September 2017 through March 2019. S. Patel administered demographic surveys and collected information from patient charts. S. Patel audited study data for quality and accuracy and entered data for food frequency questionnaires. S. Patel reviewed relevant literature, conducted statistical analyses, interpreted results and wrote the manuscript.

S. Agellon and P. Lavery were responsible for the analysis of 25(OH)D concentration in neonatal serum samples and contributed to data analyses.

O. Sotunde contributed to data analyses and critical review of the manuscript.

C. Vanstone critically reviewed the grant, study procedures, prepared ethics submissions, and critically reviewed the manuscript. She was responsible for the general coordination of the project and assisted with the development of documents for the study (i.e. parent's survey, pamphlets, consent forms, contact forms).

S. Wei was S. Patel's co-supervisor. S. Wei contributed to the research grant proposal, statistical analyses and was thoroughly involved in the review of the manuscript.

H. Weiler was S. Patel's supervisor. H. Weiler was the principal investigator of the study, designed the research and secured the grant, directed the study procedures and ethics submissions, and critically reviewed the manuscript.

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List of abbreviations

25(OH)D	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
AI	Adequate Intake
AUC	area under the ROC curve
BMI	body mass index
DBP	vitamin D binding protein
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
FFQ	food frequency questionnaire
FGF23	fibroblast growth factor 23
IGF1	insulin-like growth factor 1
IOM	Institute of Medicine
IU	international unit
PTH	parathyroid hormone
RDA	Recommended Dietary Allowance
ROC	receiver operating characteristic
RXR	retinoid X receptor
SGA	small for gestational age
SPF	sunblock protection factor
SXR	steroid and xenobiotic receptor
UL	Tolerable Upper Intake Level
UVB	ultraviolet beta radiation
VDR	vitamin D receptor
VDRE	vitamin D response element

1 Literature review

1.1 Introduction

Vitamin D is a lipid-soluble vitamin that is recognized as a prohormone and can uniquely be synthesized in the human skin when exposed to ultraviolet beta (UVB) radiation from the sun (1). It exerts its effects via a steroid receptor that is expressed in various tissues in mammals. Classical roles of vitamin D include calcium and phosphorus homeostasis and bone metabolism (2). It is becoming increasingly evident that vitamin D has a function in reducing the risk of many chronic illnesses, such as cancer, infectious diseases and cardiovascular diseases.

Along with its well established importance in supporting healthy fetal bone development, there is evidence that vitamin D plays a role in healthy pregnancy progression, fetal growth and development and immune function (3,4). Low vitamin D status is common among pregnant women (5,6), which in turn may adversely affect pregnancy outcomes and subsequently the vitamin D status of their newborns (7). Many infants in Canada are born with low vitamin D stores (8), putting them at risk of developing complications such as rickets, if untreated. Health Canada recommends daily vitamin D supplements for infants throughout the first year of life, however, not all families follow these recommendations (9). Identifying newborns that are at high risk for vitamin D deficiency may be important to providing targeted education to parents regarding supplementation for infants and improving infant health outcomes.

1.2 Vitamin D metabolism

1.2.1 General metabolism

The two major forms of vitamin D are vitamin D_2 and vitamin D_3, which are together referred to as calciferol (2). Vitamin D_2, also known as ergocalciferol, is produced by fungi, moulds and lichens, while vitamin D_3 (cholecalciferol) is synthesized by animals. Only vitamin

D_3 is endogenously synthesized in mammals, whereas both vitamin D_2 and D_3 can be obtained from consumption of foods and supplements.

In humans, keratinocytes and fibroblasts contain 7-dehydrocholesterol that is converted into pre-vitamin D_3 upon exposure to solar UVB radiation (medium wavelength, 280-320 nm) (1). Pre-vitamin D_3 isomerizes into the more thermodynamically stable vitamin D_3, while it is transferred into the extracellular space (10). The production of vitamin D_3 in the skin is a non-enzymatic process. Excessive sun exposure leads to the degradation of pre-vitamin D_3 and vitamin D_3, effectively regulating the amount of cutaneous vitamin D synthesis and preventing toxic effects due to excess vitamin D (11). From the extracellular space of the skin, vitamin D_3 diffuses into the circulation, aided by its affinity for vitamin D binding protein (DBP) in dermal capillary beds (12). DBP is a serum glycoprotein secreted by the liver, and is the primary transport protein for vitamin D metabolites in circulation of children and adults (13).

Vitamin D_2 and D_3 from the diet are incorporated into chylomicrons for absorption (12). Chylomicrons enter the lymphatic system and are released into venous circulation. Before reaching the liver, chylomicron lipids can be hydrolyzed and taken up by adipose tissue (2). Thus, some vitamin D_2 and D_3 can be stored in and later released by adipocytes. Both forms are considered inactive prohormones and require activation by two enzymatic reactions catalyzed by members of the cytochrome P450 hydroxylase family (1). Although vitamin D_2 and D_3 are converted into their own respective metabolites and active forms, both pathways are mechanistically identical and thus they are often considered together as vitamin D.

The first hydroxylation reaction takes place in the liver, where vitamin D is converted to 25-hydroxyvitamin D (25(OH)D) by 25-hydroxylases, primarily CYP2R1 (1). With a half-life of 2 to 3 weeks, 25(OH)D is the major circulating form of vitamin D (14). The subsequent hydroxylation occurs mainly in the kidneys, where 25(OH)D is converted to 1,25-

dihydroxyvitamin D (1,25$(OH)_2$D), also known as calcitriol, the physiologically active form of vitamin D. This hydroxylation reaction is catalyzed by CYP27B1, a 1α-hydroxylase that is under tight regulatory control in the kidneys. Calcitriol is transported through the circulation by DBP to various target organs, where it generally enters cells by passive diffusion. The physiological actions of calcitriol are mediated by the nuclear vitamin D receptor (VDR), which is expressed in tissues of all major organ systems (15). The VDR is a transcription factor that can regulate many different genes in a cell-specific manner. The specific interaction of calcitriol with VDR is complex involving other receptors and cofactors, in addition to gene response elements as discussed in detail in Section 1.3.

The endocrine production of calcitriol is tightly regulated at the level of 25(OH)D conversion to calcitriol by renal CYP27B1. This is primarily mediated by three hormones: parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23) and calcitriol (1). PTH stimulates CYP27B1 when serum calcium levels are low, whereas FGF23 suppresses it in response to elevated phosphate levels. Calcitriol limits its own production through negative feedback by inhibiting PTH and promoting FGF23 production (16). These regulatory mechanisms are tightly linked to calcium and phosphate homeostasis. CYP27B1 is also expressed in bone marrow stromal cells, where its expression can be upregulated by insulin-like growth factor 1 (IGF1) in a feed-forward mechanism to promote osteoblast differentiation (17).

Calcitriol regulates its own concentrations through a multifaceted negative feedback loop. Through VDR signaling, calcitriol induces the expression of CYP24A1, a 24-hydroxylase, in all its target tissues (18). CYP24A1 mediates the multi-step catabolism of both calcitriol and 25(OH)D into inert metabolites that are readily eliminated via biliary excretion and eventually into the feces.

While the kidneys are the main source of circulating calcitriol, CYP27B1 is also expressed in some extrarenal tissues, such as the skin, lungs, intestine, immune cells, osteoblasts, chondrocytes and endocrine glands including the parathyroid gland, thyroid, testes and ovaries, where calcitriol is important for autocrine and paracrine functions (1). These functions are well established in the immune system, where calcitriol is involved in regulating innate immune cell activity as well as cell proliferation and differentiation for adaptive immune responses (19). Other autocrine and paracrine functions of calcitriol include regulating inflammation and thrombosis in vascular endothelial and smooth muscle cells (20), promoting trophoblast invasion and decidual immune function during pregnancy (21,22), as well as regulating chondrocyte and osteoblast activity at the growth plate during endochondral ossification (23). The mechanisms for extrarenal regulation of calcitriol metabolism are different than those mentioned above, and generally not linked to calcium homeostasis.

1.2.1.1 Transport of vitamin D metabolites in the circulation

Vitamin D and its metabolites are lipophilic and thus require protein carriers to be efficiently transported in circulation. Approximately 90% of circulating vitamin D metabolites are bound to DBP, while 10% are bound with a low affinity to the non-specific carrier, albumin. Less than 1% exists in a free form (24), and the free levels of vitamin D metabolites are influenced by DBP concentration and their binding affinity or dissociation constants (25). Calcitriol has a lower affinity for DBP than does 25(OH)D, which is a contributing factor to the longer half-life of 25(OH)D as it remains in the circulation for longer (26). Free 25(OH)D and calcitriol are able to readily diffuse across cell membranes of target tissues to exert their effects and are thus considered the most highly bioavailable fractions (25).

1.2.1.2 Epimerization

Major vitamin D_3 metabolites can be epimerized at the C3-hydroxy group of the A-ring, and these C3-epimers can be further metabolized by the same hydroxylase enzymes involved in the standard metabolic pathway (27). *In vitro* studies have shown that the C3-epimers of $25(OH)D_3$ and calcitriol (3-epi-$25(OH)D_3$ and 3-epi-$1\alpha,25(OH)_2D_3$, respectively) have an altered biological activity relative to $25(OH)D_3$ and calcitriol. The relative binding affinities of 3-epi-$25(OH)D_3$ and 3-epi-$1\alpha,25(OH)_2D_3$ for DBP and VDR, respectively, are significantly weaker than $25(OH)D_3$ and calcitriol. Additionally, the transcriptional activity on the human osteocalcin gene, as well as anti-proliferative and differentiation-inducing activities of 3-epi-$1\alpha,25(OH)_2D_3$ are 70-90% lower than calcitriol *in vitro* (27).

C3-epimers of major vitamin D_3 metabolites have been identified in the serum of neonatal, pediatric and adult populations (28). Higher relative concentrations of these epimers have been observed in neonates, decreasing with age, and with minimal concentrations detected in adults. Some studies have reported 3-epi-$25(OH)D_3$ concentrations accounted for over 40% of the total serum $25(OH)D_3$ measured in neonates (29–31), with a weighted mean of 21.4% across studies (28). The large proportion of C3-epimers in infant serum can complicate interpretation of total $25(OH)D_3$ or reduce the reliability and validity of the measurement when using traditional liquid chromatography tandem-mass spectrometry (LC-MS/MS) procedures (31). While traditional LC-MS/MS methods did not distinguish the epimers from their same molecular weight counterparts, current methods have been adapted to exclude the epimers (32). Presently, epimers are not included in the assessment of vitamin D status (29). Further research is required to ascertain the physiological importance and clinical relevance of C3-epimers.

1.2.2 Metabolism during pregnancy

In the non-pregnant state, calcitriol concentrations are tightly regulated by feedback mechanisms involving renal CYP27B1 and CYP24A1. During pregnancy, calcitriol concentrations seem to be uncoupled from these mechanisms regulating calcium homeostasis (33). While the kinetics of 25(OH)D synthesis remain unchanged during pregnancy, there is a two- to three-fold increase in the conversion of 25(OH)D to calcitriol, primarily in the placenta and kidneys. By 12-weeks gestation, maternal serum calcitriol concentrations are at least double the levels of a non-pregnant woman, and continue to increase (33,34). Many studies have suggested that the increase in calcitriol functions beyond calcium homeostasis during pregnancy, and may have important roles in immunomodulation and epigenetics (35), which affect maternal, fetal and neonatal outcomes.

Renal CYP27B1 expression is upregulated during pregnancy, largely contributing to the increased circulating calcitriol (36,37). Rather than being tightly controlled by calcium homeostasis mechanisms, renal CYP27B1 activity in pregnant women has been shown to be driven by substrate availability (33). Analyses of calcium, phosphorus and PTH during pregnancy confirm that they do not control this increase in calcitriol (33,37). Despite calcitriol concentrations increasing to levels that would cause hypercalcemic toxicity in non-pregnant individuals, this increase is not associated with hypercalcemia or hypercalciuria during pregnancy, but rather is necessary for healthy progression of pregnancy (33).

Decidual and placental tissues produce calcitriol during pregnancy, which is also independent of calcium homeostasis (33,37). This production does not significantly contribute to circulating maternal concentrations, but rather serves autocrine and paracrine functions in the fetal-placental unit (38). During the first trimester of pregnancy, CYP27B1 is expressed in maternal decidual cells leading to production of calcitriol by the placenta (21,33). Expression of

CYP27B1 is significantly higher in first trimester vs. third trimester decidual cells. Along with immune cell culture studies, this suggests a local immunoregulatory role for calcitriol, allowing trophoblast implantation without triggering maternal immune responses and regulating other acquired and innate immune responses at the fetal-maternal interface (21,22). Additionally, placental CYP24A1 genes are hypermethylated unlike in other tissues of the body (39). *In vitro* studies have confirmed that methylation diminishes basal gene expression of CYP24A1 and inhibits its inducibility by calcitriol. The placenta-specific reduced catabolic capacity is thought to maximize the bioavailability of calcitriol at the fetal-maternal interface (39).

1.2.3 Metabolism in the fetus and infant

The evidence regarding transplacental transfer of maternal calcitriol is unclear, however transfer of maternal 25(OH)D is better established (39). The developing fetus does not produce 25(OH)D on its own, but rather relies on maternal 25(OH)D to produce calcitriol. Maternal 25(OH)D concentrations, especially closer to the time of delivery, are highly correlated with cord and neonatal concentrations (8,40). Although correlated, cord concentrations of 25(OH)D and calcitriol are consistently lower than maternal concentrations (41), suggesting passive diffusion through the placenta (13,42). Passive diffusion is also likely considering that DBP concentrations are significantly lower in the fetus compared to maternal serum (13). *In vitro* assays demonstrated that binding to DBP was a strong determinant for the transfer rates of 25(OH)D and calcitriol across the placenta (43). The lower relative DBP concentration in the fetal compartment is thought to be unfavorable to materno-fetal transfer of 25(OH)D during gestation (44), contributing to lower infant serum 25(OH)D concentrations at birth. This may not necessarily indicate low tissue stores; since DBP concentrations are significantly lower in neonates, it is unclear how serum 25(OH)D relates to storage pools in infants.

1.3 Roles of vitamin D in the body

The majority of physiologic effects of calcitriol are induced by binding to the VDR (45), although several nongenomic actions of calcitriol have been identified (46). The VDR is part of the steroid hormone nuclear receptor family, and therefore shares similar properties and mode of action to other members of the family such as the thyroid hormone receptor. Upon ligand binding, the VDR heterodimerizes with the retinoid X receptor (RXR) and translocates to the nucleus (20). The VDR-RXR heterodimer binds to vitamin D response elements (VDREs), which are specific DNA sequences located in the promoter region of target genes (45). This binding triggers the recruitment of coregulatory complexes to the VDR, which ultimately mediate changes in gene expression. These complexes may include general transcription machinery, chromatin remodeling complexes and enzymes such as acetyltransferases, deacetylases, methyltransferases and demethylases (45,47). The recruited complexes are gene or cell-specific, allowing specificity for the actions of calcitriol in transcriptional activation or repression of different target genes.

1.3.1 Classical roles

The most well established functions of vitamin D are its endocrine effects on calcium absorption and metabolism, phosphate homeostasis and bone health. Calcitriol, PTH and calcitonin function to maintain concentrations of circulating calcium within a narrow physiological range necessary for normal functioning of the neuromuscular junction, vasodilatation and neuronal transmission (2). Maintenance of plasma calcium and phosphate concentrations is also necessary to promote bone mineralization. Calcitriol increases plasma calcium levels by mainly acting on epithelial cells of the small intestine and kidneys and in bone. In enterocytes, calcitriol promotes calcium absorption by enhancing expression of calcium channels, which allow uptake of calcium ions from the intestinal lumen. It also upregulates the

expression of calcium-binding proteins, which facilitate the intracellular transport of calcium ions to the basolateral membrane of enterocytes and the subsequent extrusion of the ions into the circulation. Calcitriol also enhances the expression of sodium-phosphate cotransporters in enterocytes to increase the net absorption of phosphate. During pregnancy, physiological adaptations allow the efficiency of intestinal calcium absorption to double in order to meet the demands for fetal skeleton development (36). *In vivo* studies suggest that given sufficient calcium intake, this doubling of calcium absorption during pregnancy may occur independently of calcitriol (48).

In the kidneys, calcitriol promotes the resorption of calcium and phosphate from the distal and proximal tubules via similar respective mechanisms in enterocytes. In bone, calcitriol induces the expression of receptor activator of nuclear factor-κB ligand (RANKL) on osteoblasts. RANKL is a membrane-associated ligand that binds to its receptor (RANK) on preosteoclasts, stimulating their differentiation into mature osteoclasts as well as promoting their activity to mobilize calcium and phosphorus from the bone (47). This effect of calcitriol on bones is necessary for bone modelling and subsequent remodelling that occurs throughout life.

1.3.2 Non-classical roles

The widespread distribution of the VDR and presence of extrarenal CYP27B1 support the potential for its effects outside of bone mineral metabolism. The regulation of calcitriol production in extrarenal tissues seems to be substrate dependant (49), highlighting the importance of maintaining adequate serum 25(OH)D concentrations for functions beyond classical actions.

The role of vitamin D in immune regulation has become an area of interest in recent years. Antigen-presenting cells (APCs), such as macrophages and dendritic cells, as well as $CD4^+$, $CD8^+$ T cells express the VDR and CYP27B1 (50). Treatment of innate immune cells with calcitriol increases their expression of antimicrobial peptides (51,52) and enhances the

phagocytic capacity of macrophages in response to intracellular bacteria (50). Observational studies have associated low vitamin D status with increased risk of upper respiratory tract infections (53,54).

Researchers have hypothesized that vitamin D deficiency during pregnancy may alter immune programming in the fetus and influence asthma-related risk factors and outcomes in childhood (55). Several observational studies have shown a link between asthma-related outcomes in children and low vitamin D intake during pregnancy (56–58) or neonatal vitamin D deficiency (54,59). The observations of these studies are supported by a randomized, double-blind, placebo-controlled trial investigating the effects of maternal high-dose vitamin D supplementation during pregnancy on parameters of neonatal immunity (60). Maternal supplementation of 4400 IU/day resulted in enhanced neonatal proinflammatory cytokine responses of mononuclear cells to innate and mitogenic stimuli and higher expression of toll-like receptors on monocytes. It also enhanced production of IL-17A in response to T-cell stimulation, which has been shown to be specifically important in neonatal immunity and defense against pulmonary pathogens. These strong neonatal immune responses are thought to be associated with lower risk of developing asthma and improved respiratory health in early life (60). Additionally, in the children of mothers supplemented with 4400 IU/d vitamin D, a trend towards lower incidence of recurrent wheeze or asthma and reduced rates of respiratory tract infections were observed at age 3 y (61). Although not statistically significant, similar trends were reported for these clinical outcomes in an independent trial, supplementing women with 2400 IU/d during their third trimester (62). When these studies were pooled in a meta-analysis, prenatal vitamin D intake significantly lowered the risk of the offspring developing recurrent wheeze at age 3 y (63).

Other immunomodulatory actions of vitamin D include both activation and inactivation of innate and adaptive immune responses to different stimuli, which include anti-inflammatory

effects, promoting immune tolerance and supporting a Th17 regulatory response (64). Considering this potential role, observational studies have suggested the implication of vitamin D in the development of several autoimmune diseases including multiple sclerosis, rheumatoid arthritis, Crohn's disease and type 1 diabetes (11). Maternal vitamin D deficiency has been associated with increased risk of offspring developing type 1 diabetes (65), however the effect of vitamin D supplementation on preventing offspring type 1 diabetes is unclear (64).

Observational studies have also associated vitamin D deficiency with type 2 diabetes as the β-cells of pancreatic islets express VDR and CYP27B1 (66). Low vitamin D status is associated with poor glycemic control, reduced insulin secretion and increased risk for developing type 2 diabetes (67) and gestational diabetes (68,69), while vitamin D supplementation may improve insulin sensitivity in type 2 diabetes (70). Calcitriol promotes insulin secretion *in vitro*, both directly via VDR signalling in β-cells and indirectly through the normalization of extracellular calcium (67).

Potential cardiovascular effects of vitamin D were suggested after the identification of the VDR in the heart and blood vessels, as well as its involvement in cardiovascular risk factors such as hypertension and insulin resistance (20). Animal studies suggest that VDR activation suppresses the expression of renin, which is involved in increasing arterial hypertension, and modifies expression of other genes to promote vasodilation and vessel relaxation. Meta-analyses have associated low serum 25(OH)D concentrations with an increased risk of cardiovascular events (71) and related mortality (72). In particular, maternal vitamin D concentrations < 50 nmol/L during pregnancy are associated with an increased risk of preeclampsia and its associated increase in maternal and perinatal morbidity and mortality (4). The mechanisms behind this association may include the role of vitamin D in hypertension described above, as well as its involvement in immune dysfunction (64), placental implantation (22) and angiogenesis (20). In

addition to adverse maternal outcomes, cases of dilated cardiomyopathy have been observed in children with vitamin D deficiency rickets (73). Treatment with vitamin D alone or with calcium rapidly restores normal cardiac function, further supporting a role for vitamin D in normal cardiac function.

1.4 Recommendations

1.4.1 Vitamin D status and definitions

Serum 25(OH)D concentration is used as an indicator of vitamin D status because it reflects vitamin D produced cutaneously as well as that consumed from food and supplements; and it has a half-life of 2-3 weeks, making it the major circulating metabolite of vitamin D with a relatively stable concentration (14). There is no universally accepted definition of vitamin D deficiency, population health policy and professional organizations have established different cut-offs to define levels of vitamin D status based on circulating 25(OH)D concentration.

The IOM established cutoffs for 25(OH)D relative to bone health, where less than 30 nmol/L is considered at risk for deficiency, 30-49.9 nmol/L is at risk for inadequacy and at or above 50 nmol/L is considered sufficient (2). Recommendations set by the IOM are intended for both the United States and Canada, and are thus supported by Health Canada. These cutoffs are recommended for healthy people of all age groups and life stages, and are based on several functional outcomes, including intestinal calcium absorption, bone mineral content and risk of rickets, fractures and osteomalacia. Serum 25(OH)D concentrations below 30 nmol/L during infancy increase the risk of developing nutritional rickets, while this risk is minimal between 30 and 50 nmol/L (2). When the IOM published their recommendations in 2011, they stated that there was insufficient evidence to link vitamin D intake or status with extraskeletal health outcomes.

The American Academy of Pediatrics supports the IOM cutoffs for infants and pregnant or nursing women (74), however the definitions of desirable serum 25(OH)D concentration targets are debated within in the scientific community and different levels are recommended by researchers and other health organizations (Table 1.1). The Endocrine Society considered minimizing PTH production, reducing risk of fractures and efficiency of intestinal calcium absorption when developing their recommendations (75). They stated that their recommendations are based on maximizing bone health, and that it was not clear if their recommendations also adequately support the potential nonskeletal health benefits associated with vitamin D. They define below 50 nmol/L as deficiency, 50 to 75 nmol/L as insufficiency and at or above 75 nmol/L as sufficiency, for all ages and life stages. Similarly, the Canadian Paediatric Society considers at or above 75 nmol/L as optimal, however 25 to 75 nmol/L is defined as insufficient and less than 25 nmol/L is deficient (5). They base their definition for sufficiency on the concentrations of 25(OH)D that minimize bone resorption and PTH production as well as stabilize intestinal calcium absorption.

Table 1.1. Vitamin D status as defined by organizations based on serum 25(OH)D (nmol/L), applicable to all age groups and life stages

Vitamin D status	Institute of Medicine[1]	Endocrine Society	Canadian Paediatric Society
Deficient	< 30	< 50	< 25
Insufficient	30 – 49.9	50 – 74.9	25 – 74.9
Sufficient	≥ 50	≥ 75	75 – 225
Potentially toxic	≥ 125	-	≥ 500

[1]Joint recommendations with Health Canada (2), supported by the American Academy of Pediatrics (74) Endocrine Society (75); Canadian Paediatric Society (5)

Although definitions from these organizations are all based on maintaining bone health, they have come to different conclusions partly based on differential inclusion and interpretation of studies. An additional challenge is that there is large inter-assay and inter-laboratory variability in measurements of serum 25(OH)D, which affects the comparability of results from published studies and the conclusions drawn from them (76). The IOM and other groups also have different goals when developing their recommendations. The IOM aims to guide government and public policy, and therefore may appear more conservative in their recommendations so as to reflect the needs of a broader population (77). The Endocrine Society and the Canadian Paediatric Society provide guidelines for clinical practice, such as physicians advising individual patients.

1.4.2 Pregnancy and lactation

Sun exposure is the most important source of vitamin D for most people, but as it is not always reliable or advisable, the IOM has established recommendations for vitamin D intake based on limited sun exposure and consistent with maintaining healthy serum 25(OH)D concentrations (≥ 50 nmol/L) (2). A daily intake of 600 IU is recommended for healthy women of reproductive age, given that there are no known additional requirements during pregnancy and lactation to support maternal or fetal calcium homeostasis and bone outcomes (2).

Based on maintaining healthy 25(OH)D concentrations (≥ 75 nmol/L), as defined by respective organizations, the Endocrine Society recommends a daily vitamin D intake of 1500-2000 IU for pregnant and lactating women, not different from their recommendations for non-pregnant, non-lactating women (75). In order to achieve this level of intake, they recommend pregnant and lactating women consume a daily prenatal or multivitamin containing 400 IU vitamin D and an additional vitamin D supplement containing at least 1000 IU. The Canadian Paediatric Society considers prescribing 2000 IU/d of vitamin D to pregnant and lactating women to help maintain sufficient circulating 25(OH)D, especially during the winter (5). Unlike the

IOM, these groups took into consideration studies showing that vitamin D intakes of 600 IU/d have not been able to prevent deficiency (serum 25(OH)D < 37.5 nmol/L) in pregnant women (78,79). A randomized controlled trial assessing different levels of vitamin D supplementation beginning at 12-16 weeks gestation found that an intake of 4000 IU/d was safe and effective in achieving sufficient serum 25(OH)D concentrations (≥ 50 nmol/L) in all pregnant women throughout pregnancy, and that 79% of their infants were born with sufficient serum 25(OH)D concentrations (33). Higher doses of maternal vitamin D supplementation during pregnancy may put the neonate in a better position at birth in terms of circulating 25(OH)D concentrations.

Low vitamin D status is common among pregnant women (5,6), which in turn puts their neonates at particular risk. There is a growing body of evidence that maternal vitamin D supplementation during pregnancy is not only safe, but may also improve pregnancy outcomes. Meta-analyses published within the last year have found that maternal vitamin D deficiency and insufficiency during pregnancy are associated with small for gestational age (SGA) infants and preterm birth (3), and that maternal supplementation during pregnancy at doses between 800 IU/d and 2000 IU/d may reduce the risk of SGA and fetal or neonatal mortality (7). A 2016 Cochrane review evaluating vitamin D supplementation during pregnancy found that it may reduce the risk of preeclampsia, low birthweight and preterm birth compared to no supplementation or a placebo (4). A more recent systematic review by Roth et al. included studies which used standard vitamin D doses (up to 600 IU) as controls, and thus considered 43 trials in their analyses (80). They found that prenatal vitamin D supplementation > 600 IU/d increased mean birth weight and reduced the risk of SGA births and supplementation > 2000 IU/d reduced the risk of offspring wheeze at 3 years.

High-dose maternal vitamin D supplementation (4000-6400 IU/day) during lactation produces dose-dependent increases in breastmilk vitamin D and infant 25(OH)D concentrations

(81–83). Maternal supplementation with 6400 IU/day adequately supplied breastmilk with vitamin D concentrations that satisfy the nursing infant's vitamin D requirements. After 6 months, infants of mothers supplemented with 6400 IU/d vitamin D had similar serum 25(OH)D concentrations to infants directly supplemented with the recommended 400 IU/d (83). High-dose maternal supplementation has the added benefit of ensuring that mothers maintain a healthy vitamin D status, and may be an alternate strategy to directly supplementing infants. Several clinical trials have demonstrated the safety of high-dose supplementation during lactation, however compliance to this regimen, its efficacy in maintaining infant vitamin D status throughout the nursing period and subsequent infant health outcomes require further investigation. Alternatively, moderately high dose (2000 IU/day) maternal vitamin D supplementation, beginning during gestation and continued in lactation, has been found to protect exclusively breastfed (unsupplemented) infants against vitamin D deficiency (84).

1.4.3 Infancy

During gestation, maternal 25(OH)D is transferred to the fetus and subsequently contributes to circulating 25(OH)D in the neonate. Infant 25(OH)D concentrations decrease rapidly after birth, owing to its 2-3 week half-life (85). Those born with sufficient circulating 25(OH)D become deficient after 8 weeks of exclusive breastfeeding (86), unless receiving adequate sun exposure or vitamin D supplementation. Sun exposure is not advised for infants due to concerns of skin cancer. Infants are thought to be especially vulnerable to damage caused by solar UV radiation because they have lower levels of melanin, a thinner stratum corneum and a higher surface area to body mass ratio (87). These factors not only allow UVB rays to penetrate infant skin to a greater extent than adults, but also make them susceptible to percutaneous absorption of sunscreens. Health Canada recommends keeping infants out of direct sunlight for

the first six months of life, after which sunscreen can be used while avoiding excessive sun exposure (88).

Dietary and supplemental intake is assumed to be the sole source of vitamin D for the growing infant. In the 2011 IOM update for calcium and vitamin D, there was insufficient evidence to establish an EAR for vitamin D for infants under 1 year of age. A daily intake of 400 IU for infants up to 1 year of age is consistent with maintaining desirable serum 25(OH)D concentrations above 50 nmol/L (2). The AI for infants was therefore set at 400 IU/d on the basis of maintaining serum 25(OH)D concentrations close to 50 nmol/L, which prevents rickets and supports normal bone accretion in the majority of infants. While breast milk is the ideal nourishment for growing infants, it is not a significant source of vitamin D. When breastfeeding women are unsupplemented or supplemented with 400 IU/d of vitamin D, the vitamin D content of their breast milk ranges on average 10-80 IU/L (89). Infant formula is mandatorily fortified with vitamin D (90), and generally infants would have to consume 1 L of formula a day in order to receive the recommended 400 IU/d of vitamin D. This is amount is often not consumed by mixed feeding infants or formula fed infants until 6 months of age. Health Canada recommends a 400 IU daily vitamin D supplement for exclusively and partially breastfed infants, from 0 to 12 months (88). The Canadian Paediatric Society (5) and the American Academy of Pediatrics (74) add that supplementation should continue until infants are consuming at least 1 L of formula per day or 400 IU/d of vitamin D from other dietary sources.

Despite these recommendations for universal vitamin D supplementation for infants, many infants in Canada are not receiving the supplement regularly or at all. Based on the Canadian Community Health Survey (CCHS) in 2011-2012, 79% of exclusively breastfed infants received a vitamin D supplement, and of these, only 67% received it daily (91). With regards to partially breastfed infants, only 51% were receiving a daily vitamin D supplement based on a

study in Quebec (92). According to the 2011-2012 CCHS, mothers were less likely to give a vitamin D supplement to their infant if they were single, black, had a lower household income or had a lower education level (88). Poor supplementation practices may be one of the reasons that cases of vitamin D-deficiency rickets and severe symptomatic vitamin D deficiency continue to be reported in Canada (93,94).

There is currently no direct evidence supporting that targeted recommendations or practices are any more effective than universal approaches to promoting adherence to the infant vitamin D supplementation recommendations. It is clear, however, that the implementation of current recommendations is inadequate, with as many as a third of exclusively breastfed Canadian infants not receiving a daily vitamin D supplement (91). In Quebec, the public healthcare system covers the cost of vitamin D supplements for newborns, yet only 18% of prescriptions were filled before 2008 (95). Providing free supplements along with the universal recommendation for infant vitamin D supplementation is not enough to improve adherence by families. This is supported by a cross-continent study comparing European countries in terms of their differences in policies for infant vitamin supplementation and adherence to their recommendations (96). In this study, the presence of a national vitamin D supplementation policy and government covering the cost of vitamin D supplements are not associated with adherence. Factors that were strongly associated with adherence to supplementation included providing information on supplementation at discharge from neonatal units and monitoring of adherence to supplementation at follow-up visits for child health. While it may not be feasible to develop an implementation strategy to improve adherence in all families, providing targeted education at discharge or follow-up appointments to families whose infants are at highest risk for vitamin D deficiency may provide an alternate method to mitigate the risk of complications for those who are most likely to develop them.

1.5 Exogenous sources of vitamin D

1.5.1 Maternal dietary sources

Natural dietary sources rich in vitamin D are limited to foods consumed infrequently by most North Americans, such as fatty fish and liver (Table 1.2). Food fortification has thus been an important strategy to prevent overt vitamin D deficiency among Canadians (97). Low dietary vitamin D intake is still common in the Canadian population. Based on dietary intake data collected as part of the CCHS Cycle 2.2 in 2004, women of childbearing age had a mean vitamin D intake of 188 IU/d, with 93% consuming less than the current EAR of 400 IU/day (98).

Table 1.2. Natural food sources of vitamin D

Food	Recommended serving size	Vitamin D content* (IU)
Egg (yolk)	2	57-88
Beef liver	75 g	36
Cod liver oil	1 tsp	426
Sockeye salmon	75 g	394
Atlantic salmon	75 g	204-246
Pacific mackerel	75 g	347
Halibut	75 g	144
Albacore tuna	75 g	99-106
White tuna, canned with water	75 g	60

*Data from the Canadian Nutrient File, 2015 (99)

In Canada, fortification of cow's milk and margarine is mandatory, while it is voluntary for other foods and beverages such as orange juice and plant-based beverages (Table 1.3). Fortification of yogurt and cheese is currently not permitted, however certain Canadian companies produce yogurt with vitamin D-fortified milk, and therefore can contain 58-113 IU per 175 g serving, depending on the type and brand. In 2001, Agriculture and Agri-Food Canada estimated that fluid milk consumption contributed to 58% of vitamin D intake in the average Canadian diet (100). Milk is not consumed uniformly across Canada, and subpopulations that are at highest risk for vitamin D deficiency, such as non-white ethnicities, are less likely to consume milk or other fortified beverages (97). Overall, milk sales per capita have been steadily decreasing since 2004, and are now 25% lower than in 1998 (101). Future fortification policies will need to investigate what types of foods can be fortified that will increase vitamin D intake in all ethnicity groups.

Table 1.3. Vitamin D fortification or enrichment levels in Canada

Food	Fortification/ enrichment target[1]	Vitamin D content of recommended serving size
Mandatory		
Margarine	530 IU/100 g	27 IU/5 g (1 mL)
Cow's milk	41 IU/100 mL	100 IU/250 mL
Infant formula	100-400 IU/1000 kcal	400 IU/1 L[2]
Liquid, dried or frozen whole egg, egg white or yolk[3]		
Voluntary		
Plant-based beverages	35 IU/100mL	88 IU/250 mL
Goat's milk	35-45 IU/100mL	100 IU/250 mL
Yeast-leavened bread and bakery products	Up to 90 IU/100g	
Orange juice	100 IU/250 mL	50 IU/125 mL

[1]Standards defined in the Food and Drug Regulations (90)
[2]Serving size required to reach 400 IU; varies by brand
[3]Mandatory if there is a reduction in the vitamin and/or mineral content

1.5.2 Supplements

Maternal vitamin D supplementation during pregnancy is associated with improved maternal and neonatal vitamin D status (7). Vitamin D_3 is the form of vitamin D more commonly found in supplements in Canada, with the benefit of being more effective in raising and maintaining concentrations of vitamin D in the serum compared to vitamin D_2 (102). Over-the-counter prenatal supplements that are most commonly used by Canadian women (Table 1.4) typically contain 400 IU vitamin D, which is consistent with the EAR of 400 IU/d, but below the current recommended intake (RDA = 600 IU/d) for pregnant women. It is assumed that the supplement will complement a dietary intake of approximately 240 IU. Interestingly, general multivitamin formulations marketed to adults under 50 y contain at least 600-800 IU vitamin D. Prenatal supplements found at health food stores cater primarily to women interested in vegan, vegetarian or non-GMO supplementation options. Recommended doses of these supplements contain 300-1000 IU vitamin D, but require consuming more than 1 tablet to achieve this dose.

Most prenatal supplement tablets are relatively large in size, which is associated with lower compliance of taking daily recommended dosages in women experiencing morning sickness (103). The Pregvit® formulations (600 IU vitamin D_3) consist of smaller tablets taken twice daily, and have similar compliance rates to Nestlé's Materna® (400 IU vitamin D_3), a single large tablet, likely owing to the convenience of a single daily administration versus twice daily.

Table 1.4. Vitamin D content of common multivitamin supplements available in Canada

Supplement	Number of tablets/ capsules recommended	Vitamin D content (IU) in recommended dose[*†]
Drugstore prenatal supplements		
Nestlé Materna	1	400
Jamieson Prenatal	1	400
Kirkland Prenatal	1	400
Centrum Prenatal	1	600
PregVit & PregVit Folic 5[‡]	Blue evening pill	600
Vegan/vegetarian prenatal supplements		
Nordic Naturals	2	300
Progressive Prenatal Formula	3	400
Sisu Multi Expecting	2	500
MegaFood Baby & Me	4	600
Pure Food Women's Prenatal	2	1000
Drugstore women's multivitamin supplements		
Jamieson 100% Complete Women	1	800
Kirkland Women	1	800
Centrum Women	1	800
Swiss Natural's Total One Women	1	800
Women's One a Day	1	1000

[*]All supplements contain vitamin D_3

[†]Data obtained from Nutrition Facts Table on packaging of respective supplements

[‡]Available by prescription only

Common types of over-the-counter vitamin D_3 supplements for infants are olive or coconut oil suspensions, such as Ddrops®. The recommended dose is one drop and can be placed on the mother's finger, a pacifier or nipple. Other liquid formulations available for infants are water-based emulsions, such as Enfamil D-Vi-Sol®, requiring 1 mL of the supplement to be dispensed in the infant's mouth. Many mothers report infants spitting up the 1 mL liquid formula (104), leading to a lower dose of vitamin D consumed by the infant.

1.6 Factors affecting vitamin D status

Factors that may affect an individual's vitamin D status include those that impact cutaneous synthesis, vitamin D intake, absorption and metabolism. Neonatal 25(OH)D concentrations are dependent on maternal-fetal transfer during pregnancy. Vitamin D status at birth is thus largely determined by and highly correlated to maternal status around the time of delivery (8,40). As such, many of the risk factors for low vitamin D concentrations in the general population described below are factors that affect maternal vitamin D status before delivery, and thus also are predictors of neonatal vitamin D status.

1.6.1 Endogenous production

1.6.1.1 Sun exposure and environmental factors

The availability of solar UVB radiation is dependent on solar zenith angle (SZA), which is a function of latitude, time of day and day of the year (105). The SZA is inversely associated with the amount of solar UVB reaching the Earth's surface. The amount of UVB radiation that reaches the Earth's surface is limited when the SZA is large, at higher latitudes, in early morning and late afternoon, and during the winter months at latitudes greater than 45° N.

Solar UVB rays have short wavelengths (290-315 nm), which are easily scattered by atmospheric molecules. Stratospheric ozone plays an important protective role in limiting the amount of solar UVB transmission to the Earth's surface, absorbing as much as 99% of UVB

radiation. As the path length of solar UVB travelling through the atmosphere increases, less UVB will reach the Earth's surface. Therefore at latitudes greater than 45° N, such as in Montreal and Edmonton, the intensity of UVB rays are insufficient for cutaneous vitamin D production for at least half the year (106). Conversely, vitamin D can be produced rapidly from solar UVB radiation throughout the year at latitudes less than 20° N. In cities such as Montreal and Edmonton, vitamin D production is limited before 10 AM and after 3 PM (105). Taking winter vacations to sunny destinations have been found to increase 25(OH)D concentrations by 15-20 nmol/L in adults living at higher latitudes in Europe (107,108).

At a given solar zenith angle, UVB intensity increases at higher altitudes as the path length that UVB travels to the Earth's surface is shorter (109). Clouds and air pollution attenuate the amount of UVB reaching the Earth's surface depending on their density and composition (105). In industrial countries, such as China, air pollution can attenuate UVB radiation reaching the Earth's surface as much as 50% (110). Based on a study in Belgium, women living in urban areas with higher tropospheric ozone levels, despite spending more time in the sun, had 2-fold lower 25(OH)D concentrations than women living in rural areas with less air pollution (111). Canadian urban cities generally have lower levels of air pollutants than international urban areas in the USA, Europe, Australia and China (112). Residing in urban areas is often associated with lower 25(OH)D concentrations, which may be explained by the predominance of indoor occupations (113), higher rates of obesity and greater number of high-rise buildings inhibiting the amount of UVB reaching the surface (114).

Risk factors for low maternal vitamin D status are similarly associated with low neonatal vitamin D status (8). Factors such as intensity and duration of sun exposure during pregnancy, are known to affect maternal vitamin D status, however they may be difficult to accurately measure by questionnaire (115). Maternal outdoor behavior is also likely to change according to the

seasons. Thus, infants born in the winter and spring tend to have lower vitamin D status than those born in the summer and fall (78,116–119). Of the women who deliver in the summer or fall, those who spend more time in the sun during their pregnancy tend to have infants with better vitamin D status than those who have less to no sun exposure.

1.6.1.2 Sunscreen and clothing

Since the early 1980s, stratospheric ozone concentration has declined 5% and more UVB radiation is therefore able to reach the Earth's surface than in the past (120). Ozone depletion and increasing awareness of skin damage and skin cancer risk has prompted several governmental and health organizations to launch campaigns promoting sunscreen use and protective clothing (121–123). Using sunscreen with a sun protection factor (SPF) of 15 has been found to reduce cutaneous vitamin D synthesis up to 99% (11). Although theoretically sunscreen should reduce cutaneous vitamin D synthesis, a double-blind randomized controlled trial among Australian adults found no significant difference in serum 25(OH)D concentration between those using SPF 17 sunscreen or a placebo cream after 7 months (difference, 0.99 nmol/L; 95% CI: -7.0 to +5.0) (124). Compliance with sunscreen use was verified every 2 months using a diary to record frequency of application and weighing the amount of cream left in the participants' containers. Despite intensive instruction on proper sunscreen application, participants in both groups applied the cream inadequately on numerous occasions. Many people who use sunscreen do not apply an adequate quantity, apply it to all exposed skin or reapply it appropriately (125), so the effect of sunscreen on vitamin D synthesis may not be significant in reality in the general population. Additionally, those who use sunscreen are likely to compensate by spending more time in the sun, allowing for exposure to more UVB radiation and vitamin D synthesis (126).

Depending on the fabric, clothing can completely inhibit UVB radiation from reaching covered skin (127). Those who choose to keep the majority of their body covered with clothing

when outdoors (e.g. only face and hands exposed), for religious or other reasons, may have little to no UVB exposure and are thus primarily dependant on exogenous sources of vitamin D (128,129). Studies have shown that the area of irradiated body surface is only important at small UVB doses (< 30 min/day of sun exposure on a clear summer day at latitudes greater than 45°N) (130). At higher doses of UVB radiation, the increase in 25(OH)D depends mainly on UVB dose and is not affected by area of the irradiated body surface, so long as a minimum of face and hands are exposed to the UVB radiation.

1.6.1.3 Lifestyle and age

The majority of North American adults live and work indoors, limiting the amount of solar UVB exposure, even during the summer. Based on a 2010-2011 national survey, Canadian adults spend on average 89% of their time indoors (131), which may contribute to lower endogenous synthesis of vitamin D. Additionally, older adults have been found to spend less time in the sun than young adults (131), which may further contribute to lower vitamin D status associated with aging. Increasing age is negatively associated with serum 25(OH)D concentrations, in part due to levels of 7-dehydrocholesterol in skin decreasing linearly over the lifespan (132). While this is significant over the course of an individual's lifespan, it may not be significant in women during childbearing years. Differences in nutrient intake, rather than endogenous synthesis, likely play a more important role in women of childbearing age, as maternal age at delivery is directly and independently associated with nutrient intake (133) and 25(OH)D concentration (118).

1.6.1.4 Skin pigmentation

Melanin pigment in the skin absorbs UVB radiation and prevents it from penetrating the epidermis (134). Individuals with darker colored skin therefore have reduced cutaneous vitamin D synthesis. When black (Fitzpatrick Skin Type 5) and white (Skin Type 2) adults were exposed

to the same amount of UVB radiation in a tanning bed, vitamin D_3 blood concentrations of white adults increases 30-fold, while concentrations in black adults did not increase significantly (134). This difference in circulating 25(OH)D concentrations is less extreme with repeated exposures to tanning beds (135) or solar UVB radiation (136), however concentrations are still lower in individuals with more skin pigmentation. Several studies have shown that infants born to non-white mothers are more likely to have low vitamin D status than those born to white mothers (78,137,138).

1.6.2 Vitamin D intake

1.6.2.1 Socioeconomic status

Lower levels of education and income are independently associated with lower 25(OH)D concentrations (118,139). Lower income families may have less access to or less education about the importance of sun exposure, fortified foods, fatty fish and vitamin supplements (140).

1.6.2.2 Supplementation and dietary intake

Maternal vitamin D intake from diet and supplements during pregnancy has been found to be associated with neonatal vitamin D status. Women who consumed more servings of fortified dairy products on a daily basis during their pregnancy (118), or those that reported taking a prenatal supplement containing vitamin D gave birth to infants with higher 25(OH)D concentrations and more likely to be in the healthy range, $\geq$ 50 nmol/L (118,141,142). Intake from vitamin supplements may account for 60% of total vitamin D intake in pregnant women (143), making it an important predictor of their vitamin D status. Supplements can be especially important in improving vitamin D status in women residing at more northern latitudes and during winter months when sun exposure is minimal (144). It may be beneficial for women to begin vitamin D supplementation before the onset of pregnancy, as it may take up to 3 months to build and achieve a healthy vitamin D status.

1.6.3 Metabolism

1.6.3.1 Parity

Several studies have found a negative association between maternal parity and neonatal vitamin D status (119,137,145). Nulliparous and primiparous women have been observed to have higher vitamin D status than multiparous women (118,145,146). This may be due to depleted vitamin D stores that have not been replenished before a subsequent pregnancy (145), or possibly due to less care taken in terms of diet and supplementation after the first pregnancy.

1.6.3.2 Body mass index and gestational weight gain

Maternal vitamin D status is inversely associated with body mass index (BMI) (118,119,139), possibly through volume dilution in larger individuals (147) or sequestration of the fat-soluble vitamin in abundant adipose tissues (148). Some studies have found a positive association between physical activity and maternal vitamin D status (118,149), although this may be due to its association with BMI and time spent outdoors (150).

Based on the 2006-2007 Canadian Maternity Experiences Survey (MES), 21.0% and 13.6% of women were overweight and obese, respectively, prior to pregnancy (151). The IOM and Health Canada have established gestational weight gain guidelines based on pre-pregnancy BMI (Table 1.5) (152), however nearly half of all women gain more weight than recommended for their respective BMI category (151). Furthermore, overweight and obese women are more likely to exceed their gestational weight gain recommendations than women with a normal BMI. Since fat and fluid accumulation are large components of gestational weight gain (152), it is possible that gaining excess amounts of weight during pregnancy can further dilute or sequester vitamin D in maternal tissues. This may lead to reductions in bioavailable vitamin D metabolites, reducing maternal and subsequently neonatal vitamin D status.

1.6.3.3 Smoking

Smoking may be associated with lower vitamin D status in women during pregnancy (119,153–155), however the mechanisms behind this association are still unclear. Smoking alters the liver's metabolic enzymes, and therefore it is possible that it may alter hepatic metabolism of 25(OH)D (21). Nicotine is known to constrict uterine and placental blood vessels, reducing blood flow to the fetus (156) and potentially decreasing maternal-fetal transfer of 25(OH)D. Several studies have also shown that compared to non-smokers, smokers are more likely to have poor quality diets that are low in essential nutrients, fiber, fruits, vegetables and milk (133,157–159). It is therefore plausible that smokers may have lower dietary intakes of vitamin D due to poor dietary habits.

1.6.3.4 Medications

The steroid and xenobiotic receptor (SXR) is expressed in the gastrointestinal tract, kidneys and liver, and is involved in detoxifying xenobiotics and drugs from cells (160). Like the VDR, the SXR is in the nuclear steroid receptor family, but it is activated by a variety of drugs, including antiepileptics, glucocorticoids, antivirals and some herbal medicines. Activation of SXR by these drugs upregulates several cytochrome P450 enzymes, including CYP24A1, which increases the degradation of 25(OH)D and calcitriol (160).

Table 1.5. Recommended gestational weight gain for singleton pregnancies based on pre-pregnancy BMI

Pre-pregnancy BMI	Recommended weight gain*	
< 18.5 (underweight)	12.5 – 18 kg	28 – 40 lbs
18.5 – 24.9 (healthy weight)	11.5 – 16 kg	25 – 35 lbs
25.0 – 29.9 (overweight)	7 – 11.5 kg	15 – 25 lbs
≥ 30.0 (obese)	5 – 9 kg	11 – 20 lbs

*Health Canada & Institute of Medicine (152)

1.6.3.5 Disease conditions

Conditions that lead to intestinal fat malabsorption, such as cystic fibrosis, celiac disease and Crohn's disease, impair the associated absorption of vitamin D (11). Measures to ensure sufficient circulating 25(OH)D include adequate sun exposure, use of tanning beds, a 10,000 IU/d vitamin D_3 supplement and water-miscible vitamin D formulations (11,161).

Liver and kidney enzymes are involved in metabolizing vitamin D into its hormonal active form, and therefore patients with chronic hepatic or renal diseases tend to have lower serum 25(OH)D or calcitriol concentrations due to impaired vitamin D metabolism (162,163). Intrahepatic cholestasis of pregnancy is a common liver condition in pregnant women, occurring due to the increase in pregnancy hormones. Impaired bile acid secretions in women with intrahepatic cholestasis of pregnancy result in lower circulating 25(OH)D and subsequent calcitriol concentrations, compared to pregnant women without this disease (164,165).

1.7 Vitamin D deficiency

1.7.1 Consequences of deficiency during infancy

As placental transfer of calcium ceases at birth, neonates become dependent on circulating calcitriol and PTH to maintain calcium homeostasis (85). Infants born with healthy vitamin D concentrations (> 50 nmol/L) deplete their stores within 1-2 months of life (88). As a result, rickets and symptoms of severe vitamin D deficiency typically appear during periods of rapid bone growth between 3 and 18 months, although is it is not uncommon for deficiency to manifest at later ages up to 36 months (166).

Complications of vitamin D deficiency in infancy may be acute, such as hypocalcemic seizures, or more chronic such as delayed dentition, delayed gross motor milestones and rickets (86), whose consequences may persist into childhood and onwards. They may also include long-term adverse health outcomes, such as impaired developmental outcomes, suboptimal bone

mineralization and increased risk of fractures (86,167,168), vulnerability to respiratory infections (141), asthma (56,58) and type 1 diabetes (65).

1.7.2 Prevalence of low vitamin D status in newborns

Based on a large, predominantly white cohort in Quebec City, 24% of newborns are born with low vitamin D status (25(OH)D < 50 nmol/L) (169). Findings from a Pittsburgh cohort revealed that 46% of black and 10% of white neonates had serum 25(OH)D < 37.5 nmol/L (78). In a sample of 92 neonates in Calgary, 28% had serum 25(OH)D < 50 nmol/L (170). Among a sample of 59 neonates in Winnipeg, 30% had serum 25(OH)D < 27.5 nmol/L (171).

Infants with low vitamin D concentrations at birth are at further risk for developing the complications of low vitamin D status, as their stores will continue to be depleted if they do not receive vitamin D supplements (172). Daily supplementation is important to help newborns build and maintain their vitamin D stores. The Canadian Paediatric Surveillance Program reported 12 cases of vitamin D deficiency-rickets per 100 000 infants between age 2-4 years (93). In an updated survey in 2015, they reported that vitamin D-deficiency rickets and severe symptomatic vitamin D deficiency remain a problem in Canada (94). This may in part be explained by non-compliance with infant vitamin D supplementation and low infant vitamin D status at birth.

1.8 Assessment of vitamin D status

1.8.1 Assays

Common types of assays used to measure serum 25(OH)D are classified as ligand-binding assays or liquid chromatography (LC)-based methods. Ligand-binding assays are typically used to measure total 25(OH)D, while LC-based methods are able to distinguish between different metabolites (173).

1.8.1.1 Ligand-binding assays

The first assay developed to detect serum 25(OH)D in the early 1970s was a competitive protein-binding assay (CPBA) based on the displacement of a ^{3}H-labeled 25(OH)D_3 reporter from DBP (174). Original CPBA methods required laborious procedures involving extraction and chromatographic purification, and are thus rarely used today (175). Immunoassays were developed in the 1980s and provided a more rapid method for measuring total 25(OH)D as they do not require extraction or prepurification of samples. Radioimmunoassays (RIA) use radiolabeled reporters and antibodies that are generally cospecific for 25(OH)D_2 and 25(OH)D_3 (175). RIA formed the basis for the development of chemiluminescent assays (CLIA), which do not require the handling and disposal of radioactive materials. CLIA uses reagents that have a longer shelf-life than those needed for RIA, and has a shorter running time (176). Automated CLIAs were developed to increase the throughput of these assays and are widely used in clinical and research laboratories (32). A common limitation for ligand-binding assays is that they also measure relatively inactive metabolites, such as 24,25$(OH)_2$D and other hydroxylated vitamin D metabolites, leading to a 10-20% overestimation of 25(OH)D concentrations (175).

1.8.1.2 Chromatography-based assays

High-performance liquid chromatography (HPLC) with UV spectrophotometry uses refined extraction and chromatographic steps to separate 25(OH)D from other polar metabolites, followed by quantification based on the UV absorbance of 25(OH)D (173). HPLC is reliable and allows separate measurements of 25(OH)D_2 and 25(OH)D_3 in serum, but is labor-intensive and requires more time than other methods. HPLC measurements also requires relatively large blood samples ($\geq$ 1 mL), which may be difficult to obtain from neonates (177). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is now considered the gold-standard technique because it is highly accurate and precise when properly validated (173). LC-MS/MS procedures can be

optimized to reliably quantify vitamin D metabolites including $25(OH)D_2$, $25(OH)D_3$, $24,25(OH)_2D$, $1,25(OH)_2D$ and 3-epi-$25(OH)D_3$. Concentrations of 3-epi-$25(OH)D_3$ may account for up to 60% of $25(OH)D_3$ measured in infants (28), and thus may lead to an overestimation of $25(OH)D_3$ concentrations in infant populations measured by LC-MS/MS if not resolved separately. Since $25(OH)D_3$ and its 3-epimer form have the same molecular mass, specific chromatographic techniques are required to separately quantify 3-epi-$25(OH)D_3$ and $25(OH)D_3$ when using LC-MS/MS (31). This is not a concern when using most commercial immunoassays because the antibodies used do not cross-react with 3-epi-$25(OH)D_3$ (29). LC-MS/MS the choice method of reference laboratories, but is not generally used in clinical laboratories as it requires a high level of technical expertise and training in addition to being time-consuming (173). Procedures for LC-MS/MS can now be semi-automated, however still have lower sample throughput than automated immunoassays.

1.8.2 Prediction using screening survey tools

There is conflicting evidence regarding the cost versus benefits of routine screening for vitamin D status during pregnancy (75,178). The Endocrine Society recommends universal screening for pregnant women (75), however this recommendation is not supported by the American College of Obstetricians and Gynecologists (179) or the Society of Obstetricians and Gynaecologists of Canada (180). Simple screening questionnaires and predictive models have been designed and validated to predict vitamin D deficiency in populations at high risk for fracture such as postmenopausal women and the elderly, as well as in otherwise healthy adult populations (Table 1.6). Developing a similar survey tool to identify neonates at high risk for vitamin D deficiency would be valuable.

Table 1.6. Summary of previously developed predictive models for low 25(OH)D

First author	Sample size	25(OH)D cut-off used	Predictors included	Results
Young adults				
Bolek-Berquist et al., 2009 *(U.S.A.)*	184	< 40 nmol/L	3	79% sensitivity 78% specificity
Mitchell et al., 2012 *(U.S.A.)*	634	< 50 nmol/L	5	AUC = 0.76
		< 75 nmol/L	5	AUC = 0.80
Postmenopausal women				
Millen et al., 2010 *(U.S.A.)*	2472	< 25 nmol/L	6	3% accuracy
Nabak et al., 2014 *(U.S.A.)*	609	< 50 nmol/L	6	82% sensitivity 56% specificity
Elderly adults				
Annweiler et al., 2015 *(France)*	1924	≤ 25 nmol/L	16	65% sensitivity 89% specificity AUC = 0.835 83% accuracy
		≤ 50 nmol/L	16	87% sensitivity 70% specificity AUC = 0.867 82% accuracy
		≤ 75 nmol/L	16	98% sensitivity 81% specificity AUC = 0.938 96% accuracy
Tran et al., 2013 *(Australia)*	644	< 25 nmol/L	8	74% sensitivity 73% specificity AUC = 0.82
		< 50 nmol/L	8	AUC = 0.73
Sohl et al., 2014 *(Netherlands)*	2609	< 30 nmol/L	10	62% sensitivity 81% specificity AUC = 0.80
		< 50 nmol/L	13	61% sensitivity 82% specificity AUC = 0.78

AUC: area under the receiver operating characteristic curve

Studies that aimed to identify low vitamin D status using a threshold of serum 25(OH)D < 75 nmol/L were slightly more successful than those to identify inadequacy or deficiency, likely because a larger proportion of the population falls within this category. Among 2472 postmenopausal women that participated in the Women's Health Initiative Calcium plus Vitamin D Clinical Trial between 1993 and 2000 (181), 21% of the variance in serum 25(OH)D concentrations was explained by a predictive model that included total vitamin D intake, waist circumference, physical activity, race-ethnicity, mean annual regional solar irradiance and age. The success rate of the predictive model to correctly classify women as severely deficient (serum 25(OH)D < 25 nmol/L), moderately deficient (≥ 25 to < 50 nmol/L) and insufficient (≥ 50 to < 75 nmol/L) was 3%, 59% and 64%, respectively. While this study was unsuccessful in identifying vitamin D deficiency (25(OH)D < 25 nmol/L), another large study among 1924 adults aged ≥ 65 years used a 16-question clinical diagnostic tool to predict hypovitaminosis D ≤ 25 nmol/L with 83% accuracy and a sensitivity and specificity of 65% and 89%, respectively (182). The predicting variables included questions relating to physical and emotional state, medical history and medication usage, but surprisingly did not include variables relating to vitamin D intake, sun exposure or skin color. Among 644 Australian adults ≥ 60 years of age who were essentially all Caucasian, 21% of the variance in serum 25(OH)D concentrations was explained by a predictive model that included factors related to sun exposure, vitamin D intake, age and BMI (183). The area under the receiver operating characteristic (ROC) curve for predicting serum 25(OH)D < 25 nmol/L was 0.82. In a sample of 2609 older Dutch adults, a model predicted concentrations < 30 nmol/L with a sensitivity, specificity and area under the ROC curve (AUC) of 84%, 66% and 0.8 (184). They included variables such as alcohol consumption, smoking habits, outdoor activities, poor appetite and living without a partner.

A study in Wisconsin used a short three-question survey to predict 25(OH)D < 40 nmol/L in 184 young adults (185). Their significant predictors were sun tanning in the last 12 months, tanning booth use in the past year and daily consumption of two or more servings of milk. Using as few as 3 simple and easily assessable risk factors, this questionnaire was moderately successful, with a sensitivity of 79% and specificity of 78%. A similar study among 634 adults in Massachusetts predicted 25(OH)D ≤ 50 nmol/L with an AUC of 0.76, and included 5 variables: sex, age, self-identified race, season and multivitamin use (186). A vitamin D and sun (VIDSUN) questionnaire had a sensitivity of 89% and a specificity of 35% for predicting 25(OH)D < 50 nmol/L among 609 postmenopausal women in Wisconsin (187). The six questions used were: black vs. other races, BMI ≤ vs. > 28 kg/m^2, sun tanning in the past year, sun exposure in the past three months, sunscreen use and supplemental vitamin D intake.

Among the studies described above, sample sizes ranged from 184 to 2609. Studies with larger sample sizes often separated their participants into 'developing/training' and 'testing/validating' subsamples for the predictive models. The majority of studies used backwards elimination with logistic regression to develop predictive models. One study used a novel approach of feed forward artificial neural networks (182), with successful results. Sensitivity and specificity of models predicting low vitamin D concentrations were 61-98% and 35-90%, respectively; AUC ranged from 0.73 to 0.94. Predictive variables that reappeared frequently among studies were anthropometric measures, supplement intake, sun exposure and skin color. Based on the different target populations, it is evident that including some risk factors that are specific to the population generate a more successful screening tool than those which included only general predictors such as supplementation and sun exposure.

2 Rationale and objectives

Low vitamin D status is common amongst newborns in Canada, putting many at risk for complications if left untreated (5). Daily supplementation is recommended for all breastfed and partially breastfed infants for the first year of life in order to maintain healthy vitamin D stores (88). Supplements are especially important to improve vitamin D status in neonates born with inadequate or deficient vitamin D stores. Only two thirds of breastfed newborns in Canada are receiving the recommended daily vitamin D supplement of 400 IU (91), which may be one of the reasons that cases of vitamin D-deficiency rickets and severe symptomatic vitamin D deficiency continue to be reported in Canada (94). In Quebec, the public healthcare system covers the cost of vitamin D supplements for newborns, yet the rate of compliance with supplementation recommendations is similar to that in the rest of the country (92). Free supplements may not be sufficient to improve infant vitamin D supplementation practices. There is some evidence that targeted education can improve infant vitamin D status. A randomized controlled trial in Norway provided educational pamphlets and vitamin D supplements to immigrant mothers of 6-week old infants (188). After 7 weeks, the mean increase in serum 25(OH)D was 28 nmol/L higher for the infants in the intervention group than the control group receiving standard care. In order for healthcare professionals to provide targeted education to families whose infants are vitamin D deficient or to follow-up with them in a meaningful way, it will be important to identify those who are at high risk for deficiency. While routine vitamin D screening is not part of the standard blood panel, nor is it generally recommended, simple survey tools have been developed to identify individuals at high risk for vitamin D deficiency. Providing results from a validated screening tool that indicate an infant is at high risk for deficiency may also motivate parents to follow supplementation guidelines and subsequently improve infant health outcomes.

The literature is lacking in vitamin D status screening tools for neonates and infants. Therefore, the objectives of this study were to: 1) identify predictors of neonatal vitamin D deficiency; and 2) develop and test the validity of a screening survey tool to identify newborns at high risk for vitamin D deficiency in a sample of healthy, racially diverse neonates from Montreal, Quebec. It is hypothesized that maternal sun exposure during pregnancy, maternal vitamin D supplementation, skin color and season of delivery will be important predictors that are needed for the screening tool to have a high sensitivity and specificity.

3 Manuscript

Validity of a screening survey tool to identify neonates at high risk for vitamin D deficiency

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3.1 Abstract

Background: Many Canadian infants are born with low vitamin D stores, putting them at risk of developing complications such as rickets, if untreated. Newborns are not routinely screened for vitamin D deficiency (25-hydroxyvitamin D (25(OH)D) < 30 nmol/L), and not all parents give their infants supplements. Identifying those at high risk may facilitate targeted education to parents regarding supplementation for infants.

Objective: The objective was to develop a screening survey tool based on risk factors that can be used to easily identify term newborns at high risk for vitamin D deficiency.

Design: Healthy mother-infant pairs (n = 1112) were recruited at the Lakeshore General Hospital, Montreal, from March 2016 to March 2019. Parental demographic and lifestyle factors were surveyed. Newborn serum 25(OH)D was measured from blood samples collected < 36 h after birth. Logistic regression models were used to identify key variables associated with vitamin D deficiency. Receiver operating characteristic (ROC) curves were used to demonstrate sensitivity and specificity of the screening tool against known vitamin D status.

Results: Mothers (age 32.4 ± 4.5 y) were mostly white (58%), 83% had completed some post-secondary education and 91% reported consuming a prenatal supplement during their pregnancy. Neonates were term born (39.4 ± 2.6 wk gestation), appropriate weight for gestational age (3394 ± 393 g) and 51% were female. Mean neonatal serum 25(OH)D concentration was 44.5 ± 19.9 nmol/L, with 23% (95% CI 0.20, 0.25) below 30 nmol/L. Six risk factors were most predictive of neonatal vitamin D deficiency, including: minimal supplementation during pregnancy (< 2-3 times/wk), non-white skin color and delivery from October through April (P < 0.001). The screening tool had a sensitivity of 68.8% and specificity of 70.0%. Area under the ROC curve was 0.712 (95% CI 0.679, 0.745; P < 0.001).

Conclusions: This screening survey tool consisting of 6 easily assessable risk factors identified newborns with serum 25(OH)D < 30 nmol/L with moderate accuracy. It may be useful to identify neonates at high risk for vitamin D deficiency, however it will need to be validated in other populations before being used in clinical practice.

3.2 Introduction

Low vitamin D status is common amongst newborns in Canada, putting many at risk for complications if left untreated (5). Neonatal vitamin D status, measured using serum 25-hydroxyvitamin D (25(OH)D) concentration, is dependent on maternal-fetal transfer during pregnancy. As such, factors that affect maternal vitamin D status before delivery are also predictors of neonatal vitamin D status. At latitudes greater than 45° N, such as in Winnipeg and Montreal, the intensity of UVB rays are insufficient for cutaneous vitamin D production for at least half the year (106). At these greater latitudes, vitamin D production is additionally limited before 10 AM and after 3 PM (105). Lack of UVB radiation from sun exposure is one of many risk factors for low vitamin D status, especially in Canada.

Daily supplementation is recommended for all breastfed and partially breastfed infants for the first year of life in order to maintain healthy vitamin D stores. Supplementation is especially important to improve vitamin D status in neonates born with inadequate or deficient vitamin D stores. Only two thirds of breastfed newborns in Canada receive the recommended daily vitamin D supplement of 400 IU (91), which may partly explain why cases of vitamin D-deficiency rickets and severe symptomatic vitamin D deficiency continue to be reported in Canada (94).

Complications of vitamin D deficiency in infancy may be acute, such as hypocalcemic seizures, or more chronic such as delayed dentition, delayed gross motor milestones and rickets (86), whose consequences may persist into childhood and onwards. They may also include long-term adverse health outcomes, such as impaired developmental outcomes, suboptimal bone mineralization and increased risk of fractures (86,167,168), vulnerability to respiratory infections (141), asthma (56,58) and type 1 diabetes (65). These complications are severe, and yet can be largely avoided if infants are given a daily 400 IU vitamin D supplement to ensure they are maintaining healthy vitamin D stores.

In Quebec, the public healthcare system covers the cost of vitamin D supplements for newborns, yet the rate of compliance with supplementation recommendations is similar to that in the rest of the country (92). Free supplements may not be sufficient to improve vitamin D supplementation practices. There is some evidence that targeted education can improve infant vitamin D status (188). In order to provide targeted education or recommendations to families whose infants are vitamin D deficient, it will be important to identify those who are at high risk for deficiency. While routine vitamin D screening is not part of the standard blood panel, nor is it generally recommended, simple survey tools have been developed to identify individuals at high risk for vitamin D deficiency (182,185,187). A screening tool may serve to inform clinicians and parents on the newborn's risk of vitamin D deficiency. Providing results from a validated screening tool may also encourage parents to follow supplementation guidelines and subsequently improve infant health outcomes.

The literature is lacking in screening tools for vitamin D status in neonates and infants. Therefore, the objectives of this study were to: 1) identify predictors of neonatal vitamin D deficiency; and 2) develop and test the validity of a screening survey tool to identify newborns at high risk for vitamin D deficiency in a sample of healthy, racially diverse neonates from Montreal, Quebec. It is hypothesized that maternal sun exposure during pregnancy, maternal vitamin D supplementation, skin color and season of delivery will be important predictors that are needed for the screening tool to have a high sensitivity and specificity.

3.3 Subjects and Methods

3.3.1 Study population

Between March 6, 2016 and March 6, 2019, 3917 participants were assessed for eligibility within 36 hours of delivery at the Lakeshore General Hospital in Montreal, QC (Figure 3.1). Inclusion criteria for screening are singleton pregnancies, healthy infants born to term (37-

42 weeks gestation) and of appropriate weight for gestational age (2500-4300 g for boys; 2400-4200 g for girls). Additionally, mothers of all ages, skin color and pre-pregnancy BMI were recruited for screening. Exclusion criteria were maternal preeclampsia, Crohn's disease, celiac disease and liver or kidney disease, as well as small and large weight for gestational age as all of these factors relate to altered maternal-fetal transfer of nutrients. Hospital staff identified 3205 as eligible, and of those, 1381 healthy mother-infant pairs agreed to participate. Signed consent was obtained from mothers before beginning screening, which involved the administration of a questionnaire and collection of a blood sample from the newborn.

3.3.2 Demographic/lifestyle questionnaire and pregnancy history

After obtaining consent, researchers conducted a brief survey of parental demographics and lifestyle characteristics. Researchers were trained to ensure uniformity in the questioning process and to minimize interviewer bias. Mothers were surveyed on: intent to breastfeed, use of prescription medication during pregnancy, vitamin and mineral supplement use before and during pregnancy, smoking habits before and during pregnancy, exposure to second-hand smoke during pregnancy, physical activity before and during pregnancy, sun exposure and travel to a sunny destination during pregnancy and previous vitamin D recommendations by health professionals. Information for both parents regarding ethnicity, race, income, immigration status and education were also surveyed, using descriptors as defined by Statistics Canada. Finally, hospital charts were used to collect mothers' pre-pregnancy weight, weight at delivery, height, type of delivery, birth complications, gravidity and parity, as well as infant gestational age at birth, birth weight, length and head circumference and APGAR scores. All data was collected and managed using REDCap (Research Electronic Data Capture, v7.4).

3.3.3 Blood sample collection and analysis

Nurses collected a 500 µl capillary blood sample (Capiject, Terumo Corp.) from infants at the same time as routine phenylketonuria testing, between 24-36 hours after delivery. Samples were stored in a fridge at 4 °C until transported to the Mary Emily Clinical Nutrition Research Unit, McGill University. Blood samples were centrifuged at 4000 RPM for 15 minutes at 6 °C to obtain serum for biochemical analysis. Serum that was not analyzed immediately was stored at -80 °C until analysis. Total 25(OH)D in infant serum samples was measured using an automated chemiluminescent immunoassay (Liaison, Diasorin Inc.). This assay has a lower limit of detection of 10 nmol/L, and agrees well with liquid chromatography tandem mass spectroscopy based on a subset of samples (CLIA: 45.6 ± 19.7 vs. LC-MS/MS: 45.9 ± 16.9 nmol/L, n = 211). The mean intra-assay coefficient of variation was < 10%. The laboratory is certified by the Vitamin D External Quality Assessment Scheme (DEQAS) to ensure reliability of the assay and uses National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 972a Level 1-4 quality control samples to confirm accuracy of measurements. The measured 25(OH)D concentrations were standardized according to Vitamin D Standardization Program (VDSP) protocols (189), and calibrated using Deming regression to fit the originally measured 25(OH)D values to NIST reference measurement procedures.

3.3.4 Screening tool design

Neonatal serum 25(OH)D concentrations were classified as being at high risk of deficiency (< 30 nmol/L) or not deficient (≥ 30 nmol/L), as defined by the Institute of Medicine (2). Questions for the screening survey tool were developed based on risk factors for neonatal vitamin D deficiency according to the literature. Therefore, content validity of the screening tool was based on the following 21 potential predictors: age at delivery, pre-pregnancy BMI, gestational weight gain according to pre-pregnancy BMI, maternal and paternal skin color, parity,

maternal and paternal education, recent maternal immigration to Canada, household income, prenatal supplement intake before and during pregnancy, vitamin D supplement (≥ 1000 IU/d) intake before and during pregnancy, prior vitamin D recommendations by healthcare professionals, season of delivery, sun exposure during the 3rd trimester (if April through October), travel to a sunny destination during the 3rd trimester, sunscreen use, maternal smoking during pregnancy, second-hand smoke exposure during pregnancy. Each risk factor was transformed into a simple question with a dichotomous answer. Answers to questions were assigned either '0', for low risk of vitamin D deficiency, or '1', for high risk of vitamin D deficiency. Data collected from study participants was recoded into this binary model for analysis.

Annual family income was analyzed based on the 2015 median total income of Canadian households and low-income thresholds for families (190). Excessive gestational weight gain is determined based on pre-pregnancy BMI according to weight gain guidelines established by the IOM and Health Canada (152). Self-reported race was used as a surrogate for maternal skin color. In order to minimize errors in assumption that this may create, mothers were classified as white or non-white depending on whether they identified themselves as 'white' or any other race. Other studies have had success using simplified race categories as a proxy for skin pigmentation (181,186,187). Season of delivery was explored according astronomical seasons using the equinox and solstice dates, Northern meteorological seasons using the first day of the months that include the equinoxes and solstices, as well as by individual month.

3.3.5 Ethical approval

The study was reviewed and approved by the St. Mary's Hospital Research Ethics Committee that oversees multiple hospitals including the recruiting site. All data used for this study were collected as part of the screening phase of an ongoing double-blind randomized controlled trial (clinicaltrials.gov identifier NCT02563015).

3.3.6 Statistical analyses

A total of 1112 mother-infant pairs were included in the analyses. All statistical analyses were performed using SAS University Edition (v9.4, SAS Institute Inc., Carry, NC, US). Statistical significance was be set at $P < 0.05$ for all tests. For descriptive statistics, data are presented as mean and standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. Differences in proportions of participants were tested using Fisher's exact test.

All variables were dichotomized, assigning a value of "1" to risk factors of vitamin D deficiency, and "0" to others. For categorical variables with no clearly defined cut-off, performance characteristics were explored at different cut-offs in order to determine the level at which the variable best predicted vitamin D deficiency. For variables that were continuous rather than categorical, receiver operating characteristic (ROC) curves were used to determine an appropriate cut-off value.

Potential risk factors were tested for multicollinearity with variance inflation factors (VIF). If several risk factors were deemed highly correlated with each other, only one was considered for further analysis. Odds ratios (OR) for vitamin D deficiency given each of the potential risk factors were calculated using logistic regression analysis. The final predictive model was obtained using backwards elimination of non-significant variables. Regression coefficients (β) for each risk factor in the final regression model were transformed into individual risk scores by multiplying by an appropriate constant and rounding to the nearest integer. The individual risk scores for each participant were summed to give the participant a total risk score. The average total risk score was used as the cut-off to classify participants as at high or low risk for vitamin D deficiency. Previous studies have used similar approaches to develop their predictive models (182–187).

Finally, the ability of the survey tool to correctly predict neonatal vitamin D deficiency was determined using ROC curves to demonstrate sensitivity and specificity of the tool against known vitamin D status.

3.4 Results

From the 1381 participants recruited for the study (Figure 3.1), 242 were excluded from analysis because the newborn blood sample was not collected or the sample obtained was of insufficient volume to measure 25(OH)D concentration. An additional 27 participants were excluded due to missing data such as maternal age at delivery and pre-pregnancy BMI. Characteristics between those included and excluded in analysis did not differ in terms of maternal age, skin color, education or income, but did differ in terms of supplementation during pregnancy, sun exposure and smoking habits (Table 3.1).

Mothers were mostly white (58%), 40% were overweight and only 5% smoked during their pregnancy. Most mothers (83%) had completed some post-secondary education and only 28% had annual household incomes below the Canadian median. Prenatal supplementation was common, with 60% not consuming a supplement before pregnancy and only 7% not consuming a supplement during pregnancy. Neonates were term born (39.4 ± 2.6 wk gestation), appropriate weight for gestational age (3394 ± 393 g) and 51% were female (Table 3.2). Mean neonatal serum 25(OH)D concentration was 44.5 ± 19.9 nmol/L, with 23% < 30 nmol/L and 65% < 50 nmol/L.

Maternal characteristics were significantly different between groups (Table 3.3), particularly in terms of maternal age, skin color, family income, vitamin D intake before and during pregnancy, season of delivery, sun exposure and sunscreen use. Half the women in both groups had never been recommended a vitamin D supplement for themselves or their neonate by

a healthcare professional. Rates of smoking during pregnancy and exposure to second hand smoke were similarly low in both groups.

The majority of the risk factors were individually associated with a higher risk of neonatal vitamin D deficiency (Table 3.4). Maternal sunscreen use was associated with a lower risk of neonatal vitamin D deficiency, while no prior vitamin D recommendations, smoking and second-hand smoke exposure did not significantly affect the odds of deficiency.

Testing for multicollinearity led to the exclusion of 3 variables that were highly correlated with others: paternal skin color, education and immigration status. Thus only 18 variables were considered in the logistic regression analyses. After backwards selection in the multivariate regression model, 6 risk factors were predictive of neonatal vitamin D deficiency (Table 3.6). In this model, mothers who were not white or did not take a prenatal supplement during pregnancy had the greatest odds of having a vitamin D deficient infant. The model was moderately successful in predicting neonatal vitamin D deficiency, with an AUC of 0.712 (95% CI 0.679, 0.745; $P < 0.001$), a sensitivity of 68.8% and specificity of 70.0% (Table 3.6).

Table 3.1. Comparison of characteristics with participants excluded from analysis

Maternal Characteristics*	Included (n = 1112)	Excluded (n = 269)	P†
Age at delivery (y)			
< 25.0	75 (7%)	29 (11%)	0.538
< 26.0	106 (10%)	34 (13%)	0.764
< 27.0	146 (13%)	43 (16%)	0.971
Pre-pregnancy BMI (kg/m^2)			
< 18.5 or ≥ 25	475 (43%)	110 (48%)	0.159
≥ 25	440 (40%)	100 (37%)	0.468
≥ 30	161 (14%)	33 (12%)	0.330
Excessive gestational weight gain	482 (43%)	108 (40%)	0.340
Skin color, Non-white	464 (42%)	113 (42%)	0.933
Parity			
≥ 2	641 (58%)	152 (57%)	0.736
≥ 3	216 (19%)	55 (20%)	0.709
Education			
≤ High school	184 (17%)	48 (18%)	0.617
< University	465 (42%)	127 (47%)	0.112
Annual family income			
< $30,000	79 (7%)	24 (9%)	0.340
< $70,000	310 (28%)	80 (30%)	0.548
Recent immigration (last 5 years)	138 (12%)	41 (15%)	0.188
Supplement use 3 mo before pregnancy			
None	663 (60%)	173 (64%)	0.153
< 2-3 times/week	670 (60%)	175 (65%)	0.142
< Almost everyday	699 (63%)	183 (68%)	0.107
Supplement use during pregnancy			
None	83 (7%)	37 (14%)	0.005
< 2-3 times/week	96 (9%)	39 (14%)	0.012
< Almost everyday	161 (14%)	54 (20%)	0.036
No vitamin D supplement use (≥ 1000 IU/day)			
3 mo before pregnancy	1036 (93%)	258 (96%)	0.055
During pregnancy	1055 (95%)	261 (97%)	0.081
Month of delivery, October through April	628 (56%)	132 (49%)	0.030
Sun exposure during 3rd trimester			
≤ 15 min/day	849 (76%)	191 (71%)	0.081
≤ 30 min/day	855 (77%)	195 (72%)	0.144
≤ 60 min/day	982 (88%)	230 (86%)	0.234
Sunscreen use	190 (17%)	49 (18%)	0.666
No winter travel to sunny destination	439 (39%)	88 (33%)	0.036
No prior vitamin D recommendations	554 (50%)	139 (53%)	0.375
Smoked during pregnancy			
≥ Occasionally	55 (5%)	23 (9%)	0.049
Daily	42 (4%)	18 (7%)	0.075
Second-hand smoke exposure	85 (8%)	25 (9%)	0.397

Note: BMI = body mass index

*Data analyzed and presented based on maternal characteristics at different cut-off levels

†Fisher's exact test

Table 3.2. Descriptive characteristics of neonates (n = 1112)

Characteristics	Mean ± SD or n (%)
Gestational age at birth (wk)	39.4 ± 2.6
Birthweight (g)	3394 ± 393
Female	568 (51%)
APGAR score, 1 min	8.8 ± 1.3
5 min	9.4 ± 0.8
Serum 25(OH)D concentration (nmol/L)	44.5 ± 19.9
< 30 nmol/L	254 (23%)
< 50 nmol/L	718 (65%)
Season of blood collection, Summer	285 (26%)
Fall	263 (24%)
Winter	270 (24%)
Spring	294 (26%)

Table 3.3. Comparison of maternal characteristics based on neonatal vitamin D status

Maternal Characteristics*	Serum 25(OH)D Concentration		P†
	< 30 nmol/L (n = 254)	≥ 30 nmol/L (n = 858)	
Age at delivery (y)			
< 25.0	32 (13%)	43 (5%)	<0.001
< 26.0	46 (18%)	60 (7%)	<0.001
< 27.0	56 (22%)	90 (10%)	<0.001
Pre-pregnancy BMI (kg/m^2)			
< 18.5 or ≥ 25	133 (52%)	342 (40%)	<0.001
≥ 25	125 (49%)	315 (37%)	<0.001
≥ 30	41 (16%)	120 (14%)	0.407
Excessive gestational weight gain	121 (48%)	361 (42%)	0.119
Skin color, Non-white	157 (62%)	307 (36%)	<0.001
Parity			
≥ 2	155 (61%)	486 (57%)	0.212
≥ 3	62 (24%)	154 (18%)	0.032
Education			
≤ High school	54 (21%)	130 (15%)	0.033
< University	114 (45%)	351 (41%)	0.264
Annual family income			
< $30,000	32 (13%)	47 (5%)	0.002
< $70,000	98 (39%)	212 (25%)	<0.001
Recent immigration (last 5 years)	48 (19%)	90 (10%)	0.002
Supplement use 3 mo before pregnancy			
None	185 (73%)	478 (56%)	<0.001
< 2-3 times/week	188 (74%)	482 (56%)	<0.001
< Almost everyday	193 (76%)	506 (59%)	<0.001
Supplement use during pregnancy			
None	43 (17%)	40 (5%)	<0.001
< 2-3 times/week	50 (20%)	46 (5%)	<0.001
< Almost everyday	77 (30%)	84 (10%)	<0.001
No vitamin D supplement use (≥ 1000 IU/day)			
3 mo before pregnancy	246 (97%)	790 (92%)	0.001
During pregnancy	249 (98%)	806 (94%)	0.001
Month of delivery, October through April	179 (70%)	449 (52%)	<0.001
Sun exposure during 3rd trimester			
≤ 15 min/day	220 (87%)	629 (73%)	<0.001
≤ 30 min/day	218 (86%)	637 (74%)	<0.001
≤ 60 min/day	240 (94%)	742 (86%)	<0.001
Sunscreen use	24 (9%)	166 (19%)	<0.001
No winter travel to sunny destination	129 (51%)	310 (36%)	<0.001
No prior vitamin D recommendations	129 (51%)	425 (50%)	0.775
Smoked during pregnancy			
≥ Occasionally	11 (4%)	44 (5%)	0.592
Daily	9 (4%)	33 (4%)	0.821
Second-hand smoke exposure	23 (9%)	62 (7%)	0.363

Note: 25(OH)D = 25-hydroxyvitamin D; BMI = body mass index

*Data analyzed and presented based on maternal characteristics at different cut-off levels

†Fisher's exact test

Table 3.4. Identification of cut-off thresholds for key variables associated with neonatal vitamin D deficiency

Maternal Characteristics*	Crude OR† (95% CI)	P
Age at delivery (y)		
< 25.0	2.99 (1.82, 4.92)	<0.001
< 26.0	3.14 (2.06, 4.79)	<0.001
< 27.0	2.51 (1.73, 3.65)	<0.001
Pre-pregnancy BMI (kg/m^2)		
< 18.5 or ≥ 25	1.66 (1.25, 2.20)	<0.001
≥ 25	1.67 (1.26, 2.22)	<0.001
≥ 30	1.18 (0.80, 1.74)	0.391
Excessive gestational weight gain	1.25 (0.95, 1.66)	0.116
Skin color, Non-white	2.90 (2.18, 3.88)	<0.001
Parity		
≥ 2	1.20 (0.90, 1.60)	0.215
≥ 3	1.48 (1.06, 2.06)	0.023
Education		
≤ High school	1.51 (1.06, 2.15)	0.022
< University	1.18 (0.89, 1.56)	0.26
Annual family income		
< $30,000	2.49 (1.55, 3.99)	<0.001
< $70,000	1.91 (1.42, 2.57)	<0.001
Recent immigration (last 5 years)	1.99 (1.36, 2.92)	<0.001
Supplement use 3 mo before pregnancy		
None	2.13 (1.57, 2.90)	<0.001
< 2-3 times/week	2.22 (1.63, 3.03)	<0.001
< Almost everyday	2.20 (1.60, 3.03)	<0.001
Supplement use during pregnancy		
None	4.17 (2.64, 6.58)	<0.001
< 2-3 times/week	4.33 (2.82, 6.64)	<0.001
< Almost everyday	4.01 (2.83, 5.69)	<0.001
No vitamin D supplement use (≥ 1000 IU/day)		
3 mo before pregnancy	2.65 (1.25, 5.58)	0.011
During pregnancy	3.21 (1.27, 8.13)	0.014
Month of delivery, October through April	2.17 (1.61, 2.94)	<0.001
Sun exposure during 3rd trimester		
≤ 15 min/day	2.10 (1.43, 3.09)	<0.001
≤ 30 min/day	2.36 (1.59, 3.48)	<0.001
≤ 60 min/day	2.68 (1.51, 4.75)	0.001
Sunscreen use	0.43 (0.28, 0.68)	<0.001
No winter travel to sunny destination	1.82 (1.38, 2.42)	<0.001
No prior vitamin D recommendations	1.04 (0.79, 1.38)	0.775
Smoked during pregnancy		
≥ Occasionally	0.84 (0.43, 1.65)	0.607
Daily	0.92 (0.43, 1.95)	0.824
Second-hand smoke exposure	1.28 (0.77, 2.11)	0.336

Note: BMI = body mass index

*Data analyzed at different cut-off levels in order to identify the threshold at which the variable is most strongly associated with neonatal vitamin D deficiency for inclusion in final screening tool.

†Odds ratios calculated using univariate logistic regression analysis

Table 3.5. Risk profile for neonatal vitamin D deficiency

Maternal Characteristics	Adjusted OR* (95% CI)	Regression coefficient	Risk score
Age at delivery, < 26.0 y	2.509 (1.586, 3.969)	0.9199	6
Pre-pregnancy BMI, < 18.5 or ≥ 25	1.65 (1.214, 2.243)	0.5009	3
Skin color, Non-white	3.065 (2.245, 4.183)	1.1199	7
Supplement use < 2-3 times/wk			
3 mo before pregnancy	1.676 (1.195, 2.351)	0.5163	3
During pregnancy	3.761 (2.341, 6.04)	1.3246	8
Month of delivery, October through April	2.548 (1.838, 3.531)	0.9352	6

*Odds ratios calculated using multivariate logistic regression analysis

Table 3.6. Validity of the 6-question screening tool to predict neonatal vitamin D deficiency

Parameter	% or value
Sensitivity	68.8%
Specificity	70.0%
Positive predictive value	39.9%
Negative predictive value	88.6%
Area under the curve (AUC)	0.712

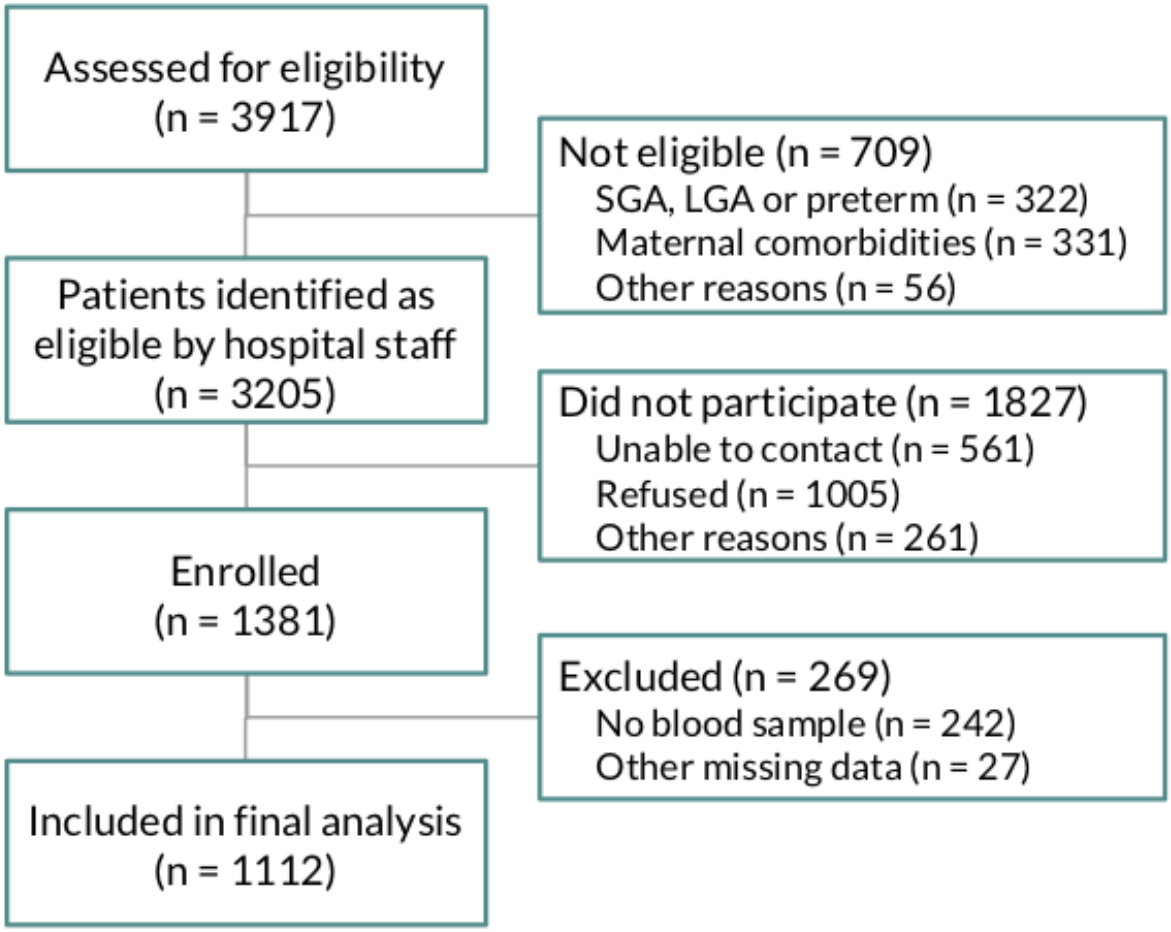

Figure 3.1. Recruitment flow diagram

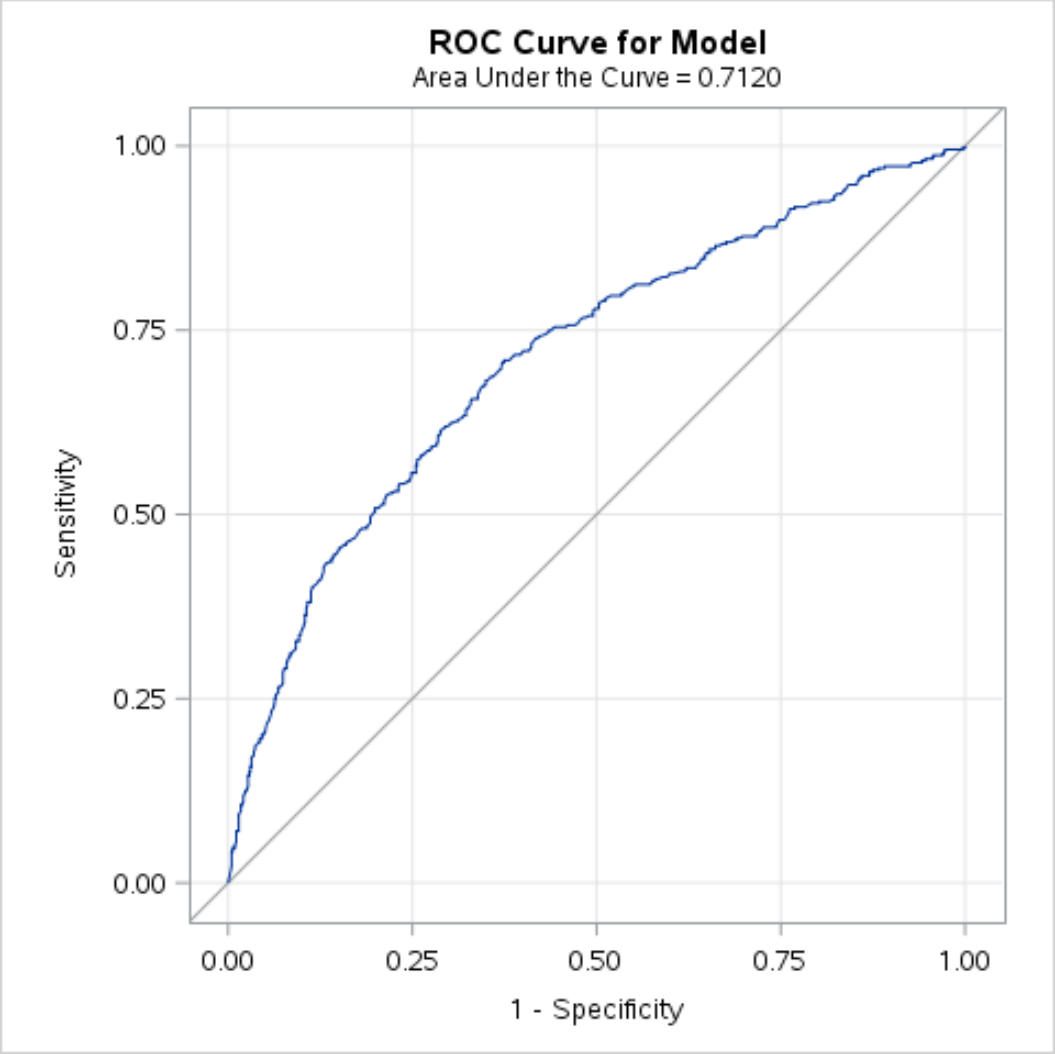

Figure 3.2. Receiver operating characteristic (ROC) curve for the prediction of neonatal vitamin D deficiency using the screening tool

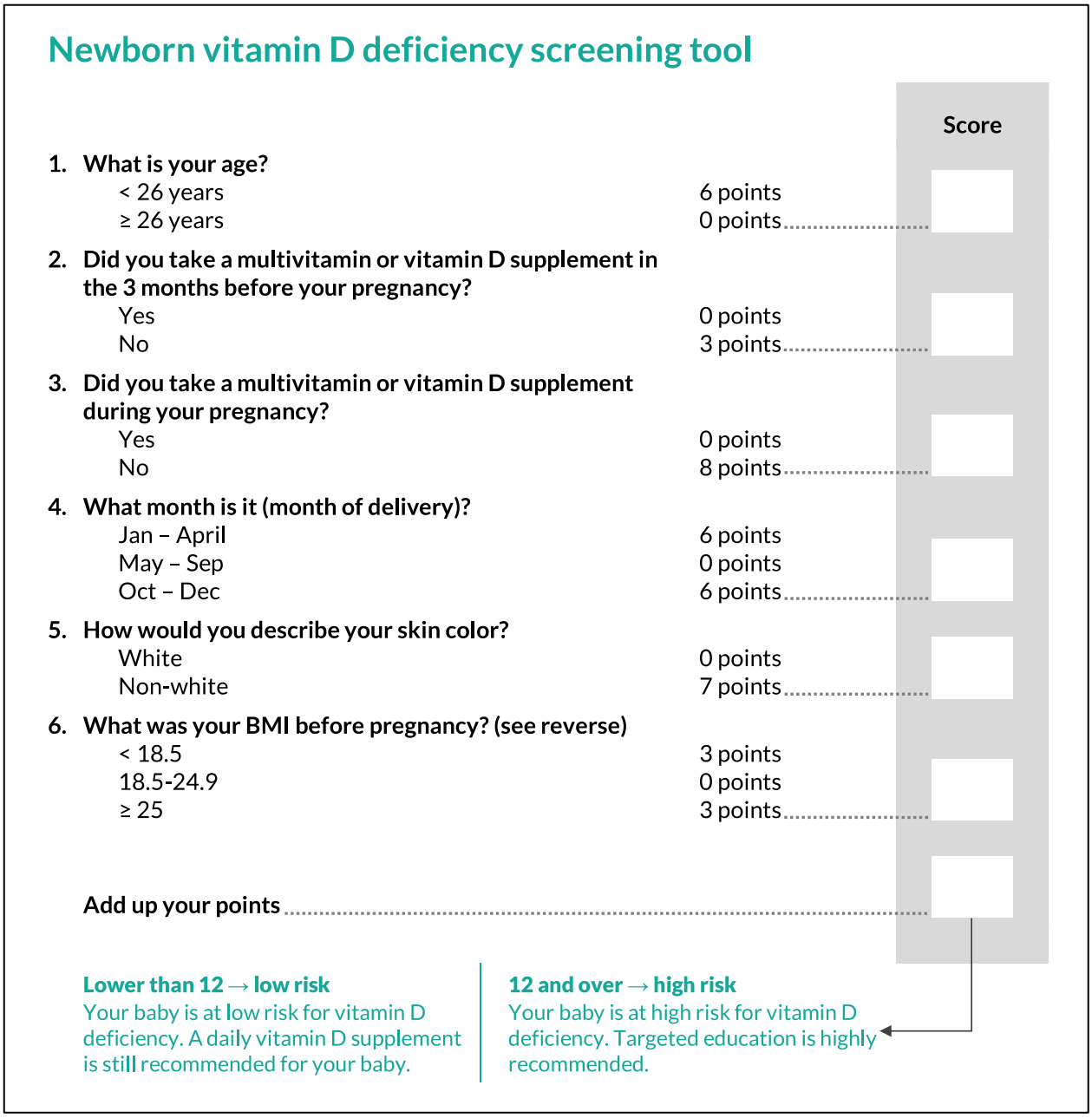

Figure 3.3. Example of a screening questionnaire to identify neonatal vitamin D deficiency

3.5 Discussion

This simple screening tool, consisting of 6 easily assessable variables, developed in this study was able to predict neonatal vitamin D deficiency (25(OH)D < 30 nmol/L) with moderate accuracy. Several studies have designed screening tools to identify low vitamin D status in adults, but this is the first to have developed a screening tool aiming to identify deficiency in newborns. Methods used in other studies involve linear regression models (184,185,187) or more complex methods, such as feed forward artificial neural networks (182) to develop their tools. While this allows for more flexibility in the interpretation of risk factors, and thus prediction with higher accuracy, the goal of the present study was to create a simple and effective tool that is accessible and easy to use in clinical practice. An example of such a tool is shown in Figure 3.3, which can be easily used by clinicians or patients themselves, and can be translated for use in French or other languages.

The Canadian Paediatric Society provides guidance to families and physicians outlining characteristics of infants at high risk for vitamin D deficiency (191). These are: infants who are breastfed, have darker skin, live in northern communities and those whose mothers have low vitamin D. The current screening tool was developed from a single hospital sample and thus doesn't include infants from northern communities, nor does it include breastfeeding status as a risk factor since it aims to identify vitamin D deficiency at birth. Infant vitamin D status at birth is largely determined by and highly correlated to maternal status around the time of delivery (8,40). The variables included in the final screening tool, such as vitamin intake, season of delivery and skin color, represent important risk factors for low maternal vitamin D status.

As expected, non-white maternal skin color, not consuming prenatal supplements and delivering in the winter and spring months (8,118) were the most important predictors of neonatal serum 25(OH)D < 30 nmol/L. Not consuming a multivitamin supplement during the 3 months

before pregnancy further increased the odds of neonatal vitamin D deficiency, presumably because consuming a supplement over this increased period of time would have allowed mothers to improve their own circulating concentrations of 25(OH)D given a longer period of time.

Older age is often associated with lower serum 25(OH)D concentrations (182,183), however, in the current study, mothers under the age of 26 were more likely to have neonates with vitamin D deficiency. It could be hypothesized that older mothers have had repeated exposure to physician advice, or are more conscientious of supplementation and healthy dietary habits during pregnancy, thus leading to better vitamin D status (133). Sunscreen use decreased the odds of neonatal vitamin D deficiency. This may be explained by increased sun-seeking behaviours in individuals who report using sunscreen (126). Sun exposure was likely not the main source of vitamin D for 76% of mothers in the study, who had < 15 min/d of sun exposure during their 3rd trimester. This proportion includes all mothers delivering during the fall and winter months (from October to April). Spending less than 30 minutes per day in the sun during the 3rd trimester was a predictor of neonatal vitamin D deficiency, however it was not significant after adjusting for other variables in the final model. Inconsistencies in reporting by mothers, as well as recall bias may affect the interpretation and thus the usefulness of this data in the screening tool.

Previous studies reporting the incidence of neonatal low vitamin D status in North American cities had predominantly white or black infants (78,169–171). While these data are extremely valuable, a strength of the current study is the racial diversity of the study population and the presence of recent immigrants, which may make it more representative of urban Canadian cities. Based on a large, predominantly white cohort in Quebec City, 24% of newborns are born with low vitamin D status (cord blood 25(OH)D < 50 nmol/L) (169). The current study showed a 65% prevalence rate for neonatal 25(OH)D < 50 nmol/L and 23% for 25(OH)D < 30 nmol/L. The

large difference in prevalence rates between studies may be attributed to the higher percentage of non-white mothers, further demonstrating that maternal skin pigmentation is an important predictor of vitamin D status. Overall, only 17% of mothers had completed high school as their highest level of education and 28% had annual household incomes below the Canadian median of $70,000. While the crude ORs for these variables indicated associations with neonatal vitamin D deficiency, they were not significant in the current model. It is possible that they may be significant independent predictors of neonatal vitamin D status in samples with mothers of more diverse educational backgrounds and lower incomes.

Previous studies have found that mothers who smoked had neonates with significantly lower 25(OH)D concentrations than non-smokers (153). No association between maternal smoking during pregnancy and neonatal vitamin D status was found in our study, however, this may be due to only 4% of the mothers being smokers. By excluding preterm and SGA infants from this study, it is likely that the confounding effects of smoking have been removed in many ways (192).

This study had several limitations. Data regarding maternal dietary intake was not available in all participants and may have contributed significantly to total vitamin D intake in certain mothers. Previously developed questionnaires have successfully included "consuming ≥ 2 servings of fluid milk per day" as an easily assessable variable in their predictive models (185). Revising the questionnaire to include dietary intake, simplified in terms of consumption habits or servings of vitamin D fortified dairy products and fatty fish, might improve its accuracy.

Additional work would need to be done to evaluate the external validity of the screening tool and its feasibility of use by healthcare workers or parents. Furthermore, the tool should be validated for use in different populations with varied socioeconomic and educational backgrounds, as well as for use in neonates of all birthweight categories. A screening tool with a

higher sensitivity and specificity that is valid for use in Canadian neonates could be an important tool in the overall goal of improving infant vitamin D status. Identifying those at high risk for vitamin D deficiency, without performing additional blood tests, would be an inexpensive way to inform clinicians and parents about their child's risk of deficiency, and perhaps encourage the importance of daily vitamin D supplementation.

4 Extended discussion

The objectives of this study were to identify predictors of neonatal vitamin D deficiency and use these variables to develop a simple screening survey that can be used to identify newborns at high risk for vitamin D deficiency. As seen in previous studies, age at delivery, pre-pregnancy body mass index (BMI), maternal skin color, parity, maternal education, recent immigration to Canada, household income, prenatal supplement use, season of delivery, sun exposure during the 3rd trimester and winter travel to a sunny destination were found to be independent predictors of neonatal vitamin D status. Neonates born to women who consumed a prenatal supplement less than twice per week during pregnancy had nearly 4 times the odds of serum 25-hydroxyvitamin D (25(OH)D) < 30 nmol/L. Data regarding frequency of multivitamin intake and brand or dose was available, however, these factors were not included in the final model. While these factors were considered in preliminary analyses, they had minimal association with neonatal vitamin D status. As seen in previous studies, routine even if occasional multivitamin use (> 1/wk) provides protection against vitamin D deficiency (186). In the current study, consuming a prenatal supplement during pregnancy < almost every day, < 2-3 times/wk and not at all were associated with similar odds of neonatal vitamin D deficiency (ORs: 4.01, 4.33 and 4.17, respectively; Table 3.4). Therefore, mothers were classified as those who had reported consuming more than 1 multivitamin per week and those who consumed 1 or less per week.

Older age is often associated with lower serum 25(OH)D concentrations (182,183), however, in the current study, mothers under the age of 26 y at delivery were more likely to have neonates with vitamin D deficiency. It could be hypothesized that older mothers have had repeated exposure to physician advice, or are more conscientious of supplementation and healthy dietary habits during pregnancy, thus leading to better vitamin D status (133). The average age of

mothers at first birth in Canada is 29 years (193). As there is no defined age cut-off in the literature, ages 5 years above and below the mean (i.e. between 24 and 34 y) were all tested as potential cut-offs. Classifying women as < 26 vs. ≥ 26 y (i.e. conception at 25 y of age or lower) led to more accurate predictions of neonatal vitamin D deficiency. Most other studies considered age as a linear term in their prediction models, however in the current study, age was transformed into a dichotomous variable in order to make this tool easy to use at the bedside. As there is no logical reason for this cut-off beyond the observations in this sample, it would be necessary to see if this cut-off is valid in other populations as well. Future development of the screening tool may include the cost vs. benefit and utility of a simple web-based questionnaire or application. In this case, age could be analyzed as a linear term in the model, which may refine the prediction accuracy of the screening tool.

Mothers who were non-white had nearly 3 times the odds of neonatal 25(OHD) < 30 nmol/L. This is in agreement with several studies that have shown that infants born to non-white mothers are more likely to have low vitamin D status than those born to white mothers (78,137,138). In the current study, skin color data was converted from responses to questions about race, which used descriptors as defined by Statistics Canada (Appendix 1). People of many different skin pigmentation levels may identify themselves within the same race category, which complicates its usefulness as a surrogate for skin color. In order to minimize this error, mothers were classified as white or non-white depending on whether they identified themselves as 'white' or any other race. Other studies have had success using simplified race categories as a proxy for skin pigmentation (181,186,187), for example, white vs. black, black vs. other, white vs. Hispanic vs. black. Considering that Montreal has a highly diverse multiethnic population, skin color as a dichotomous variable may not be able to effectively predict the effects of intermediate

pigmentation on vitamin D status. Including questions specifically regarding skin color would help overcome this challenge, and may improve performance characteristics of the screening tool.

While sun exposure in the third trimester was expected to be an important predictor of neonatal vitamin D deficiency in the final screening tool, it was not significant in the final model after adjusting for other variables. Data regarding body parts exposed to the sun was also collected, however, in order to simplify questions for the purpose of the screening tool, exposed body surface area was not considered as a variable in the analyses. Sun exposure was not the main source of vitamin D for 76% of mothers in the study, who had < 15 min/d of sun exposure during their 3rd trimester. This proportion includes all mothers delivering during the fall and winter months (from October to April). The unadjusted odds of neonatal vitamin D deficiency were 2.10, 2.36 and 2.68 with maternal sun exposure < 15 min/d, < 30 min/d and < 60 min/d, respectively. Inconsistencies in reporting by mothers, as well as recall bias may affect the interpretation and thus the usefulness of this data in the screening tool. Instead, season of delivery may lend as a surrogate marker of sun exposure during the 3rd trimester, and may be more accurately assessed than sun exposure.

Delivering in the months from October through April doubled the odds of neonatal vitamin D deficiency, and was a significant predictor in the final model. Seasons were explored according astronomical seasons using the dates of equinoxes and solstices, Northern meteorological seasons using the first day of the months that include the equinoxes and solstices, as well as by individual month. After exploring these various dates, neonates born between October 1st and April 30th had a significantly higher risk than those born in the months of May through September. In relation to 25(OH)D synthesis, these months may be specific to areas around Montreal, as other locations may vary slightly in the length of their days and solar UVB intensity around the season changes. Of the variables that relate to endogenous synthesis of

vitamin D, skin pigmentation and season best captured risk of vitamin D deficiency in the newborn. Both lend well to a bedside survey and are not as subject to recall error as actual UVB exposure would be.

The relatively large sample size and data set collected over the course of 3 full years permitted the consideration of many potential predictors of neonatal vitamin D deficiency and with data representative of seasonal differences. The sample was racially diverse and more representative of the multiethnic population of Montreal. Another strength is that the 25(OH)D measurements were performed in a Vitamin D External Quality Assessment Scheme (DEQAS)-certified laboratory with National Institute of Standards and Technology (NIST) quality controls, and 25(OH)D values were standardized to Reference Measurement Procedures (RMPs). This allows the measurements of neonatal 25(OH)D to be comparable with other studies in which measurements are standardized. While survey tools to identify vitamin D deficiency have been developed for adult populations, to the best of the author's knowledge, this will be the first tool aiming to predict neonatal vitamin D deficiency. Questions are easily assessable from hospital charts or maternal pregnancy history and calculating risk score is accomplished using simple addition. The tool can be easily translated to French, providing a bilingual tool applicable to the Canadian population as well as other languages and possibly computer applications (apps) for use at the bedside along with educational materials. As vitamin D supplementation is universally recommended for breastfed infants, special attention would need to be given to the presentation of risk categories in the screening tool so as to not mislead families' whose infants score in the 'low risk' category. While the goal would be to provide encouragement to adhere to daily supplementation practices to families whose infants are at high risk of deficiency, families who are classified as 'low risk' may be less motivated to give their infants vitamin D supplements. The tool could alternatively stratify neonates into 'at risk' and 'at high risk', or include a

statement about the limited sources of vitamin D for infants and the importance of supplementation for all.

Limitations of this study include that data was collected from a single hospital, and thus may not be completely representative of populations in highly variable regions of the country. Only appropriate weight for gestational age (AGA) babies were included as this was a requirement for the randomized controlled trial for which the data was collected. A screening tool for vitamin D deficiency should ideally be applicable to all birth weight categories, and the tool could therefore be validated using other available birth cohorts in the future. While dietary intake is an important predictor of vitamin D status, it was not included in this analysis as this data is not available for all participants. Dietary intake was included in a subgroup analysis to see if and how it would be feasible to be included in a simple screening tool.

4.1 Subgroup analyses

As mentioned earlier, assessment of maternal dietary intakes during pregnancy may have helped to refine the screening tool. Such information however is challenging to collect in a newborn unit with relatively short hospital stays. As this thesis research was conducted in tandem with recruitment for a trial beginning March 2016, a subgroup of 137 mother-infant pairs with dietary vitamin D intake during pregnancy were available and explored as an additional risk factor. Dietary vitamin D intake was measured using a modified version of the Harvard food frequency questionnaire (FFQ) that is validated for vitamin D intake. The FFQ was given to mothers within 1 month of delivery to complete at home, and they were instructed to fill it out according to their intake during pregnancy only. Servings of fluid milk and fortified non-dairy beverages (e.g. soy milk, almond milk) were calculated according to average reported daily consumption. In agreement with the main sample, 23% of infants in the subsample were born with serum 25(OH)D < 30 nmol/L. Dietary vitamin D intake during pregnancy ranged from 27 to

794 IU, with a mean of 213 ± 144 IU/d and median (IQR) of 170 (112, 268) IU. Usual intakes of milk or fortified non-dairy beverages ranged from 0 to 4 servings per day, with 45% consuming < 1 serving/day. Mothers were classified as those consuming < 1 serving or ≥ 1 serving per day, however no significant association was found with neonatal vitamin D deficiency (crude OR = 1.65; 95% CI 0.74, 3.68). This was additionally tested at cut points of 2 and 3 servings per day with no significant results. There were no significant associations between quartiles of dietary vitamin D intake and neonatal vitamin D deficiency. In terms of dietary intake, these results are in agreement with a similar study that aimed to identify predictors of vitamin D inadequacy in postmenopausal women (187). In the current study, the average daily number of servings of milk or fortified non-dairy beverages were estimated based on intake frequencies in an FFQ. Many of these frequencies required further estimation, for example, those consuming a serving: 2-4 times per week, 5-6 times per week, 2-3 times per day etc. Estimations likely contribute to further error, for example: 2-4 times per week was considered as 0.43 servings per day, while 5-6 times per week was taken as 0.8 serving per day. In other studies, more servings of dairy intake during pregnancy has been associated with improved neonatal vitamin D status (118), so future research on the screening tool should include data directly surveying mothers on habitual consumption of milk or fortified non-dairy beverages, other dairy products, other fortified foods and fatty fish.

5 Conclusion

Many Canadian infants are born with low vitamin D status and are therefore especially at risk for developing rickets and symptomatic vitamin D deficiency if they are not receiving a daily vitamin D supplement. Cases of rickets and severe symptomatic vitamin D deficiency continue to be reported across Canada, and this may be in part be due to lack of adherence to recommendations for vitamin D supplementation of infants.

Newborns are not routinely screened for vitamin D status. Although vitamin D supplements for breastfed infants are recommended by Health Canada and generally prescribed by pediatricians, not all families comply with these recommendations (91,92). Additionally, providing education to all families, including those already knowledgeable on the importance of vitamin D supplementation, would be inefficient. Identifying at risk neonates would be an important first step to educate their families on the importance of vitamin D supplementation for the infants. Using a validated and accurate tool to show parents that their child is at high risk may also encourage their adherence to supplementation guidelines.

Screening questionnaire tools to identify individuals with vitamin D deficiency are practical as they can be simple to use, easily accessible and require minimal costs. While many questionnaires or predictive models for low vitamin D status have been developed, they are mainly for identifying inadequacy or deficiency in adult populations, and some are too long for administration in a newborn unit. The simple, question-based screening tool developed in this study may be helpful to identify newborns at high risk for vitamin D deficiency. Future research in other populations and different regions are needed to validate this tool.

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Appendices

Appendix 1. Descriptors for maternal race used as a surrogate for maternal skin color

Mother: You may belong to one or more racial or cultural groups on the following list. Are you...?

Mère: Vous pouvez appartenir à un ou plusieurs groupes raciaux ou culturels sur la liste suivante. Êtes-vous...?

- ☐ White / Blanc
- ☐ South Asian (e.g., East Indian, Pakistani, Sri Lankan) / Asiatique du Sud (par exemple, Indiens, Pakistanais, Sri Lankais)
- ☐ Chinese / Chinois
- ☐ Black / Noir
- ☐ Filipino / Filipino
- ☐ Latin American / Latino-américain
- ☐ Arab / Arabe
- ☐ Southeast Asian (e.g., Vietnamese, Cambodian, Malaysian, Laotian) / Asiatique du Sud-Est (par ex, Vietnamien, Cambodgien, Malaisien, Laotien)
- ☐ West Asian (e.g., Iranian, Afghan) / Asiatique de l'Ouest (par exemple, Iranien, Afghan)
- ☐ Korean / Coréen
- ☐ Japanese / Japonais
- ☐ Other
- ☐ Refused to answer / Refuse de répondre
- ☐ Don't know / Ne sais pas