Predictors of Vitamin D Status at 4 Mos. & Change in Status in Lactating Women and their Infants from 1 to 4 Months Postpartum and Validation of a Food Frequency Questionnaire to Assess Vitamin D Intake among Lactating Women

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2. ABSTRACT

The objectives of the present study were to determine the vitamin D status at 1 and 4 months and changes since 1 month, to identify the key predictors of vitamin D status at 4 months and changes in lactating mothers and their infants, and to validate the Canadian adapted food frequency questionnaire (FFQ), adapted for Canadians, to assess vitamin D intake in lactating women.

Sun exposure, skin pigmentation, anthropometric data, body composition, supplement use, vitamin D intake and plasma 25-hydroxy vitamin D (25(OH)D) concentration were measured in lactating women and their infants (n=44). From 1 to 4 months, maternal 25(OH)D concentration decreased (73.0 ± 21.6 to 62.4 ± 18.3 nmol/L, $p<0.001$) and infant 25(OH)D concentration increased (60.2 ± 31.0 to 71.5 ± 25.4 nmol/L, $p=0.032$). Predictors of change (Δ) in maternal 25(OH)D concentration included % change in weight, the number of weeks spent in the synthesizing period, and baseline 25(OH)D concentration. Predictors of Δ in infant 25(OH)D concentration included travel to a latitude <37 ^{o}N, baseline 25(OH)D concentration, and ethnicity. Vitamin D intake was a significant predictor of maternal 25(OH)D concentration ($p<0.01$). Bland-Altman analyses indicated a satisfactory agreement between methods after energy adjustment. Cross-classification of total vitamin D intake between dietary methods classified 69% of mothers into the same tertile and weighted Kappa statistics (K_W) was 0.63. Between FFQ and 25(OH)D concentration, 45.2% of mothers were classified into the same tertile with $K_W=0.14$. These findings suggest that the Willet FFQ may be a valid tool for the assessment of vitamin D intake among lactating women which is an important contributor to vitamin D status. Furthermore, predictors are important to identify when seeking solutions to prevent declines in maternal vitamin D status and to maintain infant vitamin D status.

3. RÉSUMÉ

Les principaux objectifs de cette étude étaient de déterminer l'état vitaminique D et d'identifier les déterminers clé du changement d'état vitaminique D entre 1 mois et 4 mois postpartum chez les femmes allaitantes et leurs nourrissons, de valider le questionnaire de fréquence alimentaire de Willett (QFA) adapté pour les Canadiens, et d'évaluer l'apport en vitamine D chez les femmes allaitantes. L'exposition au soleil, la pigmentation de la peau, les données anthropométriques, la composition corporelle, la prise de suppléments, l'apport en vitamine D et le niveau de 25-hydroxy vitamine D plasmatique (25(OH)D) ont été mesurés chez les femmes allaitantes et leurs nourrissons (n=44). Durant l'étude, le niveau de 25(OH)D maternelle a diminué (73,0 ± 21,6 à 62,4 ± 18,3 nmol/L, $p<0,001$) et le niveau de 25(OH)D des nourrissons a augmenté (60,2 ± 31,0 à 71,5 ± 25,4 nmol/L, $p=0,032$). Les déterminers de changement pour le niveau de 25(OH)D maternelle incluent le pourcentage de changement de poids, le nombre de semaines passées dans la période favorable de synthèse au cours de l'étude, le niveau de 25(OH)D de base, et l'ethnicité. Les déterminers de changement pour le niveau de 25(OH)D chez le nourrisson incluent un voyage sous un climat ensoleillé, le niveau de 25(OH)D de base et l'ethnicité. L'apport en vitamine D a aussi été un déterminer du niveau de 25(OH)D maternelle ($p<0,01$). Les analyses de Bland-Altman ont indiqué un accord satisfaisant entre les méthodes. La classification croisée de l'apport total en vitamine D entre le QFA et la moyenne des rappels de 24 heures a classifié 69% des mères dans le même tercile avec les statistiques pondérées kappa (K_W)=0.63; celle entre le QFA et le niveau de 25(OH)D a classifié 45.2% des mères dans le même tercile avec K_W=0.14. Ces résultats suggèrent que le QFA de Willett pourrait être un outil valide pour l'évaluation de l'apport en vitamine D chez les femmes allaitantes, ce qui est un facteur important pour l'état vitaminique D. En outre, il est important d'identifier ces déterminers lors de la recherche de solutions pour la prévention de déclins dans l'état vitaminique D chez la mère et pour e maintien d'état vitaminique D chez les nourrissons.

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6. KEY TERMS

6.1. Abbreviations

$1,25(OH)_2D$:	1,25-dihydroxyvitamin D or calcitriol
25(OH)D:	25-hydroxyvitamin D or calcidiol
AI:	Adequate Intake
aOR:	adjusted odds ratio
ASA24:	Automated Self-administered 24 hour Dietary Recall
BIA:	bioelectrical impedance analysis
BMI:	body mass index
BSA:	body surface area
CBP:	competitive binding-protein
CDC:	Centers for Disease Control and Prevention
CI:	confidence interval
CIE:	Commission Internationale de l'Eclairage
CNF:	Canadian Nutrient File
CPS:	Canadian Paediatric Society
CT:	computerized tomography
CV:	coefficient of variation
DBP:	vitamin D binding protein
DXA:	dual-energy x-ray absorptiometry
EAR:	Estimated Average Requirement
EI:BMR:	energy intake by basal metabolic rate ratio
FFM:	fat free mass
FFQ:	food frequency questionnaire
FFQ1:	food frequency questionnaire completed at 1 month baseline visit
FFQ2:	food frequency questionnaire completed at 4 month follow-up visit
FM:	fat mass
HPLC:	high performance liquid chromatography
IOM:	Institute of Medicine

IQR:	intraquartile range
ITA:	individual typology angle
K_W:	weighted Kappa statistics
LC-MS/MS:	liquid chromatography-tandem mass spectrometry
LoA:	limit of agreement
MRI:	magnetic resonance imaging
OCP:	oral contraceptive pills
PTH:	parathyroid hormone
RCT:	randomized clinical trial
RDA:	Recommended Dietary Allowance
RIA:	Radioimmunoassay
SD:	standard deviation
SPF:	sun protection factor
TBF:	total body fat
TBW:	total body water
total vitamin D intake:	dietary and supplemental vitamin D
UVB:	ultraviolet *Beta*
VDR:	vitamin D receptors
WHO:	World Health Organization
WHR:	waist-to-hip ratio

6.2. Conversion Factors

1 ng/ml of 25(OH)D = 2.496 nmol/L

1 µg of vitamin D = 40 IU

1 pg/mL of PTH = 0.1042 pmol/L

7. CONTRIBUTIONS OF AUTHORS

A. Phan was the primary author of the manuscripts in this thesis. A. Phan assisted in the planning and organization of the study and involved in ethics revisions submission. A. Phan was involved in all aspects of recruitment and study process. A. Phan was present for the majority of visits, obtained consent, assisted in measurement of anthropometric and body composition, and performed skin pigmentation measurements. A. Phan assisted in blood procurement, centrifuged blood samples, and analysis of 25(OH)D concentration. A. Phan administered demographic surveys, general health surveys, and 24 hour dietary recalls. A. Phan entered all questionnaires and dietary data. A. Phan conducted statistical analysis, interpretation of data, reviewed relevant literature, and drafted manuscripts.

S. Gallo was the doctoral student and principal investigator of the study. S. Gallo submitted to the Institutional Review Board under the supervision of Dr. Weiler. S. Gallo was involved in all aspects of the study and audited the data and reviewed drafted manuscripts.

C. Vanstone was responsible for blood procurement, involved in aspects of participant study visits, and reviewed drafted manuscripts.

S. Agellon was involved in training and assistance of analysis of 25(OH)D concentration in the laboratory and reviewed drafted manuscripts.

C. Rodd was the co-investigator of the study. C. Rodd was involved in randomization of the vitamin D isoforms, provided statistical consultations, and reviewed drafted manuscripts.

H. Weiler was the co-principal investigator of the study and A. Phan's supervisor. H. Weiler critically reviewed the grant, study procedures, ethics submissions, and manuscripts.

8. INTRODUCTION

Vitamin D deficiency is a problem globally [1-12], including among Canadian mothers and breastfed infants [13-18]. Health Canada recommends that breastfed infants receive a daily supplement containing 400 IU of vitamin D due to its low concentration in breast milk [19]. The Canadian Community Health Survey reported that only 53% of breastfed infants received a vitamin D supplement [19]. Breastfeeding without vitamin D supplementation is associated with vitamin D deficiency [20-24]. The early postnatal period is a critical period due to rapid growth and high risk of developing vitamin D deficiency [24] which could result in infantile rickets [14, 25]. The Canadian Paediatric Society (CPS) recommends 800 IU/d of vitamin D between November 1 to March 31 [26] for infants living at latitudes >55 ^{o}N, between 45 to 55 ^{o}N in those with risk factors for vitamin D deficiency, and those with dark skin pigmentation [13].

Health Canada's recommendations for milk [27] and fish intake [28], and postnatal supplement [29] for lactating women equates to ~1400 IU/d of vitamin D depending on the type of fish consumed. The CPS recommends 2000 IU/d for lactating women to support maternal-infant transfer pre- and post-natally [13]. The Institute of Medicine (IOM) increased vitamin D recommendations to Recommended Dietary Allowance (RDA) of 600 IU/d from an Adequate Intake (AI) of 200 IU/d to prevent vitamin D deficiency [30]. Although few meet the Estimated Average Requirement (EAR) of 400 IU/d [31-36], total vitamin D intake (food and supplements) is a major contributor to vitamin D status, especially for infants or people with limited ultraviolet *Beta* (UVB) radiation. Supplements are also an important vitamin D source during lactation [37]. Therefore, accurate dietary assessment methods are necessary to estimate usual vitamin D intake due to the fact that few foods are rich in vitamin D and may not be consumed on a daily basis. The food frequency questionnaire (FFQ) could be a useful tool to capture intake over a longer period of time, is cost-effective and has low participant burden [38].

The best clinical indicator of vitamin status is 25-hydroxyvitamin D (25(OH)D) concentration. 25(OH)D concentration reflects both exogenous

sources obtained through foods and supplements and endogenous vitamin D synthesis in the skin from UVB radiation [38-41]. In Canada, vitamin D can be endogenously synthesized between April 1 to October 31 [26]. Factors that influences endogenous vitamin D synthesis include latitude, season, measures of UV exposure such as time of day, length of time, and body surface area (BSA) exposed [7, 26, 42-47], and sunscreen use [48, 49].

Due to melanin production that interferes with endogenous vitamin D synthesis by competing with UVB photon absorption [50, 51], dark skinned individuals tend to have lower vitamin D status than light skinned individuals [7, 8, 18, 43, 50-56]. However, in these studies, skin colour tends to be classified by ethnicity [7, 18, 51] but within ethnic groups, skin colour varies. The reflectance spectrophotometer can objectively measure constitutive, genetically determined skin colour [35, 53, 54, 57] and facultative skin pigmentation, which develops due to sun exposure [58]. Based on a continuum, the lighter the constitutive skin, the greater the synthesizing capacity and darkening of facultative skin should align with higher 25(OH)D concentration due to UVB exposure [59]. This association has not been explored in lactating women and their infants.

Excess adiposity can reduce circulating 25(OH)D concentration by sequestration in fat tissues [1, 60, 61]. On the other hand, loss in adiposity can increase circulating 25(OH)D concentration [62-64] by release of vitamin D from fat tissue depots [65]. With changes in weight and adiposity in both mothers and infants in the postpartum period, its association with 25(OH)D concentration remains unexplored.

Vitamin D deficiency is a concern among lactating women and their infants [5, 6, 18, 25, 43] and hypovitaminosis D is associated with increased risk of developing rickets [14, 25], osteomalacia, osteoporosis, bone fractures [66, 67], and a number of chronic diseases [67-73]. Thus, it is important to examine the extent to which potential predictors affect vitamin D status during the postpartum period. The aim of this thesis was to contribute to the limited data on vitamin D status in lactating women and their infants at 1 and 4 months postpartum and change since 1 month postpartum. The secondary objectives were to identify key

predictors of vitamin D status at 4 months and change since 1 month postpartum in lactating mothers and their infants and to validate the Willett FFQ, adapted for Canadians, to assess vitamin D intake in lactating women for use in epidemiological studies.

9. LITERATURE REVIEW

9.1. Vitamin D: Sources, Metabolism, and Function

Vitamin D is a fat soluble vitamin existing as two isoforms, vitamin D_2 and vitamin D_3. Vitamin D_2, or ergocalciferol, is a 28-carbon molecule derived from ultraviolet irradiation of ergosterol in yeast and plants. Vitamin D_3, or cholecalciferol, is a 27-carbon molecule produced from the absorption of UVB radiation on the skin and found in limited natural food sources such as fatty fish [39, 74-76]. Fortified vitamin D_3 products include milk (fluid, evaporated, powdered, goat), orange juice, margarine, and fortified vitamin D_2 mainly include plant-based beverages such as soy and rice beverages (**Table 1**) [77, 78]. Fortified milk can be used in the manufacturing of yogurt and cheeses [78]. Vitamin D can also be obtained from supplements. Vitamin D_2 supplementation is effective in preventing vitamin D deficiency, although others have found that vitamin D_3, derived from lanolin in sheep's wool, is more efficacious in increasing 25(OH)D concentration [79, 80]. However, with daily intake, there may be no biological differences in supporting vitamin D status between the isoforms [79-81]. When dietary sources of vitamin D_2 and vitamin D_3 are ingested, vitamin D is absorbed with free fatty acids in the small intestine, incorporated into chylomicrons-lipoprotein complex to be transported into the blood via the lymphatic system for further metabolism in tissues [82].

Vitamin D can be endogenously synthesized from a precursor, 7-dehydrocholesterol, or provitamin D_3, to previtamin D_3 in the plasma membrane of the epidermal cells [83] (**Figure 1**). The skin has a high capacity to synthesize vitamin D but its efficiency depends on the number of UVB photons that penetrates into the epidermis [82]. Excess endogenous vitamin D_3 and previtamin D_3 are photolyzed to biologically inactive photoproducts such as lumisterol and tachysterol thus preventing vitamin D intoxication [82-85]. Previtamin D_3 is inherently unstable and once formed by UVB radiation (290 to 315 nm), rapidly converts to vitamin D_3 by enthalpy [67, 83]. By exocytosis, vitamin D_3 is excreted from the epidermal cells into the extracellular space and enters the dermal capillary bed [82, 83] by passive diffusion [86]. In the circulation, both

endogenous and exogenous vitamin D sources bind to vitamin D binding proteins (DBP) and lipoproteins [82, 83, 87]. Vitamin D binding proteins are an α-globulin [31] and the most common genetic forms of DBP are GC*1F, CD*1S, and GC*2 which have significantly different affinity for $25(OH)D_3$ [88]. The GC*1F has the highest affinity for $25(OH)D_3$ and is found at a higher frequency in black individuals [88]. Vitamin D is deposited in adipose and muscle tissue by DBP and lipoprotein lipase action [67, 82, 83, 89]. Vitamin D is deposited in both lean and obese individuals but in obese, pregnant, and lactating women, the excess adipose tissue may enhance sequestration of endogenously synthesized vitamin D [1, 61].

If not deposited in muscle or fat tissue, vitamin D is hydrolyzed in the liver by mitochondrial and microsomal vitamin D-25-hydroxylase enzymes (P450C25 or CYP27A1) [33, 87, 90] to form 25(OH)D, the major circulating metabolite of vitamin D [67, 82, 83]. In the circulation, 25(OH)D binds to DBP (85%) and albumin (15%) or is free (0.03%) [91]. DBP-25(OH)D enters the plasma membrane of the renal proximal convoluted tubule through endocytosis [92]. In the inner mitochondrial membrane of the cells, 25(OH)D can be hydrolyzed by 25(OH)D-1α-hydroxylase (CYP27B1 or P450C1) to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol) [67, 90] or by 24-hydroxylase (CYP24A1) to 24,25-dihydroxyvitamin D [93]. 1α-hydroxylase found in the proximal convoluted tubule renal cortex and other sites throughout the nephron [92], is induced by parathyroid hormone (PTH) through a cAMP/phosphahydroxylase 4,5 bisphosphate-mediated signal transduction mechanism [94]. Calcitriol is the biologically active form of vitamin D and 24,25-dihydroxyvitamin D is the inactive form [67, 83, 90]. Finally, 25(OH)-24-hydroxylase (CYP24) degrades $1,25(OH)_2D$ to calcitroic acid, the biliary excretory formed through C24 oxidation pathway and is excreted in urine [87, 94]. Other tissues and organs (heart, prostate, breast, immune, and *β*-islet pancreatic cells) have the capacity to metabolize 25(OH)D to $1,25(OH)_2D$ [67, 70, 86, 95-97]. The mechanisms are less well understood in comparison to the kidney.

Other vitamin D metabolites of interest to infancy are C-3-α-epimer of 25(OH)D and $1,25(OH)_2D$ epimerized from 25(OH)D and $1,25(OH)_2D$ at the C-3

position by 1α-hydroxylase in renal and extrarenal tissues [98]. 3-epi-25(OH)D can also be converted to 3-epi-1,25$(OH)_2$D [98] which can also suppress PTH secretion but has reduced calcemic effects [99]. C-3 epimers contribute between 8.7-61.1% to total 25(OH)D concentration in infants <1 years of age, which could overestimate 25(OH)D concentration [98, 100]. The high rates of C-3 epimerization may be a function of immaturity of vitamin D metabolism [98].

Calcidiol has a relatively long half-life of 10 to 21 days and is an accurate indication of vitamin D stores from both recent endogenous and exogenous sources [38-41]. Since 25-hydroxylation step is unregulated, 25(OH)D is dependent on substrate availability [39, 41]. Cholecalciferol has a half-life of 24 hours and reflects recent sun exposure and vitamin D intake [41]. Serum 1,25$(OH)_2$D concentration is a poor indicator of vitamin D status because it reflects immediate physiological need, is tightly regulated, and has a short half-life of 4 to 6 hours [38, 41, 101]. In vitamin D deficient individuals, 1,25$(OH)_2$D concentration has been observed to be normal or even elevated [83, 101, 102].

The classic actions of 1,25$(OH)_2$D are to increase intestinal calcium and phosphorus absorption and inhibit PTH secretion by decreasing parathyroid gland gene expression to maintain calcium homeostasis. In the small intestine and kidneys, 1,25$(OH)_2$D binds with high affinity to vitamin D receptors (VDR) to enhance the absorption of renal and intestinal calcium to 30-40% and phosphorus to 80% [33, 67, 82, 87, 103]. In the vitamin D deficient state, only 10-15% of dietary calcium and 50-60% of dietary phosphorus are absorbed [67, 82]. Preserving serum calcium and phosphate concentrations are necessary to provide sufficient ions to mineralize the collagen matrix [104]. Circulating 1,25$(OH)_2$D reduces PTH and indirectly by increasing serum calcium [82, 83] and decreasing renal calcium excretion [31]. Hypocalcemia and hypovitaminosis D stimulates PTH secretion, resulting in hyperparathyroidism, to restore eucalcemia. Increased PTH causes renal conversion of 25(OH)D to 1,25$(OH)_2$D. PTH and VDR are found throughout the length of the nephron of the kidney and in osteoblasts but not in osteoclasts [94]. 1,25$(OH)_2$D travels to bone and activates osteoblasts. The interaction between 1,25$(OH)_2$D and/or PTH within osteoblasts results in the

stimulation of osteoclasts [94, 105]. Osteoclasts resorb bone (mobilizes calcium) from the skeleton [94, 105] and with intestinal calcium absorption elevate plasma calcium concentration [31, 38, 66]. During pregnancy and lactation, renal 25(OH)D-1α-hydroxylase is upregulated by hypocalcemia and phosphosphatemia in addition to estrogen and prolactin [106]. High estrogen levels alter the relative proportion of bound and free 25(OH)D by increased DBP in the circulation [107]. There is little information about vitamin D absorption and metabolism in healthy neonates due to examination difficulties given modern technology [108]. Theoretically, this could be examined using stable isotope labeling vitamin D followed by blood sampling.

9.2. Vitamin D Status and Associated Predictors

There has been much debate surrounding how vitamin D recommendations should be set. Criteria used to set an optimal serum 25(OH)D concentration have been based on minimal yet normal PTH concentration and when bone resorption and intestinal calcium absorption are stabilized [13]. This association has been observed in adults [109, 110], adolescents [111, 112], children [113], and infants [114]. In older adults, 25(OH)D concentration >78 nmol/L was associated with a plateau in PTH concentration [109]. Although, C-3 epimers contribute to total infant 25(OH)D concentration [98], it is not currently used to assess vitamin D status.

The IOM defines rickets as 25(OH)D concentration as <27.5 nmol/L, deficiency as <37.5 nmol/L, insufficiency as <50 nmol/L [115], and upper levels as >125 nmol/L [30]. On the other hand, the CPS defines deficiency as 25(OH)D concentration as <25 nmol/L, insufficient as 25-75 nmol/L, optimal as 75-225 nmol/L, pharmacological (potential adverse effects) as >225 nmol/L, and potentially toxic as >500 nmol/L [13]. Interestingly, 25(OH)D concentration corresponding to the RDA of 600 IU/d of vitamin D is 50 nmol/L [30] which is below that observed to minimize serum PTH concentrations [116, 117]. To achieve the 25(OH)D concentration cut-off of 75 nmol/L, dark skinned individuals with low sun exposure require 2100-3100 IU/d of vitamin D and those with high sun exposure require 1000-2250 IU/d of vitamin D [53]. Light skinned

individuals with low sun exposure require 1000-2550 IU/d of vitamin D and those with high sun exposure require no additional vitamin D but 1300 IU/d is recommended in the winter [53]. These values have been extrapolated from data where vitamin D ingestion increased vitamin D status but maintenance dosages are undetermined.

Vitamin D deficiency increases the risk of infantile rickets [14, 25], bone fractures, osteomalacia, and osteoporosis [66, 67]. Fetal bone growth depends on increased placental calcium transport capacity influenced by maternal vitamin D status [118]. Thus, infants born with limited vitamin D stores are born at lower gestational age [119], shorter knee-heel length after adjustment for gestational age [119], and lower [37] or no effect on birth weight [120]. Low maternal vitamin D status leads to inadequate 25(OH)D and/or $1,25(OH)_2D$ transferred across the placenta to the fetus with implications being reduced osteoblast activity and bone mineralization [119]. On the other hand, infant vitamin D deficiency has been associated with greater birth weight, length, and head circumference but lower whole body and femur bone mineral content relative to body weight suggesting inadequate bone mineralization [25]. In the latter study, these larger infants were not able to adequately mineralize their bones due to vitamin D deficiency [25]. There is still controversy since studies show lower, no effect, and higher birth weight.

9.2.1. Maternal Vitamin D Status

Vitamin D deficiency continues to be a problem among pregnant and lactating women globally [1-12], including among Canadian mothers [13-18]. Vitamin D deficiency ranges from 31% (25(OH)D concentration <25 nmol/L) to 81.1% (25(OH)D <37.5 nmol/L) in study participants [5, 16, 23, 121, 122]. In northern Canada, the majority of mothers post-delivery did not achieve the 25(OH)D cut-off concentration of 75 nmol/L (Caucasian 59.8 ± 29.4 nmol/L; First Nations 52.1 ± 25.9 nmol/L; Inuit 48.8 ± 14.2 nmol/L) [18]. Weiler et al. [25] conducted a study in Manitoba and found 46% of mothers had plasma 25(OH)D concentration <37.5 nmol/L just after delivery. A recent cross-sectional study in pregnant women, found that the prevalence of vitamin D deficiency (<50

nmol/L) was 40% in Newfoundland and Labrador [16]. Maternal vitamin D status tends to be low during pregnancy and continues to decline up to 6 months postpartum [123]. This suggests that low vitamin D status is likely to be found during breastfeeding thus, continued vitamin D supplementation may be necessary. The current EAR of 400 IU/d does not maintain or significantly improve maternal 25(OH)D concentration [31-35]. When supplementation exceeded 400 IU/d, there was only a modest increase in 25(OH)D concentration [52] where for every 100 IU of vitamin D ingested, 25(OH)D concentration increased by 1.5-2.8 nmol/L [62, 124]. Thus, it is important to identify predictors of Δ25(OH)D concentration and at specific time points postpartum in which 25(OH)D concentration declines in order to identify when to strategize towards improving vitamin D status.

9.2.2. Vitamin D Transfer to Infants Pre- and Post-natally

Infant vitamin D status is dependent on vitamin D transferred both pre- and post-natally [13]. The positive relationship between cord and maternal vitamin D status, with higher circulating 25(OH)D concentration in mothers, is well-documented in many countries [5, 10, 18, 43, 45, 102, 125]. During pregnancy, mothers tend to have low vitamin D status and continues to decline immediately post-natally [6, 13-15, 17, 25, 43, 123]. Preliminary data from the Canadian Health Measure Survey found that mean 25(OH)D concentration was 68 nmol/L in women of childbearing age (19 to 50 years old) [126, 127]. In another study, pregnant women (n=160) with 25(OH)D concentration <20 nmol/L were administered 800 IU/d of vitamin D (n=80) at their first antenatal visit [128]. At 36 weeks, 58 (72.5%) of these women received 1600 IU/d of vitamin D due to low 25(OH)D concentration. From the antenatal visit to delivery, 25(OH)D concentration increased from 15 to 27.5 nmol/L and 60% of these women had normal vitamin D status. The short period between delivery and doubling the dose of vitamin D from 800 to 1600 IU/d was insufficient to detect a change in vitamin D status due to the 21 to 30 day half-life of 25(OH)D [38-41]. In deficient mothers, higher amounts of vitamin D supplementation may be necessary to increase 25(OH)D concentration. Furthermore, immediately in the postpartum

period, low 25(OH)D concentration was observed in dark skinned mothers and infants (mothers: black 41.1 ± 34.3 nmol/L, white 51.3 ± 47.6 nmol/L; infants: black 26.6 ± 25.4 nmol/L, white 38.5 ±35.2 nmol/L) despite taking a prenatal multivitamin containing 400 IU of vitamin D (70%), intake of fish (90%), and milk intake of 575 mL/d [11].

Low maternal vitamin D stores increases odds of vitamin D deficiency in newborns [9, 12] with adjusted odds ratio (aOR) of 5.28 (95% CI: 2.90-9.62) [12]. Vitamin D deficiency in studies ranges between 10% (25(OH)D concentration <27.5 nmol/L) to 82% (25(OH)D <25 nmol/L) depending on whether a vitamin D supplement was administered to the infant [5, 9, 21, 23, 43, 122, 129]. Beyond the neonatal period (first 28 days of life), infant 25(OH)D concentration is uncorrelated to maternal vitamin D status [130]. However, all these infants had 25(OH)D concentration <37.5 nmol/L. Infants may no longer be able to meet their vitamin D needs from fetal stores with limited sun exposure, without supplementation, and/or limited vitamin D transferred across the placenta. In contrast, the relationship between maternal and infant vitamin D status up to four months post-delivery [12, 44, 121, 131, 132] and maternal milk and supplement intake have been documented [12, 132]. The evidence remains conflicting regarding the relationship between infant and maternal vitamin D status.

Infants born with insufficient vitamin D stores will deplete their stores and decline into deficiency unless a supplement is provided [20, 21, 24, 122, 133]. Even after receiving routine supplementation, it can take up to 90 days to improve 25(OH)D concentration [24, 34, 35, 134]. In infants receiving 200 IU/d of vitamin D commencing at birth, 25(OH)D concentration significantly increased by 4 months of age [24]. Similarly, in infants supplemented with 400 IU/d of vitamin D, 25(OH)D concentration increased from 1.5 to 3 months of age (75.6 ± 23.9 to 97.2 ± 25.9 nmol/L) [135]; however, vitamin D status remained stable thereafter between 3 to 6 months of age (92.4 ± 29.7 nmol/L) [135]. In fully breastfeed infants receiving 400 IU/d of vitamin D, 25(OH)D significantly increased between 1 and 4 months (39.9 ± 23.2 to 108.8 ± 35.2 nmol/L) and between 1 and 7 months (106.1 ± 30.2 nmol/L) demonstrating stable values between 4 to 7

months of age [129]. Predictors of Δ25(OH)D concentration was not examined thus, we cannot confirm that vitamin D supplementation was an independent predictor of change in status. However, as infants become dependent on PTH and $1,25(OH)_2D$ to maintain calcium homeostasis [136], endogenous and supplemental vitamin D become important in Canadian breastfed infants.

9.2.3. Latitude of Residence and Seasonality

Endogenous vitamin D synthesis depends on season, latitude, and time of day, length of time, and BSA exposed to UVB radiation. In the northern and southern latitudes, decreased cutaneous synthesis is due to the increased angle of penetration and passage through the atmosphere increases UVB photons absorption by the stratospheric ozone layer [42, 52, 82, 102]. In the spring, summer and fall, sun exposure between 1000 to 1500 h allows enough UVB to pass through the ozone layer for endogenous synthesis [101, 136]. At latitudes >37º (N or S), 25(OH)D concentration peaks from mid-end of summer with a nadir at the end of winter [1, 7, 42, 82, 124, 137-139]. These results been found in black and white pregnant women in Pennsylvania (40 ºN) [43]. In a study conducted in Ohio (39 ºN), there was a significant decline in infant 25(OH)D concentration in the fall-winter months compared to spring-summer months (30 vs. 57.4 nmol/L, respectively) [44]. In Newfoundland and Labrador (48 ºN), cord 25(OH)D concentration measured in the winter was significantly lower than in the summer (48.6 ± 17.5 nmol/L vs. 63.3 ± 14.1 nmol/L, respectively) [16]. Thus, infants born during the winter have lower 25(OH)D concentrations than those born in the summer (48.6 ± 17.5 vs. 63.3 ± 13.5 nmol/L, respectively) [45, 46] with increased odds of vitamin D deficiency (aOR: 3.86 [95% CI: 1.74-8.55) [12]. As latitude rises >40° (N or S) endogenous synthesis in the summer may be limited [26]. At 52 °N, photosynthesis of previtamin D_3 ceases from October to March (non-synthesizing period) because UVB exposure threshold (18 to 20 mJ/cm^2) is not reached [26].

One day of travel during the last 3 months to a destination of latitude <42 °N was associated with higher 25(OH)D concentrations [42, 140]. The number of weeks traveled abroad over a lifetime was associated with increased 25(OH)D

concentration [138]. In both studies, vitamin D status of individuals who traveled compared to those who did not travel was not provided. In reproductive women, travel to a latitude <35 ^{o}N during winter/spring for at least one day was an independent predictor of 25(OH)D concentration (β=0.20, $p<0.01$) [141]. Given the half-life of 25(OH)D is between 10 to 21 days [38-41], the difference between the last travel date and collection of blood sample is important to consider if endogenous vitamin D synthesis from the travel was captured.

UV exposure as measured by duration of sun exposure and sun-exposed BSA can be examined as individual variables or as a sun exposure factor, also known as the sun index (product of hours/week of sun exposure and fraction/percentage of BSA exposed) [142, 143]. Individuals with low sun exposure (20 minutes/d) and low BSA exposed (~18%) tend to have lower vitamin D status than those with high sun exposure (90 minutes/d) and high BSA exposed (~35%), regardless of skin pigmentation [53]. However, regression analyses indicated that sun index was only a significant predictor of vitamin D status in black individuals [142]. Sun index and BSA exposed were both related to 25(OH)D concentration at the end of the summer ($r=0.49$, $p<0.01$ and $r=0.58$, $p<0.001$, respectively). Furthermore, change in vitamin D status (late summer minus late winter 25(OH)D concentration) was also related to sun index ($r=0.67$, $p<0.001$) and BSA exposed ($r=0.66$, $p<0.001$) [143]. Therefore, greater BSA exposed or sun index in the late summer resulted in a smaller decline in 25(OH)D concentration. Duration of sun exposure was only correlated with season differences in 25(OH)D concentration ($r=0.39$, $p<0.05$) [143]. Pregnant women with adequate vitamin D status (25(OH)D concentration ≥50 nmol/L) had higher median daily sun exposure (15 [0, 240] vs. 7.5 [0,150], respectively) and sun exposure index (18.9 [0, 302] vs. 4.7 [0, 236], respectively) [123]. This relationship was not observed when mothers with adequate and inadequate vitamin D status were combined [123]. This could be due to the fact that the majority of pregnant women had no sun exposure or minimal sun exposure only in the face and hands. In March 2010, Statistics Canada reported that sunlight

exposure >1 hour/d during the summer months between 1100 to 1600 h was positively associated with higher 25(OH)D concentration [127].

The Canadian Dermatology Association advises that infants under one year of age avoid direct sunlight due to the dangers of skin damage [144]. Thus, safe levels of sun exposure have not been established [145] to maintain adequate 25(OH)D concentration in exclusively breastfed infants. Following the guidelines by the Canadian Dermatology Association, sunlight exposure would not be a predictor of infant vitamin D status [20]. Infants tend to be covered in blankets or dressed in layers of clothing [15, 44] and often only a small BSA is exposed to direct sunlight [44] due to the concerns for skin damage [77, 144, 146]. However, UVB exposure of 30 minutes/week if the infant is wearing only a diaper or 2 hours/week if the infant is fully clothed without a hat significantly increased 25(OH)D concentration [147] without vitamin D supplementation [44, 47]. At ~3.5 months of age, sun exposure was a significant predictor of infant vitamin D status [121]. In infants 1 to 8 months of age, those with more sun exposure had significantly higher 25(OH)D concentration than those with lower sun exposure (115 ± 72 vs. 63 ± 53 minutes/day, respectively and 100 ± 57.5 vs. 45 ± 35 nmol/L, respectively) [47]. UV exposure measured by minutes of sun exposure, sun-exposed BSA, and time of day exposed in mothers and infants are important to capture when assessing vitamin D status.

9.2.4. Skin Pigmentation

Melanin, the natural light-absorbing pigment in the skin, produced from melanocytes has a maximal wavelength absorption of ~300 nm [50, 51]. Melanin prevents UVB photons from reaching 7-dehydrocholesterol [8, 50-52]. Light and dark skinned individuals have the same capacity for endogenous vitamin D synthesis but may have different sun exposure requirements due to the melanin content of their skin [66]. Dark skinned individuals require between 60-72 minutes of sun exposure to achieve the same levels of 25(OH)D concentration that light skinned individuals could produce in 10-12 minutes [31, 50]. Thus, light skinned individuals often have significantly higher 25(OH)D concentration than those with darker skin [1, 7, 8, 51, 53, 142]. This relationship persists with similar

dietary and supplemental vitamin D intake, time spent in the sun, and BSA exposed between the skin groupings [142]. Pregnant, white women have significantly higher 25(OH)D concentration than black women (80.4 nmol/L vs. 49.4 nmol/L, respectively) [43]. Similarly, white infants tend to have significantly higher vitamin D status compared to First Nations and Inuit infants (41.4 nmol/L vs. 34.1 nmol/L vs. 34.6 nmol/L, respectively) [18] and between white and black infants [3, 44]. Dark skin pigmentation increases the odds of vitamin D deficiency in mothers (aOR: 2.74 [95% CI: 1.53-4.88]) and in infants (aOR: 3.36 [95% CI: 1.74-8.55]) [12]. On the other hand, Gordon et al. [20] found no association between 25(OH)D concentration and skin pigmentation in infants (8-24 months) recruited year-round. The high proportion of vitamin D deficiency and low variability in 25(OH)D concentration did not allow detection of any relationships between skin pigmentation.

Tanning due to UVB exposure redistributes melanin in the basal, middle, and upper layer of the skin. Using spectrophotometer to measure skin pigmentation, every 10^{o} lower in forearm skin colour (increased UVB exposure) calculated using individual typology angle (ITA) predicted 5 nmol/L increase in 25(OH)D concentration [54]. Women who had an decrease in 5^{o} ITA (decrease skin colour) predicted a 15.3 nmol/L increase in 25(OH)D concentration over a 15 month period [148]. In women who had no change in skin pigmentation (i.e. likely no UVB exposure), there was a predicted 9 nmol/L increase in 25(OH)D during the same period [148].

The relationship between skin colour and vitamin D status is complex and confounded by individual behavior as related to sun exposure and diet [32]. In a large sample of white and black individuals, skintype was only a significant predictor of 25(OH)D concentration in black individuals [149]. Low vitamin D status among certain ethnic groups may result from other factors than skin pigmentation [150]. Differences in vitamin D status between light and dark skinned individuals may be due to less storage of previtamin D, vitamin D, or 25(OH)D in body tissues beyond the synthesizing period in dark skinned individuals [151] or genetic differences in vitamin D metabolism [138, 152].

Further research is required to explore the genetic relationships between 25(OH)D concentration and skin pigmentation and ultimately on bone health. As black women tend to have much lower 25(OH)D concentrations than white women, but have higher BMD and fewer fractures than white women [153].

9.2.5. Sunscreen Use and Cultural and Lifestyle Practices

The common sunscreen agent, para-aminobenzoic acid, and a sun protection factor (SPF) >8 significantly interferes with endogenous vitamin D synthesis by absorbing UVB radiation [49, 154]. Sunscreen use is either positively or negatively associated with vitamin D status [141] even among women with high sun exposure (12 hours/week) [141]. In contrast, sunscreen use during the summer was associated with higher 25(OH)D concentration compared to those who did not use sunscreen (61.5 vs. 49.6 nmol/L, respectively) [148]. It is possible that there was insufficient sunscreen application in sun-exposed areas or inadequate re-application of sunscreen allowed UVB absorption [104, 148]. Sunscreen with SPF 15 allows 6% of UVB photons to penetrate the skin [104]. In addition, sunscreen use may be a marker for UVB exposure [148]. Although sunscreen use is not recommended in infants <6 months of age, sunscreen behaviours should be considered. Glass absorbs UVB photons [84] thus, skin exposed to sun through windows does not promote vitamin D synthesis. Minimal sun exposure in individuals who dress conservatively due to lifestyle or cultural practices significantly inhibits endogenous vitamin D synthesis [155-157]. Veiled women living in Denmark have lower vitamin D status compared to non-veiled women (7.1 ± 1.1 vs. 12.6 ± 2.6 nmol/L, respectively) [157]. These factors should be considered when assessing sun exposure.

9.2.6. Body Mass Index (BMI) and Body Fat Content

Fat mass changes in both mothers and infants in the immediate months postpartum. For mothers, there may be a large initial weight loss during the first 2-3 weeks postpartum followed by a mild weight and fat loss or plateau [158-165]. In the WHO Multicentre Growth Reference Study, there was a gradual weight loss from delivery to 12 months postpartum with a plateau up to 24 months in five of the six sites, except Ghana [166]. The extra energy requirements

necessary for lactation can be met by mobilizing fat stores acquired during pregnancy, reducing energy expenditure, and/or increasing energy intake [158, 159, 164, 167]. Body composition may be re-established within 6 months postpartum by utilizing the extra fat reserve [168] but weight retention has been evident up to 12 months postpartum [169]. In infants, BMI-for-age increases from 0.5 to 6 months of age suggesting greater deposition of soft tissue including fat mass [170]. Adiposity may not affect endogenous vitamin D synthesis but may increase metabolic clearance through enhanced uptake in fat tissue, decrease bioavailability by acting as a reservoir for vitamin D [1, 60, 61, 171], or alter vitamin D endocrine system with increased $1,25(OH)_2D$ production exerting a negative feedback control on the hepatic synthesis of 25(OH)D [172].

Obese subjects who were exposed to identical amounts of UVB irradiation over a 24 hour period, Δ25(OH)D concentration was less in obese than in non-obese (17.4 ± 3.6 vs. 38.5 ± 5.5 nmol/L, respectively) [61]. Individuals with higher BMI had lower vitamin D status (34.9 ± 2.9 kg/m^2; 57.3 ± 17 nmol/L) than those with lower BMI (27.5 ± 1.2 kg/m^2; 64 ± 20 nmol/L) [173]. Vitamin D status in obese individuals may be 20% lower than normal weight or overweight individuals [60]. An increase in 1 kg/m^2 in BMI predicted 1.0-1.21 nmol/L decrease in 25(OH)D concentration [60, 174]. An increase of 1% in percent TBF (total body fat) predicted 1.15 ± 0.55 nmol/L decrease in 25(OH)D concentration [62]. Vitamin D status was lower in individuals in the highest tertile of TBF (>33%, 47.8 ± 17.3 nmol/L) compared with those in the 1^{st} and 2^{nd} tertiles (69.0 ± 22.2 vs. 69.4 ± 23.8 nmol/L, respectively; p=0.001) [175]. The inverse relationship between 25(OH)D concentration and adiposity, specifically percent TBF [1, 7, 61, 63, 171, 173, 174, 176-178], is stronger than between BMI, waist circumference, waist-to-hip ratio (WHR) [63], and skinfold thickness [176]. This implies that direct measures of adiposity rather than proxies of obesity such as BMI are more useful indictors when assessing the relationship with 25(OH)D concentration [1, 176]. Endogenously synthesized and dietary vitamin D can both be stored in subcutaneous and visceral adipose tissue [173, 176]. Thus, 25(OH)D concentration may not be a good indicator of vitamin D stores in obesity.

Sequestration may occur more in obese individuals due to greater adipose mass [61]. Vitamin D supplementation can correct vitamin D deficiency in obese individuals; both obese and non-obese adults receiving 50000 IU of vitamin D showed a similar peak in 25(OH)D concentration [61].

It has been suggested that the inverse relationship between 25(OH)D and adiposity is stronger in white than black women (% TBF: 33.9 vs. 37.4%) [55]. After adjusting for %TBF, 25(OH)D concentration was higher in white compared to black women (76.7 vs. 47.1 nmol/L, respectively) [55]. The β coefficient was larger in white compared to black women (-0.475 vs. -0.130, respectively) after controlling for age, physical activity, month of blood collection, smoking status, oral contraceptive pills (OCP), dietary vitamin D intake, frequency of milk or cereal consumption, and vitamin-mineral supplement use [55]. These results suggest that the interaction between skin pigmentation and vitamin D status is stronger than the interaction between adiposity and 25(OH)D concentration.

The release of vitamin D from fat depots is slow and proportional to vitamin D concentration in adipose tissues and the rate of re-entry is not yet understood [65, 179]. There is a trend for an inverse relationship between Δweight ($r=-0.37$, $p=0.065$) and ΔBMI ($r=-0.376$, $p=0.059$) with Δ25(OH)D concentration [63]. This relationship is significant in obese children [180] and adults [63, 64, 181]. In a 20 week weight loss study, 10% weight loss and 12% TBF loss was associated with 34% increase in 25(OH)D concentration (38.5 ±15.0 vs. 47.8 ± 12.8 nmol/L, respectively) with PTH levels unaffected (5.7 ± 2.9 vs. 5.4 ± 2.0 pmol/L, respectively) [63]. Results were not controlled for confounding variables and interactions. After 5% fat mass loss, there was a significant increase in 25(OH)D concentration [63]. Increasing tertiles of 25(OH)D concentration (median for tertiles = 36.2, 52.9 ± 4.7, and 75.4 nmol/L) was associated with greater decrease in weight (-3.1 ± 5.7, -3.8 ± 4.4, and -5.6 ± 6.6 kg, respectively; between-group effect $p=0.013$) [64]. In addition, increasing tertiles of Δ25(OH)D concentration (-23, -6.5, +6.3 nmol/L) was associated with greater weight loss (-2.5 ± 4.9, -4.0 ± 5.3, -5.8 ± 7.0 kg, respectively; between-group effect $p=0.009$) [64]. These studies suggest a threshold effect between 25(OH)D concentration

and weight and fat loss. Lastly, maternal BMI is a risk factor for both maternal and infant vitamin D deficiency [2, 12]. These results suggest that increased maternal adiposity may impede sufficient transfer of vitamin D to the infant during pregnancy and lactation. Changes in adiposity in lactating women and infants could contribute to vitamin D status.

9.3. Breastfeeding Practices in Canada

The World Health Organization (WHO) recommends to exclusively breastfeed infants from birth to 6 months postpartum to promote optimal growth, health, and behavioural development [22]. In 2003, 85% of mothers reported that they attempted to breastfeed their infants [182]. This is a significant improvement from 25% in the mid-1960s [183] and 79% in the mid-1990s [184]. From 2003 to 2005, infants who were exclusively breastfed for 6 months increased from 14.2% to 16.4% [185]. Optimal nutritional status is important to support breastfeeding and support maternal-infant nutrient transfer, including vitamin A and B_{12}, iron, zinc, [22], and vitamin D [13, 22]. Maternal diet may be low in these nutrients, and with low concentration in breast milk, results in low intakes in their infants. This could be overcome by improving the mothers diet and/or supplementing the infant directly, as is the case for vitamin D [22]. Indeed, breast milk contains 1-10 IU/250 mL of vitamin D and 25(OH)D [77, 186, 187]. Due to the limited transfer from plasma to breast milk [187], ~ 20-30% [135, 188], breastfed infants without vitamin D supplementation are at high risk for developing vitamin D deficiency [20, 189]. In Canada, exclusively or partially breastfeeding for longer period [183, 190] is associated with lower infant 25(OH)D concentration [191]. Thus, it is important to ensure adequate vitamin D stores in both mothers and infants.

9.4. Vitamin D Recommendations for Lactating Women

The IOM increased vitamin D recommendation to RDA of 600 IU/d [30] from an AI of 200 IU/d for lactating women emphasizing the importance of vitamin D on bone health [115]. The RDA is set to meet the needs of >97.5% of the population, the EAR corresponds to the median intake needs of the population, and the AI is an alternative recommendation when there is insufficient evidence to develop an EAR or RDA [30, 192]. However, meeting the EAR of

400 IU/d may not maintain or significantly improve vitamin D status [31-35] without UVB exposure [35, 193]. The Canadian Paediatric Society suggests vitamin D supplementation of 2000 IU/d for pregnant and lactating women to support maternal-infant transfer pre- and post-natally [13]. This may increase maternal 25(OH)D concentration by 55 nmol/L over 1 year of supplementation [62]. Higher doses of vitamin D supplementation up to 6000 IU/d is also being researched in this population [35, 129, 194, 195], although no recommendation has endorsed such high intakes.

Health Canada's recommendations for milk, fish, and supplement intake in lactating women emphasized the importance of vitamin D to bone health (**Table 1**). In Eating Well with Canada's Food guide, lactating women should include 2 servings of milk and alternatives with 2 to 3 extra food guide servings daily [27]. These extra food guide servings may inclusively be from milk and alternatives (milk, soy beverage, yogurt) [27]. Therefore, milk and alternatives can contribute ~500 IU/d of vitamin D to lactating women [27]. In March 2010, Statistics Canada reported that milk consumption more than once a day was positively associated with higher 25(OH)D concentration [127]. On the other hand, there were no significant differences in the vitamin D status of individuals who drank milk and who did not (87.5 ± 25 vs. 82.5 ± 25 nmol/L, respectively) [139]. However, vitamin D status was high in this sample population and the amount of milk consumed was insufficient to increase 25(OH)D concentration.

Health Canada also recommends consumption of at least 2 servings (150 g)/week of cooked fish including among lactating women [28]. Based on the Canadian Nutrient File (CNF), this would translate into 1398 IU/week of vitamin D if both servings were sockeye salmon, 492 IU/week if both were wild Atlantic salmon, 288 IU/week if both were canned pacific sardines with bones contains, 156 IU/week if both were Atlantic Mackerel, and 72 IU/week if both were light canned tuna in oil [196]. Vitamin D intake from fish could range from 10-200 IU/d of vitamin D depending on the type of fish consumed. Predatory fish such as fresh and frozen tuna and shark should be avoided due to high levels of methyl mercury that crosses the placenta and harms the developing infants' brain [28].

Regardless of latitude and ethnicity, few women meet the vitamin D intake recommendations. In pregnant women, mean daily vitamin D intake was 229 IU/d [123, 141]. For mothers in northern Canada, vitamin D intake in First Nations and Inuit was 136 ± 100 IU/d and in Caucasians was 232 ± 172 IU/d [18]. There were no significant differences between vitamin D intake in white mothers with sufficient (25(OH)D ≥32.5 nmol/L) and insufficient vitamin D status (284 ± 224 vs. 172 ± 164 IU/d, respectively) [197]. First Nation and Asian mothers also had low vitamin D intake (132 ± 152 IU/d vs. 152 ± 180 IU/d, respectively) [197]. Since there is a lack of Canadian data on vitamin D intake of lactating women, the best comparator would be data from the Canadian Community Health Survey for women of reproductive age (18-50 years). Their mean intake of vitamin D from food was 200 IU/d [182]. Therefore, dietary vitamin D intake of lactating women and those of reproductive age both fall short of what is now considered the RDA.

Health Canada recommends that pregnant and breastfeeding women take a multivitamin which contains 150 or 400 IU of vitamin D/tablet [29]. Although dietary supplement intake tends to be lower during lactating than pregnancy [45, 198], pre- and post-natal supplements are important sources of vitamin D [37]. With limited information on supplemental intake in lactating women, vitamin D intake in pregnant women provides insight regarding dietary supplementation behaviours in this population. With supplements, vitamin D intake in First Nations and Inuit mothers was 324 ± 220 IU/d and in Caucasian mothers was 528 ± 236 IU/d [18]. Although, there is a positive association between 25(OH)D concentration and total vitamin D intake (dietary and supplemental) [8, 18, 43, 45], this relationship is not seen in dark pigmented women [35] or in the winter months [32]. However, fish intake was not assessed. Prenatal supplement use ≥5 times/week in the third trimester may be protective against maternal (aOR 0.37 [95% confidence interval (CI) 0.20-0.69]) and infant vitamin D deficiency (aOR: 0.30 [95% CI: 0.16-0.56]) [12]. In addition, those who took a multivitamin had significantly higher vitamin D status than those who did not (92.5 nmol/L vs. 72.5 nmol/L, respectively) [139]. Among women taking a daily prenatal supplement containing 400 IU of vitamin D, 7.5% had 25(OH)D concentration <37.5 nmol/L

and only 49.2% had 25(OH)D concentration ≥75 nmol/L [45]. The IOM recommends that lactating women obtain nutrients from a well-balanced, varied diet rather than supplements [199]. However, even when supplements were considered, the majority of women did not achieve the recommended vitamin D intake as proposed by various organizations.

Overall, vitamin D food sources are primarily mammalian products. Thus, omnivores have significantly higher dietary vitamin D intake compared to vegetarians (165 vs. 119 IU/d, respectively) [142]. Omnivores tend to have significantly higher 25(OH)D concentration than vegetarians and vegans [142, 200]. Similarly, vegetarian, lactating women have lower 25(OH)D concentration than non-vegetarian, lactating women (49.9 ± 12.5 nmol/L vs. 94.9 ± 12.5 nmol/L, respectively) [186]; this is most apparent during winter and spring months [186, 200]. However, regardless of the source of vitamin D (mammalian or plant-based), if total vitamin D intake was similar between omnivores and vegetarians (403 vs. 351 IU/d, respectively), there were no significant differences in 25(OH)D concentration (78.6 vs. 76.8 nmol/L, respectively) [142].

9.5. Vitamin D Recommendations for Infants

The IOM increased vitamin D recommendation from an AI of 200 to 400 IU/d for infants [30, 33]. This is aligned with Health Canada's recommendations since 1967 [19] that all breastfed, healthy term infants receive a daily vitamin D supplement of 400 IU at birth and continue until their food intake contains 400 IU/d [77]. The CPS recommends an increase to 800 IU/d between October to April for those living in latitudes >55 ^{o}N, between 45-55 ^{o}N in individuals with vitamin D deficiency risk factors, and those with dark skin pigmentation [13].

Due to the rapid body weight increments of infants between birth and 12 months of age, it has been suggested that vitamin D recommendations be based on weight [201]. In breastfed infants, aged 1 to 4 months, receiving 400 IU/d of vitamin D, there was a significant increase in 25(OH)D concentration (40 ± 23.3 to 109 ± 35.3 nmol/L) despite a significant decrease in vitamin D intake/kg (88.9 ± 10.5 IU/kg to 59.7 ± 6.6 IU/kg) [129]. Between 4 to 7 months of age, 25(OH)D concentration remained unchanged but infants were receiving 50.5 ± 6.0 IU/kg

[129]. For infants consuming both breast milk and formula, 400 IU/d of vitamin D is recommended if intake of vitamin D fortified formula is <500 mL/d [146].

9.6. Compliance of Infant Vitamin D Supplementation

Infants without supplements are at higher risk for developing vitamin D deficiency [20, 21]. However, compliance in administering vitamin D supplements to infants in study samples varies between 2% in Boston, Massachusetts [20], 16% in Washington, DC [202], 64% in Switzerland [203], and 82% in New Orleans, LA [24]. When pediatricians recommended vitamin D supplementation, <50% of parents administered the supplement to their infant [202]. In Canada, 53% of breastfed infants received a supplement containing vitamin D however, the frequency and quantity was not collected [19]. Newfoundland and Labrador found that 34% of infants were given vitamin supplements [16]. In a recent Montreal survey, 74% of exclusively breastfed infants received 400 IU/d of vitamin D [204]. Overall it appears that compliance in administration of vitamin D supplements to infants varies globally.

9.7. Measured Outcomes of Vitamin D Predictors

9.7.1. Total 25(OH)D Concentration

The best clinical assessment of vitamin D status is serum 25(OH)D concentration. From the Bland-Altman plot, adult capillary samples is ~19 nmol/L higher compared to venous samples (limit of agreement (LoA) between capillary and venous samples were -5.6 to 43 nmol/L) [205]. This relationship has not been examined in infants. Both the plasma and serum reflects recent dietary intake and nutrients being transported to the tissues accurately reflecting nutrient status [38]. It is preferred to perform vitamin D assays on serum but ethylenediaminetetraacetic acid and lithium-heparin treated plasma is satisfactory [206]. Vitamin D metabolites are unaffected by up to 4 freeze-thaw cycles [207] and when stored at -20°C for up to one year [41]. During procurement, collection time should be standardized [208].

Various clinical assays have been developed since 1971 to measure 25(OH)D concentration (**Table 2**). Assays include radioimmunoassay (RIA) (uses antibodies that recognize both $25(OH)D_2$ and $25(OH)D_3$), competitive binding

protein (CBP) (which utilizes a reagent that separates vitamin D from DBP), and direct detection measurements such as gas chromatography/mass spectrometry, high performance liquid chromatography (HPLC), and liquid chromatography tandem mass spectroscopy (LC-MS/MS) [74]. Chromatographic separation is a suitable reference method. The HPLC with UV detection and LC-MS/MS are considered the gold standard [41, 83]. Due to problems with reproducibility and accuracy when analyzing small quantities of samples, LC-MS/MS tends to be used by high-volume reference laboratories with highly trained operators [39, 209]. Until recently, it was accepted that these methods were unsuitable for routine, clinical and laboratory use due to its complexity and cost [41]. Since 25(OH)D binds to DBP (85%), albumin (15%), and is free (0.03%) [41], an extraction step is required to release 25(OH)D from DBP [41]. Assays must be able to detect both $25(OH)D_2$ and $25(OH)D_3$. DiaSorin RIA, immunodiagnostic systems Gamma-B, and Nichols Advantage assays underestimate $25(OH)D_2$. Although, DiaSorin RIA method was improved with ^{125}I-labelled tracer to equally detect both vitamin D isoforms, it has been found to underestimate $25(OH)D_2$ [41]. Nichols Advantage CBP assay overestimates total 25(OH)D concentration by an average of 85% when $25(OH)D_3$ is the dominant form in the serum and underestimates by 27% when $25(OH)D_2$ is the dominant form [209]. Total 25(OH)D detection is necessary and under-detection of $25(OH)D_2$ is a major limitation, if vitamin D_2 supplements are administered. The most recent candidate reference method utilizes liquid-liquid or liquid-solid pre-sample clean-up with UV detection after column separation [41]. Due to the significant serum 3-epi-25(OH)D concentration found in infants, serum 25(OH)D should be measured with an assay that does not cross-react with 3-epi-25(OH)D or allows unequivocal separation of 3-epi-25(OH)D from 25(OH)D concentration [98].

Recently, chemiluminescence assays have utilized both DBP and/or antibody-based binding for 25(OH) detection [41, 206]. The LIAISON® 25(OH)D assay has been validated and is a rapid, accurate, and precise tool for measuring 25(OH)D concentration [206] utilizing small volumes, 25 µL [39, 41]. The equivalent cross-reactivity of $25(OH)D_2$ and $25(OH)D_3$ is 100% and range of

detection is between 7.5-375 nmol/L [206]. When compared against HPLC, there are no significant differences between total recovery of 25(OH)D using RIA or LIAISON®, or total recovery that were analyzed first by HPLC and then by LIAISON® [206]. The analytical sensitivity (≤10 nmol/L) and precision of the intra- (4%) and inter- (6%) assay is comparable to results reported by RIA [206]. Comparison between assays remains a problem due to variability in VDP interactions , recognition of vitamin D metabolites [41], and calibration errors [132]. When HPLC with UV detection or LC-MS/MS is unavailable, comparison of vitamin D status among different assays must be examined with caution.

9.7.2. Dietary Assessment

Measurement of total vitamin D intake (dietary and supplemental) is necessary to evaluate vitamin D status. To accurately assess the relationship between vitamin D intake and vitamin D status, it is important to select an assay with equivalent cross-reactivity for $25(OH)D_2$ and $25(OH)D_3$ to reflect dietary and supplemental sources that contain vitamin D_2 or D_3. The 24 hour food recall utilizes the multiple-pass technique and provides relatively precise information about recent food intake [38, 75, 208, 210, 211]. Due to individual and day-to-day variation, multiple 24 hour recalls are necessary to reflect usual intake of foods and nutrients and be administered throughout the year to account for seasonal food variations [38, 75, 210]. However, there are no published reports indicating the necessary number of recalls to estimate usual vitamin D intake. The 3- and 7-day food records are commonly used to capture vitamin D intake [38, 75, 208, 210, 211]. However, food records require high participant motivation resulting in low response rates, and the act of recording may alter the diet [75, 211].

For micronutrients such as vitamin D that are derived from relatively few food sources, a FFQ representing a long period of time (i.e. 1 month) may be the best estimate of usual intake [208]. FFQs can be qualitative (Willett) or semi-quantitative (Block) and provide retrospective information on the patterns of dietary intake during longer periods [38]. FFQs can be completed within 15-30 minutes imposing less participant burden [38]. Reproducibility of any FFQ is necessary to evaluate as it will reflect both the performance of a FFQ and the true

change in diet [208]. Validation of a FFQ is important to evaluate the degree to which the FFQ reflects aspects of the diet it was designed to measure [38, 208, 211, 212]. FFQ are commonly validated against another dietary assessment method. Few validate against both dietary method and biomarkers [212]. Biomarkers are considered the gold standard to validate FFQs because measurement errors should be uncorrelated to errors from self-reported dietary assessment methods [208].

The use of correlation coefficients in validation studies is controversial but could be useful in conjunction with the Bland-Altman method, a graphical form to assess the agreement between methods [213]. The means of the intake determined by the two methods are plotted against the difference [212]. Additional statistical analyses include weighted Kappa (K_W) statistics and cross-classification. The equation for K_W is $[P_{O(W)} - P_{e(W)}]/[1- P_e]$ where P_O is the observed proportion of agreement and P_e is the expected proportion of agreement by chance [214]. K_W >0.80 indicates very good agreement, between 0.61 to 0.80 is good agreement, between 0.41 to 0.60 is moderate agreement, between 0.21 to 0.40 is fair agreement, and <0.20 is poor agreement [215]. Cross-classification utilizes a grid-like table with three (tertile), four (quartile), or five (quintile) categories to identify the percentage of individuals correctly classified into the same, adjacent, and opposite category of intake by the FFQ and reference method [216]. The percentage misclassified is likely impact of measurement error and the percentage of agreement is likely actual agreement that could also be by chance [216].

Issues that must be considered regardless of method chosen are underestimation of unhealthy foods, overestimation of healthy foods, day-to-day variability in eating patterns, seasonal food variations, and incomplete information on the nutrient content of certain foods [208]. Estimating and evaluating nutrient intake in Canada can be analyzed using nutrient analysis software using the latest version of the CNF [8, 18, 25].

9.7.3. Sun Exposure Assessment

Estimating UVB exposure requires measurement of latitude, personal ambient, and anatomical distribution of exposure. Ambient exposure is measured

quantitatively by dosimetry which assesses UVB exposure and accounts for environmental influences [217]. A dosimeter will continue to measure sun exposure regardless of cutaneous vitamin D synthesis ability. Personal ambient exposure is determined by amount and time of sun exposure, clothing worn outside, and sunscreen use [20, 35, 217]. This information does not consider age and skin colour [217, 218]. Sun exposure questionnaires tend to be a poor proxy for vitamin D status due to imprecise UVB estimates [217]. The sun index is the product of hours of sun exposure per week and fraction of sun-exposed BSA [142, 143]. BSA exposed can be calculated using adapted burn exposure charts described by Hall et al. [53], O'Sullivan and Schmitz [219], and the rule of nines [53, 219]. For both adults and infants <12 months of age, the fractions of BSA that are similar included neck (0.02), chest (0.02), back (0.13), shoulders (0.02), upper arms (0.06), lower arms (0.06), hands (0.05), and feet (0.07). In infants, the fractions of BSA for the face is 0.08, upper legs are 0.11, and lower legs are 0.10 [219]. In adults, the fractions of BSA for the face is 0.04, upper legs are 0.19, and lower legs are 0.14 [219]. The sun index is also known as sun exposure factor has been used to assess sun exposure in infants [44, 134]. UVB exposure can be assessed using the reflectance spectrophotometer from changes in skin colour.

9.7.4. Skin Pigmentation Assessment

Skin pigmentation is a more sensitive index of vitamin D status than racial origin [45]. Classification systems such as "light" or "dark" [6, 20], Munsell colour-order system [220], and Fitzpatrick skin type (I-VI) [221] have been used. Skin type I always burns, never tans; type II always burns, tans less than average or with difficulty; type III sometimes mildly burns, tans average; type IV does not burn, has marked tan; and type V and VI do not sunburn or tan [221]. These methods provide subjective classification of skin pigmentation.

Skin colour can be classified as constitutive skin, genetically determined colour such as underarm [35, 53, 54, 57], inguinal area [58], and upper thigh [35], or facultative skin, which develops due to sun exposure [58] such as forearm [35, 54] and forehead [57, 58]. The forehead may not be a suitable facultative site due to hair bangs and hats [222]. Skin colour can be objectively measured with

reflectance UV spectrophotometer, colour analysis, or reflectance Commission Internationale de l'Eclairage (CIE) colorimetry [220, 223] following the European Society of Contact Dermatitis guidelines [223]. The CIE 3-dimensional colour system of L* (black to white), a* (green to red), and b* (blue to yellow) values is widely accepted [222, 223]. The spectrophotometer is an objective, reproducible method to determine sun exposure and allows measurement of skin colour changes due to UV radiation [224]. The spectrophotometer measures degree of skin pigmentation on a continuous scale from 0 to 100, where 0 is absolutely black and 100 is absolutely white [35, 225, 226]. Increased L*, a*, and b* values are quantified as more white, more red, and more yellow, respectively [222]. L* values are influenced by pigmentation and blood volume of the skin, a* values are markers of blood volume of the skin, and b* values are influenced by cutaneous factors other than pigmentation [223]. Decreasing L* or increasing b* values are indicative of skin colour changes associated with tanning due to UVB exposure [222]. Skin classification can be determined by ITA (ArcTangent (L-50/b)*180/π) into 6 different skin types: very light >55^{o}> light >41^{o}> intermediate >28^{o}> tanned >10^{o}> brown >-30^{o}> dark [227, 228]. The tanning effects of UV exposure is a result of increased and redistribution of melanin from the basal to upper layers [55, 56, 87] and is important to examine its effect on endogenous vitamin D synthesis. Melanin index (100*log(1/red reflectance)) is a specific marker of skin pigmentation [223]. Melanin index ranges from low 20s, correlating to the lightest skin tone, to more than 100, correlating to the darkest skin tone [8]. When utilizing these assessment tools to measure skin pigmentation and sun exposure, important considerations are its ability to quantify skin colour, changes in sun exposure, and inter- and intra-rater reliability [222].

Infant skin undergoes a dynamic process of adaptation and maturation during the first 3 months of life particularly increased epidermal desquamation (skin turnover) on the forehead and forearm [229] and increased skin thickness [230]. Although, these methods of assessment have been used determine skin pigmentation in infants, careful interpretation of infant skin pigmentation is necessary as spectrophotometer may not be reliable in infants.

9.7.5. Body Composition Measurement

Body composition techniques have not been validated in lactating women to measure short term changes [231]. Despite this limitation, skinfold thickness measurements, bioelectrical impedance analysis (BIA), ultrasound techniques, and magnetic resonance imaging (MRI) have been utilized to determine body composition in this population [231, 232]. Height and weight are used to measure nutritional status [38, 208] but does not provide direct information about TBF or regional fat distribution [38]. BMI (kg/m^2) evaluates the appropriateness of weight for height and correlates with many measures of TBF content [211]. WHR assesses subcutaneous and visceral fat depots [38] and WC measures intra-abdominal visceral fat and correlates better with abdominal fat content and TBF than WHR [38, 211]. WHR and WC does not reflect adipose depots and are not suitable for postpartum women [233]. Measurements of triceps, midaxillary, subscapular, suprailiac, and midthigh skinfolds are indicators of subcutaneous fat but not for visceral fat, which is closely related to health [38, 75, 211]. With higher variability in subcutaneous fat distribution, intra- and inter- measurements [208], higher hydration, and distortion of the skin in postpartum women, skinfold thickness may be inappropriate and does not reflect subcutaneous fat [233].

Body composition can be assessed with tissue conductivity techniques (total body electrical conductivity, near-infrared interactance, BIA). These methods utilizes the conductivity of hydrated tissues to estimate FM and fat free mass (FFM) [38, 75]. Thus, TBF estimates are affected by changes in hydration, electrolyte concentration, and recent eating, drinking, and exercise [38, 75]. The near-infrared interactance is based on the principles that FFM reflects light and FM absorbs light. Based on the infrared light reflected back after penetration in underlying tissues, %TBF is calculated based on predicted equations [75]. Both the near-infrared interactance and BIA are non-invasive, portable, and inexpensive [233] but near-infrared interactance is not as accurate as other methods [75]. The BIA is based on the principle that electrical resistance is inversely proportional to the total body water (TBW) content and electrolytes concentration [231]. Although equations to convert impedance data to estimate

body composition are not specific to lactation women [231], primary values from the BIA (model BIA-103) have been applied to women during a 3 month lactation period [231]. Resistance values remained unaltered and since these values are associated with TBW, weight loss during this period reflected fat loss rather than TBW loss [231]. It is still debated whether the BIA is an appropriate measure during lactation since the comparable method used was skinfold thickness which is not an accurate measurement in lactating women. BIA correlates well to different density measurement techniques [233]. The foot-to-foot BIA (Tanita, TBF 300 GS, US) have been used in a vitamin D study involving adults [60].

Density measurements such as hydrodensitometry and air displacement plethysmography estimates body composition from the deduction of volume of water displaced or volume of air displaced, respectively [38]. These techniques do not provide information on regional fat distribution [211] and tend to overestimate fat in pregnancy [233]. Thus, density measurements may overestimate fat during lactation. Imaging techniques include dual-energy x-ray absorptiometry (DXA), MRI, computerized tomography (CT), and ultrasonography. DXA estimates BMD, FM, and lean body mass by passing two x-ray beams with differing energies through the body which involves a small amount of radiation exposure [75, 211]. Although CT provides a very accurate indication of visceral and subcutaneous abdominal fat, there is significant radiation exposure. MRI provides accurate and precise measurements of intra-abdominal, retroperitoneal, and subcutaneous adipose tissue [75, 211]. Both MRI and ultrasound scanning are considered safe in postpartum women and do not involve radiation exposure [233] but the equipment is expensive and require trained personnel to administer and interpret the data.

The WHO Child Growth Standards can be used to estimate TBF in infants by using BMI-for-age, triceps skinfold-for-age, and subscapular skinfold-for-age curves [170]. BMI-for-age increases from 0.5 to 6 months of age suggesting greater deposition of soft tissue including fat mass [170]. Triceps skinfold decreases from 3 to 20 months and increases up to 60 months [170]. Boys have gradual decrease in subscapular skinfold from 3 to 24 months and plateaus

whereas, girls have a decrease from 3 to 21 months and increases beyond 21 months [170]. For infants, adiposity may increase metabolic clearance of vitamin D through enhanced uptake in fat tissue and/or decrease bioavailability by sequestration in fat tissue [1, 60, 61, 171]. Adiposity in infancy may be an important consideration in assessing changes in vitamin D status in early infancy.

9.7.6. Infant Growth Assessment

Functional indicators such as skeletal growth patterns, bone mineral content, BMD, and PTH and calcium concentrations are important markers of vitamin D status. Measurements of infant growth include weight, recumbent length, and head circumference. Crown rump is also an indirect measure of spine growth. These anthropometric measurements, except crown rump, can be plotted on age- and sex-appropriate standardized growth charts and z-scores can be calculated to directly assess infant growth patterns and indirectly assess nutrition. The 2000 US Centers for Disease Control and Prevention (CDC) Growth Charts and the 2006 WHO Child Growth Standards are widely utilized [234]. The CDC Growth Charts provide percentile curves for weight-for-age, length-for-age, weight-for-length, and head circumference-for-age [75]. These growth charts were derived from the proportion of formula and breastfed infants in the population where only one-third of all infants were breastfed for ≥3 months [235]. Exclusively breastfed infants tend to gain weight more rapidly in the first 2 to 3 months and from 6 to 12 months gain less weight than formula fed infants [235].

The WHO Child Growth Standards are appropriate to assess growth in predominately breastfed infants. Their standards were developed based on data collected from the WHO Multicentre Growth Reference Study in six countries to reflect an international standard [234] on how children should grow based on current breastfeeding recommendations [170]. In addition, WHO growth charts also monitors body composition [170]. More importantly, the WHO growth curves capture rapid growth patterns in early infancy [234].

9.8. Conclusion

In summary, hypovitaminosis D is a concern among Canadian lactating women and their infants despite public health policies. There is limited

information on the vitamin D status of this population. The potential predictors of vitamin D status have not been comprehensively evaluated and measured in lactating women and their infants in the postnatal period. Thus, assessing vitamin D status and the extent to which these predictors contribute to status may assist, if warranted, in the development of strategies to improve vitamin D status.

Table 1. Dietary Sources of Vitamin D from CNF 2010b [196]

Food ***(Food Code)***	**Per serving**	**Vit D (IU)**
Meat and alternatives		
Salmon, sockeye (red), baked or broiled (*3053*)	75 g	699
Salmon, sockeye (red), canned, solids with bone and liquid (*3223*)	75 g	597
Salmon, Atlantic, wild, baked or broiled (*3156*)	75 g	246
Salmon, Atlantic, farmed, baked or broiled (*3183*)	75 g	204
Trout, rainbow, wild, baked or broiled (*3206*)	75 g	210
Trout, rainbow, farmed, baked or broiled (*3187*)	75 g	192
Herring, Atlantic, baked or broiled (*3015*)	75 g	161
Trout, mixed species, baked or broiled (*3215*)	75 g	150
Sardine, Pacific, canned in tomato sauce, drained with bones (*3054*)	75 g	144
Tilapia, baked or broiled (*5967*)	75 g	121
Egg, chicken, whole, scrambled or omelet (*133*)	2	88
Mackerel, Atlantic, baked or broiled (*3022*)	75 g	78
Sardine, Atlantic, canned with oil, drained with bone (*3203*)	75 g	70
Tuna, light, canned with oil (*3214*) or water (*3131*), drained, unsalted	75 g	36
Beef, liver, pan fried (2657), braised (*2656*)	75 g	36
Pork, cured, ham, rump, bone-in, separable lean fat, heated, roasted *(6207)*	75 g	24
Haddock, baked or broiled (*3199*)	75 g	9
Chicken, broiler, thigh, meat and skin, water chill, roasted *(851)*	75 g	8
Milk and alternatives		
Milk, evaporated, skim, canned, undiluted, 0.2% M.F. (*112*)	250 mL	216
Milk, fluid, chocolate, partly skimmed, 1% (*4711*), 2% MF (*70*)	250 mL	105
Milk, fluid, partly skimmed, 2% M.F. (*61*) or skim (*114*)	250 mL	103
Milk, fluid, whole, pasteurized, homogenized, 3.3% MF (*113*)	250 mL	103
Milk, dry, skim, powder, regular, prepared (*134*)	250 mL	103
Milk, fluid, goat, enriched, whole (*72*)	250 mL	100
Beverage, soy, enriched, all flavours (*6720*) or reduced fat (*6784*)	250 mL	87
Yogurt, fruit variety, fat free, vitamin A and D added, with sucralose, SOURCE, YOPLAIT (*6293*)	100 g	31
Yogurt, fruit bottom, 1% to 2% MF (*144*)	100 g	trace
Yogurt, stirred/Swiss style, fruit/vanilla flavours, 2-4% MF (*6295*)	100 g	49
Yogurt, plain, 2% to 4% MF (141)	100 g	49
Fruits and vegetables		
Orange, juice, chilled, includes from concentrate, fortified with added calcium and vitamin D (*6203*)	250 mL	100
Mushroom, shiitake, cooked (*2125*)	100 mL	17
Orange, juice, chilled, includes from concentrate (*1620*)	250 mL	0
Margarine, tub, non-hydrogenated		
Canola and safflower oils, BECEL (*6009*)	5 mL	25
Canola oil, Healthy Attitude Omega-3, LACTANTIA (*6017*)	5 mL	25
Olive oil, OLIVINA (*6014*)	5 mL	25

Eating Well with Canada's Food Guide recommendations: fish: 150 g/week; meat and alternative: 150 g/day ; milk and alternatives: 2 servings/day + possible 2-3 extra serving inclusively from milk and alternatives [27]

Table 2. Commercially Available Assays for Measuring 25(OH)D Concentration [39, 41]

Assay type and manufacturer	Sample type and volume	Extraction	Range of detection (nmol/L)	Sensitivity (nmol/L)	Intra-assay CV (%)	Inter-assay CV (%)	Assay time
RIA							
DiaSorin	Serum or plasma, 50 µL	Acetonitrile, then I-labeled 25(OH)D and antibody, second antibody as precipitating agent	0-100	≤6	<8	<12	2.2 h
IDS Inc	Serum or plasma, 50 µL	Two step reagent extraction (acetronitrile and cellulose)	4-400	≤3	6.8	8.9	3 h
ELISA							
IDS Inc.	Serum or plasma, 25 µL	None	6-360	≤5	<6	<9	3 h
Immunodiagnostic	Serum or plasma, 30 µL	Proprietary extraction reagent	6.3-250	2	10	8	Overnight
CPB							
Immunodiagnostic	Serum or plasma, 50 µL	Acetonitrile	6.4-250	65	11	13	4.5 h
HPLC							
Immunodiagnostic	Serum, 500 µL	Acetonitrile and C_{18} cartridge extraction	Up to 1250	4	5.2	8.4	20 minutes
Chemiluminescence							
DiaSorin Liaison	Serum or plasma, 25 µL	Automated (incubated with anti-vitamin D coated microparticles and isoluminol derivative-conjugated 25(OH)D)	7.5-375	≤10	4	6	40 minutes

25(OH)D: 25-hydroxyvitamin D; CV: coefficient of variation; h=hour(s)

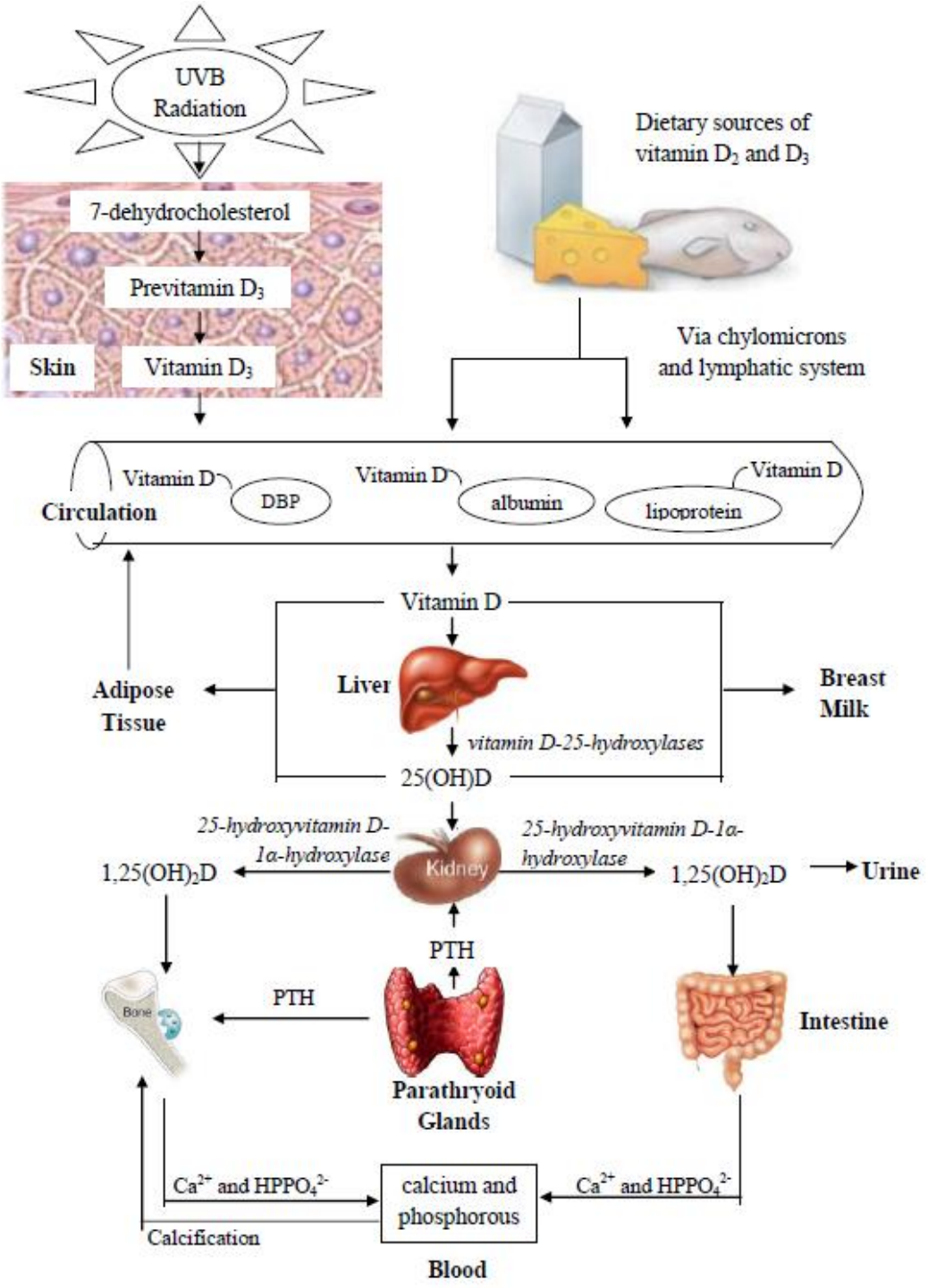

Figure 1. Synthesis, Absorption, and Metabolism of Vitamin D [82, 103]

10. RATIONALE AND OBJECTIVES

Vitamin D is fundamental to the development and maintenance of bone health. Vitamin D deficiency is a concern in both breastfeeding mothers and their infants. Declines in maternal vitamin D status during pregnancy and post-delivery limit vitamin D stores transferred to infants pre- and post-natally. Thus, it is important to determine and examine the extent to which potential factors such as vitamin D intake (dietary, supplemental, and total as dietary and supplemental), season, latitude, sun exposure, sunscreen use, skin pigmentation, and adiposity contribute to vitamin D status. To date, studies examining maternal and infant vitamin D status tend to be cross-sectional and of the limited longitudinal studies, few examined predictors of status beyond delivery and changes in status during lactation. Total vitamin D intake (dietary and supplemental) is a major contributor to vitamin D status especially for infants and when UVB exposure is limited. Since vitamin D is found in limited food sources and may not be consumed on a daily basis, the FFQ is a useful tool to capture intake over a longer time interval. Due to differences in physiological metabolism and intake, FFQ should be validated in the population in which it will be utilized.

The objectives of this thesis were to: 1) determine vitamin D status at 1 and 4 months postpartum and changes since 1 month in lactating women and their infants, 2) identify key predictors of vitamin D status at 4 months and change since 1 month postpartum in lactating mothers and their infants, and 3) validate a FFQ, adapted for Canadians, to assess vitamin D intake among lactating women.

The results of this research will contribute to the growing body of knowledge and the current limited research concerning the relationship between vitamin D predictors and vitamin D status in breastfeeding women and their infants at 1and 4 months of age and Δ25(OH)D concentration since 1 month. The identified predictors in this unique population can assist with developing preventative strategies and knowledge transfer to healthcare policymakers and professionals to prevent vitamin D deficiency. Lastly, the results of this study can provide the basis for future larger studies such as randomized clinical trials (RCT) in this understudied population.

11. MANUSCRIPT 1

Baseline 25-hydroxyvitamin D and UV Exposure are Common Predictors of Change in Vitamin D Status in Lactating Women and their Infants from 1 to 4 Months Postpartum

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11.1. Abstract

Vitamin D deficiency is a concern in lactating mothers and their infants. However, there is limited information on the predictors of vitamin D status and change in status of lactating women and their infants during the lactating period. The primary objective of this study was to determine vitamin D status of lactating women and their infants at 1 and 4 months postpartum and change since 1 month postpartum. The secondary objectives were to identify key predictors of vitamin D status at 4 months and change since 1 month postpartum in lactating mothers and their infants (n=44). Total 25(OH)D concentration was analyzed using competitive chemiluminescence assay (LIAISON®, DiaSorin, Stillwater, MN). Participants were recruited as part of a RCT where breastfed infants received 400 IU/d of vitamin D. Demographics, anthropometry, body composition, sun exposure, skin pigmentation, supplement use, and nutrition information were collected to identify predictors of vitamin D status. From 1 to 4 months postpartum, there was a decrease in maternal vitamin D status (73.0 ± 21.6 to 62.4 ± 18.3 nmol/L, $p<0.001$) and an increase in infant vitamin D status (60.2 ± 31.0 to 71.5 ± 25.4 nmol/L, $p=0.032$). Due to the differences observed in vitamin D status from 1 to 4 months postpartum, the predictors of change in status were examined. Predictors of maternal 25(OH)D concentration at 4 months postpartum included UV exposure (the number of weeks spent in the synthesizing period), vitamin D intake, and % change in TBF; predictors of maternal Δ25(OH)D concentration included % change in weight, the number of weeks spent in the synthesizing period, and 25(OH)D concentration at 1 month postpartum. Predictors of infant vitamin D status at 4 months of age included UV exposure (travel to a latitude <37 ^{o}N) regardless of season, facultative skin pigmentation, and maternal 25(OH)D concentration at 4 months; predictors of infant Δ25(OH)D concentration included travel to a latitude <37 ^{o}N , 25(OH)D concentration at 1 month of age, and ethnicity. These predictors are important to identify to prevent declines in vitamin D status that are associated with bone-related as well as a number of chronic diseases in lactating mothers and their infants.

Key words: vitamin D, lactation, infants, predictors, 25-hydroxyvitamin D

11.2. Introduction

Vitamin D is fundamental in the development and maintenance of bone health and the prevention of rickets [14, 25], osteoporosis, bone fractures [66, 67], and several chronic diseases [67-73]. Vitamin D deficiency is a problem globally [1-3, 5, 6, 8, 10], as well as among Canadian mothers and breastfed infants [13-18]. Maternal vitamin D stores tend to be low [6, 13-15, 17, 25, 43, 123] and can continue to decline throughout pregnancy and up to 6 months postpartum [123]. The IOM increased the recommended vitamin D intake to a RDA of 600 IU/d from an AI of 200 IU/d in lactating women with an EAR of 400 IU/d [192]. However, 400 IU/d does not maintain or significantly improve maternal vitamin D status [31-35]. Low maternal vitamin D stores limit transfer of vitamin D to infants pre- and post-natally. This reinforces the importance of directly supplementing the infant to ensure adequate vitamin D status for calcium homeostasis and bone mineralization [67, 136, 236].

Health Canada recommends that breastfed infants receive 400 IU/d of vitamin D at birth [77]. The Canadian Community Health Survey reported that only 53% of breastfed infants received a supplement containing vitamin D [19]. Serum 25(OH)D concentration, the best clinical indicator of vitamin D status [38, 39, 41], will decline in infants born with limited vitamin D stores and who do not receive supplementation [20, 189]; correspondingly post-natal rickets (25(OH)D concentration as <25 nmol/L) has not been eradicated in Canada [13]. The Canadian Paediatric Surveillance Program confirmed 104 cases of rickets between 2002 to 2004 [13]. Infantile rickets is diagnosed as early as two weeks of age [15] with maximum frequency occurring between 8-32 weeks of age [82].

Vitamin D status reflects both exogenous sources, obtained from food and supplements, and endogenous vitamin D synthesis, stimulated by skin exposure to UVB radiation. Total vitamin D intake (food and supplement), in adults, pregnant, and postpartum women, is positively associated with 25(OH)D concentration [8, 12, 18, 43]. Latitude >37 °N is associated with distinct seasons (winter, spring, summer, and fall) and 25(OH)D concentration peaks in the middle of summer with a nadir at the end of winter in infants and adults [1, 7, 13, 42, 68, 82, 134,

137]. Dark skinned individuals often have lower 25(OH)D concentration than light skinned individuals [1, 7, 8, 12, 51, 53]. Sunscreen with a sun protection factor >8 [49, 154], increased indoor hours, and veiled or long clothing are inversely related to 25(OH)D concentration [12, 155-157]. Adiposity is inversely related to 25(OH)D concentration [7, 61, 63, 173, 174, 176-178] by decreasing vitamin D bioavailability as a result of sequestration in fat tissue [1, 60, 61]. Thus, weight loss in obese individuals is associated with increased 25(OH)D concentration [63, 64, 180, 181] by releasing vitamin D from fat tissues [65].

When examining the predictors of vitamin D in breastfed infants, it is important to examine the predictors mentioned as well as maternal characteristics and vitamin D status. It has been well-documented the relationship between cord and maternal 25(OH)D concentration [5, 10, 18, 43, 45, 102, 125]. Breastfeeding without receiving vitamin D supplementation [20, 21] and for longer periods are associated with vitamin D deficiency [191]. However, the predictors of vitamin D status beyond delivery have not been comprehensively examined.

By 1 month postpartum, breast milk composition is stabilized [167] and maternal loss in adiposity is mild or plateaus [158-165]. From 1 to 4 months postpartum, infant and maternal supplementation and sun exposure behaviours may change. Based on the 21 d half-life of 25(OH)D, a standardized 3 month period would readily capture changes in vitamin D status. There is evidence of vitamin D deficiency in lactating women and their infants and the early postnatal period to 4 months of age presents a highly vulnerable period for developing vitamin D deficiency [24]. With physiological growth during this period, breastfed infants not receiving a vitamin D supplement have the potential to be unable to maintain calcium homeostasis for bone mineralization [67, 136, 236]. With limited information on vitamin D status and extent to which potential factors predict vitamin D status, the primary objective of this study was to determine vitamin D status of lactating women and their infants at 1 and 4 months postpartum and change since 1 month postpartum. The secondary objectives were to identify key predictors of vitamin D status at 4 months and change since 1 month postpartum in lactating mothers and their infants

11.3. Subject and Methods

11.3.1. Study Design and Subjects

Lactating women and their infants were recruited as part of a RCT comparing vitamin D_2 and vitamin D_3 supplements in breastfed infants. Mother-infant pairs were recruited from a primary care and birthing centre from the West Island of Montreal. Mothers and infants enrolled at 1 month of age because vitamin D transferred prenatally begin to decrease at 3 weeks due to the half-life of 25(OH)D and infants rely on their own vitamin D stores [38, 41]. The final study visit was scheduled at 4 months postpartum as the majority of Canadian infants are breastfed until 3 to 6 months of a ge [183] and this allowed ample time to observe Δ25(OH)D concentration [24]. Inclusion criteria were predominately breastfed (>80% of milk feeds) infants and mothers who delivered a healthy, singleton, term (between 37 to 42 weeks) infant with birth weight between 3rd and 97th percentile (±2 SD) for age and sex according to the WHO Growth Charts [170]). Breast milk samples were not collected as typical vitamin D content is low, 4-40 IU/L, and was considered negligible [77, 186]. Infant exclusion criteria included non-singleton births, non-term births, infants <3rd and >97th percentile for weight, or receiving >20% of feeds by formula. Premature infants tend to have lower vitamin D status and higher needs due to reduced transfer of stores, decreased fat absorption and bile acids for intestinal vitamin D absorption [237], have immature liver and kidney for hydroxylation of vitamin D metabolites [238], or increased utilization for bone mineralization [237]. Maternal vitamin D supplementation ≥2000 IU/d influences infant vitamin D status and were excluded [239]. Mothers diagnosed with diabetes, kidney, liver disease, Crohn's, celiac, or other diseases and medications that may affect vitamin D bioavailability were excluded [240]. Maternal diagnosis of gestational diabetes [70, 241, 242], hypertension, or pre-eclampsia [243] were excluded as these mothers and infants have lower vitamin D status during pregnancy and at delivery.

Participants were recruited year round from May 2010 to April 2011 to account for seasonal differences in endogenous vitamin D synthesis. In Montreal, latitude 45.5 ^{o}N, vitamin D synthesizing period is between April 1 to October 31

and non-vitamin D synthesizing period is between November 1 to March 31 [26]. To capture endogenous vitamin D synthesis, a maximum of 3-6 mother-infant pairs were recruited each month. At the baseline visit, mothers were provided with an oil-based vitamin D supplement that was prepared in either the vitamin D_2 or D_3 isoform (Baby Ddrop^{TM}, Ddrops® Company, CA). Instructions were to administer a single drop dose per day that delivered 400 IU of vitamin D/drop, the current recommendation for exclusively breastfed infants [19, 77]. Both vitamin D_2 and vitamin D_3 isoforms reflect products available in North America and vitamin D_2 has been found to be equally as effective as vitamin D_3 in maintaining 25(OH)D concentration in adults [81]. Compliance was monitored based on change in bottle weight from before and after the trial and self-reported total number of missed doses during the study period.

11.3.2. Anthropometry and Body Composition Assessment

Self-reported gestational age and infant birth weight, length, and head circumference was obtained from the vaccination booklet as part of the screening process. At both visits, infant weight was measured to the nearest 0.1 gram using an electronic pediatric scale with a movement program (model SB 16001, Mettler-Toledo Inc., Greifensee, Switzerland). Standardized diapers and gowns were worn by each infant and this weight was subtracted from the total weight. Recumbent and crown-rump length was measured to the nearest 0.1 cm using an infant length board (O'Learly Length Boards, Ellard Instrumentation Ltd., Seattle, USA). Head circumference was measured to the nearest 0.1 cm using a flexible, non-stretched tape (model 212, Seca, Hanover, USA). Z-scores for all anthropometric data, except crown rump, were generated using WHO software (Anthro 3.2.2, Geneva, Switzerland).

Maternal body weight was measured to the nearest 0.1 kilogram (kg) in light clothing without shoes and %TBF was measured using a BIA (TBF-310, Tanita, Illinois, USA). Standing height was measured to the nearest 0.1 cm using a stadiometer (Seca, Hanover, USA). Total gestational weight gain was collected and pre-pregnancy BMI (kg/m^2) was calculated based on self-disclosed pre-pregnancy weight. Weight and TBF were also categorized based on mean value.

11.3.3. Dietary Assessment

Three 24 hour dietary recalls were completed at the baseline, midpoint (2.5 months postpartum), and final visit using the online National Cancer Institute Automated Self-administered 24 hour Dietary Recall (ASA24^{TM}) [244]. Each participant has a unique ASA24^{TM} username and password. The baseline and follow-up recalls were interview-administered on-site (primarily scheduled on Mondays or Fridays) and midpoint recall was completed off-site by mothers (on a convenient day). This allowed us to capture at least one weekend and one weekday of intake. Mothers received a midpoint study reminder telephone call and email to complete the midpoint 24 hour recall online. The choice of the 24 hour recall was based on the assumption that the response rate would be higher than food records [38]. The ASA24^{TM} utilizes the multiple pass method [244]. To assist with portion size estimations, ASA24^{TM} included photos of various foods and beverage portion sizes. Participants were also, able to input food items that were missing from the database. After completion of the recall, the created food list was retrieved and checked for completeness. Mothers were contacted for any clarifications. There were no significant differences in dietary vitamin D intake between mothers who completed 2 or 3 recalls ($p=0.975$). Thus mean vitamin D intake of 2 or 3 recalls were used in the analyses. Supplementation use during pregnancy and post-delivery including brand, frequency, dose, and date initiated and/or stopped of each supplement was documented. Among the 44 participants, 75% (n=33) completed three 24 hour recalls, 20.5% (n=9) completed two recalls, and 4.5% (n=2) completed one recall.

Nutrient analyses for naturally occurring and fortified vitamin D content of foods were estimated using the Nutritionist Pro^{TM} Software (Axxya systems, Stafford, TX) utilizing the 2010b CNF. After extraction of nutrient analysis, data were audited by a Registered Dietitian and vitamin D sources (dairy, fish, and fortified foods) were checked for accuracy of vitamin D content to reflect current Canadian fortification trends. Food items with missing vitamin D content were inputted from Canadian manufacturer websites. Vitamin D content from mixed food recipes were entered based on estimated serving size. Manufacturer

specifications were used to estimate nutrient content of supplements. Dietary vitamin D was adjusted for energy intake using the nutrient density approach [208, 245]. Supplement intake was not energy adjusted. Total vitamin D intake was sum of dietary and supplemental vitamin D and was categorized as the proportions below and equal or greater than the EAR and RDA.

At the 4-month follow-up visit, all infants were included in the final analysis regardless of feeding method to examine the natural behavior and progression of the population. At each visit, infant feeding status (exclusively breastfed, mixed, or exclusively formula feed [246]) was collected. Exclusively breastfeeding was defined as being fed breast milk and not receiving anything else except drops of vitamins, minerals, or medicines [247]. If infants were receiving formula, date formula was introduced, the estimated daily amount consumed, and the formula brand was documented.

11.3.4. Sun Exposure and Skin Pigmentation Assessment

Maternal sun exposure during pregnancy, from delivery to baseline visit, and between baseline and follow-up visit was self-reported. Mothers reported infant sun exposure during the first 4 months of life. Detailed sun exposure questions included time of day, duration of sun exposure, BSA exposed to direct sunlight, the number of weeks spent in the synthesizing period, and the above UV exposure questions in addition to location and number of days travelled to a warm and sunny climate (latitude <37 ^{o}N). Fraction of body surface area exposed was calculated by adapted burn exposure charts described by Hall et al. [53], O'Sullivan and Schmitz [219], and the rule of nines [53, 219]. For both mothers and infants <12 months of age, the fractions of BSA explored were neck (0.02), chest (0.02), back (0.13), shoulders (0.02), upper arms (0.06), lower arms (0.06), hands (0.05), and feet (0.07). In infants, the fractions of BSA for face was 0.08, upper legs were 0.11, and lower legs were 0.10 [219]. Maternal BSA fraction for face was 0.04, upper legs were 0.19, and lower legs were 0.14. Sun index was then calculated (product of hours/week of sun exposure and BSA exposed) [143].

For mothers and infants, skin pigmentation and sun exposure were also, measured using a portable, computerized narrow band reflectometer (Konica

Minolta Spectrophotometer CM-600D, Osaka, Japan). Our lab has conducted UV spectrophotometer quality control assessments with no significant differences between intra- and inter-measurers. The equipment was calibrated prior to use with a reference tile and each measurement takes <1 second without any discomfort to the person being measured. Three measurements were performed on the upper inner arm representing constitutive skin pigmentation, genetically determined skin colour and on the flat area of mid-forehead, outer upper forearm, and exterior mid-lower leg representing facultative skin pigmentation sites, which develops due to sun exposure, [58]. An average of the three values was calculated for each measurement site and a combined value of sun-exposed sites was calculated to represent composite facultative skin. An alcohol swab was provided to mothers to remove any make-up, lotion, or face cream on the measurement sites. All measurements were taken in the same room between 0900 to 1300 h and followed the European Society of Contact Dermatitis guidelines [223]. Measurements were expressed as ITA (ArcTangent (L-50/b)*180/π). Constitutive and facultative skin colour were classified as very fair or fair skintype (>41^{o}) or medium, olive, dark, or very dark skintype (<41^{o}) [227, 228]. Difference in ^{o}ITA within the same sites was an indirect measure of UV exposure or tanning.

11.3.5. Total 25(OH)D Concentration

Fasted (8 hours) maternal venous blood and non-fasted infant capillary blood were collected between 0900 h and 1200 h to standardize protocols. After warming, heel or finger was lanced, depending on the age of the infant, and 1 mL of whole blood was collected in heparinized centrifuge tubes. Maternal whole blood, 5mL, was collected in lithium heparinized tubes. Samples were immediately separated into plasma and erythrocyte fractions by centrifugation at 3000 g, 4 ^{o}C for 20 minutes and subsequently frozen at -80 ^{o}C until analysis.

Plasma 25(OH)D concentration was analyzed in batches using the “25-OH Vitamin D TOTAL” competitive chemiluminescence assay on the automated LIAISON® analyzer with 100% cross-reactivity for 25(OH)D_2 and 25(OH)D_3 (DiaSorin, Stillwater, MN). Controls provided by the manufacturer were measured in duplicate, yielding values within the specified acceptable range and

with an intra-assay coefficient of variation (CV) of 1.1%. Intra-assay variability was also determined from samples measured in either duplicate or triplicate yielding an average CV of 3.0%. This assay has a detection range between 10-375 nmol/L. The change in maternal and infant vitamin D status was assessed. Vitamin D status at 1 and 4 months postpartum was examined in view of the various suggested targets proposed by the IOM and CPS. The IOM defines deficiency as 25(OH)D concentration as <37.5 nmol/L and insufficiency as <50 nmol/L [30]. The CPS defines deficiency as 25(OH)D concentration as <25 nmol/L ad insufficiency as 25-75 nmol/L [13].

11.3.6. Demographic Variables

Interviewer-administered questionnaires collected education, ethnicity, ancestry/tribe, family status, household income based on national median, and breastfeeding status information. Maternal age of delivery, changes to medications, caffeinated coffee/tea intake, and smoking status were documented.

11.3.7. Ethical Approval

The study was reviewed and approved by the Research Ethics Board of McGill University, received a no objection letter from Health Canada, and is part of a larger Phase III RCT registered at Clinicaltrials.gov (identifier, NCT01190137). Written informed consent was received from all participants.

11.3.8. Statistical Analysis

Statistical analysis was performed using SAS 9.2 (Cary, North Carolina). Continuous variables were expressed as mean (± standard deviation (SD)) or median (range), based on data distribution. Normality was analyzed using qq-plots and Shapiro-Wilks statistics. Categorical variables were expressed as proportion (%). Statistical significance was set at $p<0.05$. All data were screened for accuracy and outliers (>3SD). Statistical analyses without outliers did not improve or change the results.

Student's *t*-test or ANOVA and Mann-Whitney test explored potential maternal and infant predictors of vitamin D status at 4 months and Δ25(OH)D concentration since 1 month postpartum and change in vitamin D status based on normality of the data. In both mothers and infants, predictors explored were

baseline 25(OH)D concentration, season of assessment, UV measures (minutes of sun exposure, BSA exposed, sun index, travel to latitude <37 ^{o}N, number of weeks in the synthesizing period), sunscreen use, ethnicity, and skin colour (constitutive, facultative). For mothers only, vitamin D intake during pregnancy and during lactation (food, supplemental, total) and adiposity (weight, TBF, BMI, % change in weight and TBF) were examined. Likewise for infants, maternal 25(OH)D concentration (1 and 4 months, Δ25(OH)D), feeding status, z-scores for anthropometric data, age at which routine supplementation began, and dose of vitamin D missed during the study period were examined. Using multiple regression models, predictors of maternal and infant vitamin D status and change from baseline were explored with variables were associated with 25(OH)D concentration. All biologically plausible two-term interactions were explored. Interactions were removed if these did not improve the model (R^2). Potential influential observations were explored with studentized residuals, leverage, and Cook's D. Normality of residuals and heteroskedasticity were assessed (Shapiro-Wilks, qq-plots, visual examination of residual plots, Breusch-Pagan). Variance inflation factor (<5 to be conservative) was used to assess collinearity. In the case that collinearity existed between similar variables, only one of the variables was included in the final model. The final model was verified for multicollinearity. β coefficient reflects for every unit increase in the explanatory variable an estimated 25(OH)D concentration or Δ25(OH)D concentration would result after adjusting for all other variables.

11.3.9. Sample Size Calculation

Being part of a RCT, the sample size was previously determined, n=44 for mother-infant pairs. With α=0.05 and β=0.20, the standardized effect size would be 0.60. Maternal Δ25(OH)D concentration from 1 to 4 months postpartum had an effect size of 8.3 nmol/L [35]. Therefore, detection of the variability of 25(OH)D concentration was 13.8 nmol/L was possible. To achieve a power=0.80 and R^2=0.50 with six predictor variables in regression models, n=49 participants were necessary [248, 249]. Thus, in this study a maximum of 5-6 predictors/regression

equation were used. Mother-infant pairs were recruited in the vitamin D synthesizing period (n=22) and non-synthesizing period (n=22).

11.4. Results

Of the 267 mothers approached to participate in the study, 223 (83.5%) were excluded due to ineligibility, unable to contact, or refused to participate, 42 (15.7%) completed the study, and 2 (0.7%) withdrew before the follow-up visit (**Appendix 17.2**). **Table 1** presents maternal characteristics in addition to age at delivery 32.3 ± 3.8 years, pre-pregnancy BMI 23.3 ± 3.5 kg/m^2, and gestational weight gain of 14.7 ± 4.3 kg. During pregnancy, 7.1% self-reported smoking and 52.4% self-reported intake of <1 cup/d of caffeinated coffee and/or tea. The majority of mothers were living with a partner (97.6%), white (78.6%), with more than high school education (88.1%), and household income was ≥$75,000 (59.5%). **Table 2** presents infant characteristics. At birth, mean weight-for-age z-score was 0.5 ± 0.8, length-for-age z-score was 1.3 ± 1.2, and head circumference-for-age z-score was 0.2 ± 1.0. The majority of infants were exclusively breastfeed at 1 (77.3%) and 4 months (76.2%) of age. At the final visit, 2 (4.8%) were exclusively formula fed. Of the infants who enrolled and completed the study during the non-synthesizing period and without sun exposure (n=11), from 1 to 4 months, the number of infants classified as very fair to fair constitutive skin pigmentation increased from 4 (36.4%) to 6 (54.6%). From 1 to 4 months, the number of infants classified with medium, olive, dark, or very dark constitutive skin colour decreased from 7 (63.6%) to 5 (45.5%).

Daily maternal intake of selected nutrients was estimated by mean 24 hour recalls (**Supplementary Table 1**) including vitamin D intake from foods at 237 [53, 779] IU/d, supplements 326 [0, 2400] IU/d which contributed 59% of intake, and total vitamin D intake was 596 [99, 2476] IU/d. Mothers were taking a prenatal/postnatal supplement during pregnancy (95.5%) and at 1 (79.6%) and 4 months (76.2%) postpartum. The number of mothers taking supplemental vitamin D intake was similar between pregnancy (18.2%) and at 1 (15.9%) and 4 months (21.4%) postpartum. Fourteen (33.3%) mothers had total vitamin D intake <EAR and 22 (52.4%) had intake <RDA. Those with total vitamin D intake ≥400 IU/d had higher 25(OH)D concentration than those with intake <400 IU/d (66.5 ± 16.7 vs. 54.4 ± 19.4 nmol/L, p=0.042).

At both visits, no mothers had 25(OH)D concentration <25 nmol/L. At the baseline visit, 2 (4.6%) mothers had 25(OH)D concentration <37.5 nmol/L, 7 (15.9%) had 25(OH)D concentration <50 nmol/L and 26 (59.1%) had 25(OH)D concentration <75 nmol/L. At the follow-up visit, 3 (7.1%) mothers had 25(OH)D concentration <37.5 nmol/L, 9 (21.4%) had 25(OH)D concentration <50 nmol/L, and 30 (71.4%) had 25(OH)D concentration <75 nmol/L. **Table 3** shows variables associated with maternal vitamin D status at 4 months postpartum and Δ25(OH)D concentration since 1 month. At 1 month postpartum, mothers who took a prenatal/multi-vitamin before pregnancy had higher vitamin D status compared to mothers who did not (74.7 ± 19.5 nmol/L vs. 54.2 ± 8.9 nmol/L, p=0.010). At 4 months postpartum, mothers who took both a prenatal/postnatal and vitamin D supplement had significantly higher 25(OH)D concentration than those who did not (76.1 ± 16.1 vs. 59.7 ± 17.7 nmol/L, p=0.029). At 4 months, there were no significant differences in maternal 25(OH)D concentration between very fair or fair and medium, olive, dark or very dark constitutive skin pigmentation (63.6 ± 22.0 vs. 55.4 ± 21.1 nmol/L, p=0.317); no differences were observed for the same groupings for facultative pigmentation colour (63.3 ± 22.0 vs. 61.6 ± 14.7 nmol/L, p=0.770). There were no differences in Δ25(OH)D concentration based on the same groups for constitutive (-10.8 ± -12.6 nmol/L, p=0.834) and facultative skin pigmentation (-15.0 ± 14.1 vs. -7.4 ± 22.2 nmol/L, p=0.195).

Table 4 presents the models predicting maternal vitamin D status at 4 months and Δ25(OH)D concentration since 1 month postpartum. The final model for predicting maternal vitamin D status at 4 months included % change in TBF, the number of weeks spent in synthesizing period, and total vitamin D intake as statistically significant parameters after adjusting for all other variables in the final model. From 1 to 4 months, mothers who gained or did not lose more than 3.1% in TBF predicted a 14.6 nmol/L decrease in maternal 25(OH)D concentration. Every unit increase in weeks in the synthesizing period predicted a 1.2 nmol/L increase in maternal 25(OH)D concentration. Total vitamin D intake ≥EAR predicted a 17.4 nmol/L increase in maternal 25(OH)D concentration. These variables explained ~45.6% of the variance of maternal vitamin D status at

4 months. The final model for predicting maternal Δ25(OH)D concentration included baseline 25(OH)D, % change in weight, and the number of weeks spent in synthesizing period as statistically significant parameters. Every unit increase in maternal baseline 25(OH)D concentration predicted a 1.2 nmol/L decrease in maternal Δ25(OH)D concentration. From 1 to 4 months, mothers who gained or did not lose more than 1.9% in weight predicted a 9.4 nmol/L decrease in maternal Δ25(OH)D concentration. Every unit increase in the weeks spent in the synthesizing period predicted a 1.5 nmol/L increase in maternal Δ25(OH)D concentration. These variables explained ~62.2% of the variance in maternal Δ25(OH)D concentration from 1 month postpartum. The standard regression diagnostics indicated that these models were a good fit.

There was a significant correlation between maternal and infant vitamin D status at 1 (r=0.39, p=0.009) and 4 months (r=0.42, p=0.005) (**Figure 1**). From 1 to 4 months, there was a significant decrease in maternal 25(OH)D concentration (73.0 ± 21.6 to 62.4 ± 18.3 nmol/L, p<0.001) and a significant increase in infant vitamin D status (60.2 ± 31.0 to 71.6 ± 25.4 nmol/L, p=0.032) (**Figure 1–insert**).

At 1 month of age, 5 (11.4%) infants had 25(OH)D concentration <25 nmol/L, 13 (29.6%) had 25(OH)D concentration <37.5 nmol/L, 18 (40.9%) had 25(OH)D concentration <50 nmol/L, and 32 (72.7%) had 25(OH)D concentration <75 nmol/L. At 4 months of age, 1 (2.4%) infant had 25(OH)D concentration <25 nmol/L, 3 (7.1%) had 25(OH)D concentration <37.5 nmol/L, 4 (11.9%) had 25(OH)D concentration <50 nmol/L and 25 (59.5%) had 25(OH)D concentration <75 nmol/L. At 1 and 4 months, infants born in the synthesizing period did not have higher vitamin D status than those born during the non-synthesizing period (1 month: 54.8 ± 22.5 vs. 65.6 ± 37.5 nmol/L, respectively, p=0.254; 4 months: 66.8 ± 22.5 vs. 76.4 ± 27.8 nmol/L, respectively; p=0.226). BAZ was not associated with infant vitamin D status at 1 and 4 months of age (1 month: 54.5 ± 28.5 vs. 66.7 ± 32.4 nmol/L, respectively, p=0.201; 4 months: 70.4 ± 23.7 vs. 72.7 ± 27.5 nmol/L, respectively, p=0.767). **Table 5** presents variables associated with infant vitamin D status at 4 months and Δ25(OH)D concentration since 1 month of age. At 1 and 4 months, infant vitamin D status was not associated with season

of assessment, minutes of sun exposure, BSA exposed, and sun index (data not shown). Of the infants that traveled to a latitude <37 ^{o}N, 5 (83.3%) were white and 1 (16.7%) was non- white. The time frame from the last day of travel to the final visit was 24 [4, 53] days. Maternal sun exposure at 4 months was associated with change in infant vitamin D status. At 4 months, 12 (46.2%) of these mothers used sunscreen sometimes to often when exposed to sunlight compared to mothers with no sun exposure (none used sunscreen).

The final model predicting infant 25(OH)D concentration at 4 months included maternal vitamin D status at 4 months, travel to latitude <37 ^{o}N, and facultative skin pigmentation as statistically significant parameters after adjusting for all other variables in the final model (**Table 6**). Every unit increase in maternal 25(OH)D at 4 months predicted a 15.0 nmol/L increase in infant 25(OH)D concentration. Not having traveled to a latitude <37 ^{o}N predicted a 27.1 nmol/L decrease in infant vitamin D status. Every unit increase in facultative skin pigmentation (lighter skin pigmentation) predicted a 0.7 nmol/L increase in infant 25(OH)D concentration. These variables explained ~40.4% of the variance of infant vitamin D status at 4 months. The final model predicting infant Δ25(OH)D concentration included infant baseline vitamin D status, travel to a latitude <37 ^{o}N, and ethnicity as statistically significant parameters. Every unit increase in infant baseline vitamin D status predicted a 0.5 nmol/L decrease in infant Δ25(OH)D concentration. Not having traveled to a latitude <37 ^{o}N predicted a 23.5 nmol/L decrease in infant Δ25(OH)D concentration. Being a non-white infant predicted a 14.0 nmol/L decrease in infant Δ25(OH)D concentration. These variables explained ~58.5% of the variance in Δ25(OH)D concentration from 1 month of age. The standard regression diagnostics found that these models were a good fit.

11.5. Discussion

The majority of lactating women and their infants in this study achieved the 25(OH)D cut-off concentration of 50 nmol/L at 1 and 4 months postpartum. Vitamin D status in this study population was higher than reports of vitamin D deficiency, defined as 25(OH)D concentration <25 nmol/L [5, 9, 23], <27.5 nmol/L [21], <32.5 nmol/L [197], <37.5 nmol/L [43, 121], and <50 nmol/L [129]. The decline in maternal vitamin D status in this population has been observed by others of up to 30% decrease post-delivery until 24 weeks postpartum [123] or sustained at a lower level up to 21 weeks postpartum [250]. The postpartum decline in DBP remains low up to 18 weeks post-delivery [251] which may explain declines in maternal vitamin D status despite increased UVB exposure.

Prenatal supplements are an important vitamin D source during lactation [37]. In this study, supplemental vitamin D contributed ~59% to maternal total vitamin D intake at 4 months postpartum and total vitamin D intake was higher than those published in lactating women [18, 252] but was similar to pregnant women taking prenatal supplements [45]. High supplementation use could be attributed to a population of educated, white mothers [253] participating in a supplement study for their breastfed infants. It is likely that mothers are also, taking their post-natal or vitamin D supplements. There is a lack of consistency on the relationship between maternal vitamin D intake and status [11, 18, 45]. In the present study, although there was no correlation between maternal vitamin D intake and status, total vitamin D intake ≥EAR was a predictor of maternal vitamin D status at 4 months. This emphasizes the importance of capturing both dietary and supplemental vitamin D intake.

The effect of season was associated with maternal 25(OH)D concentration at 4 months and Δ25(OH)D concentration. Several studies have observed higher 25(OH)D concentration in the summer months compared to the winter months in pregnant and lactating women [18, 43, 45, 254]. These results are in accordance with evidence that at a latitude of >53 ^{o}N, between November 1 to March 31, endogenous vitamin D synthesis is inhibited [26]. UV exposure measured by BSA exposed, sun index, and the number of weeks spent in the synthesizing period

were associated with maternal 25(OH)D concentration at 4 months and Δ25(OH)D concentration. The number of weeks spent in the synthesizing period is a proxy for potential sun exposure. The association between UV exposure and 25(OH)D concentrations has been observed in adults [12, 45, 143]. Statistics Canada reported that sunlight exposure >60 minutes/d was positively associated with higher 25(OH)D concentration [127]. In the present study, minutes of sun exposure and travel to a latitude <37 ^{o}N was not associated with maternal vitamin D status or changes in status. However, the majority of mothers was in direct sunlight for <60 minutes/d and did not travel to a latitude <37 ^{o}N.

Melanin production, due to UVB exposure, interferes with vitamin D synthesis [8, 50-52]. Thus, dark-skinned mothers tend to have lower vitamin D status than those with light skin colour [12, 18, 21, 43-45, 197, 255]. In this study, there were no associations between constitutive and facultative skin colour and maternal vitamin D status. The spectrophotometer cannot capture sun exposure in fair skinned individuals who burn but do not tan and in dark skinned individuals who never tan. Thus, sun exposure can be underestimated. Due to the lack of variation in skin pigmentation in the present study, skin colour was categorized into 2 rather than 6 groups [227, 228] and may be insufficient to detect a relationship between skin colour and vitamin D status. In a study conducted by Nicolaidou et al. [254], there was a significant difference in vitamin D status between mothers with dark phototypes (III) compared to fair phototypes (I) but not with intermediate phototypes (II). The present study observed an increase in vitamin D status with a decrease in facultative skin colour, indicating UVB exposure. The increase in maternal constitutive skin colour classified as very fair or fair from 1 to 4 months postpartum suggest that the inner upper arm was exposed to direct sunlight. The tanning effects on maternal upper inner arm has been observed [45]. Epidermal hyperpigmentation due to high estrogen levels during pregnancy [256, 257] may regress post-delivery with declines in estrogen[258], whether these changes are immediate or gradual and in the upper inner arm remain to be explored [259].

There are limited longitudinal studies on the changes of body composition during lactation. However, several studies observed an inverse relationship between vitamin D status and adiposity [1, 7, 60, 63, 173, 174, 176, 260, 261] including among mothers [12, 45]. Fat tissue may increase metabolic clearance through enhanced uptake and/or decreases bioavailability by sequestration [1, 60, 61, 171, 262]. In the present study, this relationship was not observed due to the lack of variance in maternal weight, BMI, and TBF and inadequate statistical power to detect such an interaction. However, % change in weight and TBF were predictors of maternal vitamin D status and change in status, respectively.

There is a significant relationship between infant and maternal vitamin D status (r=0.32 to 0.83) [5, 9, 11, 12, 43-45, 131, 197, 254] regardless of whether cord blood was measured or if infants were tested post-natally. This relationship was observed in the present study at 1 and 4 months and maternal vitamin D status was a predictor of infant vitamin D status. This was also observed by Merewood et al. [12]. Therefore, up to 4 months of age, infants may be partially relying on maternal vitamin D stores transferred pre- and post-natally [13] and participating in an infant supplement study may positively impact maternal and infant supplementation behavior. In the present study, there was an association between prenatal supplement use and infant 25(OH)D concentration. This relationship has been observed by others [12, 45]. Thus, improved maternal vitamin D status will likely have beneficial effects on both mothers and infants beyond delivery.

Baseline vitamin D status was a negative predictor of both maternal and infant change in vitamin D status. The attenuated response in change in 25(OH)D concentration in those with higher baseline 25(OH)D concentration suggests that supplements were unable to maintain vitamin D status. Although, the mechanisms remain to be explored, there is an inverse relationship between Δ25(OH)D concentration and baseline vitamin D status [263, 264]. In the present study, mothers and infants with lower baseline vitamin D status who were taking supplements containing vitamin D [175] or following UVB exposure [263, 264]

had a greater increase in 25(OH)D concentration from 1 to 4 months than those with higher baseline vitamin D status.

In the present study, there was a significant increase in infant vitamin D status from 1 to 4 months of age. This increase was observed in infants (n=19) receiving 400 IU/d of vitamin D from 1.5 to 3 months of age (75.6 ± 23.9 to 97.2 ± 28.9 nmol/L) and remained unchanged at 6 months (92.4 ± 29.7 nmol/L) [134]. This increase was also observed in infants (n=33) receiving 400 IU/oil-based vitamin D drop/day from 1 to 4 months (39.9 ± 23.2 to 108.8 ± 35.2 nmol/L) and remained unchanged at 7 months (106.1 ± 30.2 nmol/L) [129]. Steady state was achieved ~90 days after routine supplementation [24, 34, 35, 134].

The lack of association between infant 25(OH)D concentration and season of delivery and season of assessment at 4 months and Δ25(OH)D concentration could be due to the limited sample size. There was no significant differences in 25(OH)D concentration between the vitamin D synthesizing and non-synthesizing period. This was also observed by Nicolaidou et al. [254]. However, the majority of studies observed significantly higher 25(OH)D concentration in the summer months compared to winter months in cord blood [43, 45] and infants in the postnatal period [12, 16, 21, 23, 44, 265].

In the present study, minutes of sun exposure, BSA exposed, sun index, and the number of weeks spent in the synthesizing period were not associated with infant vitamin D status or Δ25(OH)D concentration. These infants had insufficient sun exposure to significantly increase 25(OH)D concentration. However, these measures of UV exposure have been observed in infants [12, 20, 45]. It is also possible that the present study lacked statistical power to detect such an association. Infant and/or maternal sun exposure can increase infant 25(OH)D concentration [21]. In contrast, in the present study, infants of mothers without sun exposure at 4 months had a greater increase in vitamin D status from 1 to 4 months than their counterparts. The use of sunscreen in mothers exposed to UVB radiation may inhibits endogenous vitamin D synthesis. At 4 months, travel to latitude <37 ^{o}N was a significant predictor of infant vitamin D status. Since, the time frame between the last travel date and the follow-up visit was within the

half-life of 25(OH)D [38-41], endogenous vitamin D synthesis from their travel was captured. This relationship has been observed in adults who traveled to a latitude <42 or <35 ^{o}N [42, 141]. However, the relationship between traveling to latitude <37 ^{o}N should be interpreted with caution due to the limited sample size.

In the present study, infants with very fair or fair constitutive and facultative skin colour had significantly higher 25(OH)D concentration than those with darker skin pigmentation. This is in accordance with others [12, 18, 21, 43-45, 197, 255]. Only facultative skin was a predictor of infant vitamin D status which could reflect skin synthesizing capacity. This has been observed by others [45, 54]. In a study where individuals were matched by 25(OH)D concentration, there were no significant differences in Δ25(OH)D concentration between dark skinned (skintype V-VI) and fair skinned adults (skin type I-IV) in constitutive and facultative skin groupings. This suggests that change in vitamin D status is unrelated to skin colour [264]. The present study also observed that skin pigmentation was not associated with change in infant vitamin D status. In infants without sun exposure, there were more infants classified as very fair or fair skin colour and fewer classified as medium, olive, dark, or very dark skin colour from 1 to 4 months of age. This could potentially support that changes in infant constitutive and facultative skin is a result of skin development during the first 3 months of life through increased epidermal desquamation [229] and skin thickness [230]. In addition, the spectrophotometer may not be a reliable tool to measure skin colour in infants <3 months of age.

We can hypothesize that lower 25(OH)D concentration would be observed in infants with higher adiposity due to fat mass deposition similar to adults [1, 7, 60, 63, 173, 174, 176, 260, 261]. However, the present study did not observe an association between BAZ and infant vitamin D status possibility due to the lack of variation in BAZ. However, maternal loss in adiposity was associated with higher infant 25(OH)D concentration at 4 months. This inverse relationship has been documented between pre-pregnancy BMI and cord 25(OH)D concentration [2, 45] and infant vitamin D status [12]. These results suggest that maternal body

composition may impede transfer of vitamin D during pregnancy and lactation and decreased adiposity can allow sufficient vitamin D transfer to infants.

The major strength of this study is that, to our knowledge, it is the first study to comprehensively assess the predictors of vitamin D status of lactating women and their infants at 4 months and Δ25(OH)D concentration since 1 month postpartum. The spectrophotometer was used to assess UV exposure and classify skin colour rather than ethnicity. Future studies should be including photosensitive skin classification as a measurement to capture skin pigmentation, tanning ability, and sun behavior in individuals who burn but do not tan and individuals who never tan. The R^2 was relatively low which may be due to inadequate statistical power and other factors such as genetic variants of DBP or metabolism [138, 150, 152, 266]. These are important considerations when examining vitamin D insufficiency among certain ethnic groups. The use of DXA or MRI should be considered to measure adiposity, mobilization of fat stores, and lean mass. Lean mass correlates positively with vitamin D status [267] but has not been explored in lactating women. This study mainly consisted of white, higher income, and educated women. Therefore, the results cannot be generalized to reflect the diverse ethnic and socio-demographic characteristics of Canadian lactating women and their infants. Some parents reported difficulties in administering the oil-based vitamin D supplement provided thus, some infants may not have received 400 IU/d. Significant 3-epi-25(OH)D has been found in infants but the LIAISON® does not detect 3-epi-25(OH)D [98].

In conclusion, the majority of mothers and infants achieved the 50 nmol/L threshold at 1 and 4 months postpartum. Common predictors of both maternal and infant Δ25(OH)D was baseline 25(OH)D concentration and various measures of UVB exposure (the number of weeks spent in the synthesizing period and travel to latitude $<37\ ^{o}N$). Although, there were subtle differences in how these two variables behaved in regression analyses, both were related to vitamin D status. Those with high vitamin D status may not maintain 25(OH)D concentrations with current vitamin D supplementation practices. The relationship between maternal

and infant vitamin D status highlights the importance of maternal vitamin D status and behavior influencing infant vitamin D status.

Table 1. Maternal Characteristics of Relevance to Vitamin D Metabolism

Characteristics	**N**	
Baseline visit (1 month)		
Weight (kg)	44	68.3 ± 12.1
Body fat (%)	44	33.3 ± 6.9
Season of assessment, synthesizing[1]	44	17 (38.6)
Weeks spent in synthesizing[1] period	44	2.1 [0, 5.9]
Sun exposure (min/d)[2]	44	0 [0, 120]
Sun index[3]	44	0 [0, 3.5]
Facultative[4] skin (ITA°)	44	38.6 [-1.4, 58.3]
Constitutive[5] skin phototype, very fair or fair	44	28 (63.6)
Facultative[4] skin phototype, very fair or fair	44	19 (43.2)
Sunscreen use, sometimes to often	44	0 (0)
Follow-up visit (4 month)		
Weight (kg)	42	67.0 ± 11.7
Height (cm)	42	163.6 ± 6.9
% change body weight[6] (%)	42	-1.9 ± 4.3
Body fat (%)	42	32.4 ± 7.1
% change body fat[7] (%)	42	-3.1 ± 7.5
Season of assessment, synthesizing[1]	42	18 (42.9)
Weeks spent in synthesizing[1] period	42	4.4 [0, 14]
Sun exposure (min/d)[2]	42	17.5 [0, 270]
Sun index[3]	42	0.33 [0, 24.6]
Travel to latitude <37 °N	42	6 (14.3)
Sun exposure (min/d)	6	178 [60, 300]
Facultative[4] skin (ITA°)	42	39.6 [-2.7, 56.1]
Constitutive[5] skin phototype, very fair or fair	42	36 (85.7)
Facultative[4] skin phototype, very fair or fair	42	19 (43.2)
Sunscreen use, sometimes to often	42	12 (28.6)

25(OH)D: 25-hydroxyvitamin D; BMI: body mass index; SD: standard deviation
Values are presented as n (%), mean ± SD, or median [range]
[1]Synthesizing period (April 1 to October 31); [2]based on those exposed between 1000 – 1500h during synthesizing period; [3]Sun index=hours/week of sun exposure * fraction of body surface area exposed; [4]Faculatitive skin=mean of mid forehead, outer forearm, and outer lower leg (sun-exposed area); [5]Constitutive skin=upper inner arm (non-exposed area); [6]%Δ body weight=(measured weight at 4 months minus measured weight at 1 month)/weight at 1 month); [7]%Δ body fat=(measured body fat at 4 month minus measured body fat at 1 month)/body fat at 1 month)

Table 2. Infant Characteristics of Relevance to Vitamin D Metabolism

Characteristics	**N**	
Gestational age (weeks)	43	39.5 ± 1.0
Gender, female	44	23 (52.3)
Age at which routine vitamin D supplementation began (day)	44	7 [1, 43]
Season of delivery, synthesizing[2]	44	22 (50)
Baseline visit (1 month)		
Exclusively breastfeeding	44	34 (77.3)
Mixed feeding	44	10 (22.7)
Amount of formula (mL/d)	10	0 (0, 500)
Crown rump length (cm)	44	36.8 ± 1.4
WAZ	43	0.28 ± 0.80
LAZ	43	0.39 ± 1.04
HCZ	44	0.50 ± 0.81
Season of assessment, synthesizing[2]	44	17 (38.6)
Weeks spent in synthesizing[2] period	44	2.1 [0, 5.9]
Sun exposure (min/d)[3]	44	0 [0, 30]
Sun index[4]	44	0 [0, 0.5]
Constitutive[5] skin phototype, very fair or fair	44	25 (56.8)
Facultative[6] skin phototype, very fair or fair	44	20 (45.5)
Follow-up visit (4 month)		
Exclusively breastfeeding	42	32 (76.2)
Mixed feeding	42	8 (19.0)
Amount of formula (mL/d)	42	0 [0, 1419]
Vitamin D supplement missed (dose)[1]	42	4.5 [0, 66]
Crown rump length (cm)	42	42.2 ± 2
WAZ	42	0.23 ± 0.92
LAZ	42	0.50 ± 1.11
HCZ	42	0.35 ± 1.02
Season of assessment, synthesizing[2]	42	18 (42.9)
Weeks spent in synthesizing[2] period	42	4.4 [0, 14]
Sun exposure (min/d)[3]	42	0 [0, 60]
Sun index[4]	42	0 [0, 5]
Travel to latitude <37 ^{o}N	42	6 (14.3)
Sun exposure (min/d)	6	30 [0, 120]
Constitutive[5] skin phototype, very fair or fair	42	36 (85.7)
Facultative[6] skin phototype, very fair or fair	42	36 (85.7)

BAZ: BMI-for-age z-score; LAZ: length-for-age z-score; HCZ: head circumference-for-age z-score; SD: standard deviation; WAZ: weight-for-age z-score; WHZ: weight-for-length z-score

Values are presented as n (%), mean ± SD, or median [range]

[1]Vitamin D missed during standardized 3 month period; [2]Synthesizing period (April 1 to October 31); [3]Based on those exposed between 1000 – 1500h during synthesizing period; [4]Sun index=hours/week of sun exposure * fraction of body surface area exposed; [5]Constitutive skin=upper inner arm (non-exposed area); [6]Faculatitive skin=mean of mid forehead, outer forearm, and outer lower leg (sun-exposed area)

Table 3. Factors Associated with Maternal Vitamin D Status at 4 Months and Change from 1 to 4 Months Postpartum

Variables	4 month			From 1 to 4 months[1]		
	N=42	25(OH)D, nmol/L	p-value	N=42	Δ 25(OH)D, nmol/L	p-value
% change body weight[2]			0.058			0.136
Loss more than 1.9%	22	67.5 ± 20.6		22	-6.9 ± 21.1	
Gain or loss <1.9%	20	56.9 ± 13.8		20	-15.6 ± 15.5	
% change body fat[3]			0.004			0.505§
Loss more than 3.1%	25	68.4 ± 19.6		25	-9.0 ± 20.6	
Gain or loss <3.1%	17	53.6 ± 11.9		17	-14.0 ± 16.4	
Prenatal supplement intake			0.675			0.144§
Yes	32	61.8 ± 18.0		32	-13.5 ± 17.8	
No	10	64.6 ± 20.1		10	-3.0 ± 21.2	
Vitamin D supplement intake			0.012			0.014
Yes	9	75.8 ± 14.3		9	2.4 ± 18.3	
No	33	58.8 ± 17.8		33	-14.7 ± 17.6	
Calcium supplement intake			0.021			0.059
Yes	4	82.3 ± 11.4		4	5.9 ± 23.5	
No	38	60.4 ± 17.7		38	-12.8 ± 17.8	
Total[4] vitamin D intake (IU/d) from 24 hour recall			0.042			0.810§
<EAR	14	54.4 ± 19.4		14	-9.7 ± 18.1	
≥EAR	28	66.5 ± 16.6		28	-11.7 ± 19.6	
Sun exposure[5]			0.233			0.037
Yes	26	65.1 ± 18.8		26	-6.3 ± 19.2	
No	16	58.1 ± 17.2		16	-18.7 ± 16.3	
Synthesizing[6] period, weeks spent			0.006			0.002
<5.19	22	55.2 ± 16.4		22	-19.4 ± 15.1	
≥5.19	20	70.4 ± 17.4		20	-1.8 ± 18.7	
Season of visit			0.019			<0.001
Synthesizing[6]	18	69.7 ± 19.6		18	0.6 ± 18.2	
Non-synthesizing[7]	24	56.8 ± 15.3		24	-19.7 ± 14.5	
BSA[8]			0.028			0.015
<0.11	19	55.7 ± 17.3		19	-18.7 ± 14.9	
≥0.11	23	68.0 ± 17.6		23	-4.7 ± 19.8	
Sun index[9]			0.011			0.032§
<0.39	25	56.7 ± 17.2		25	-16.6 ± 14.1	
≥0.39	17	70.9 ± 16.9		17	-2.7 ± 22.3	
% change facultative[10] skin pigmentation			0.001			<0.001
Decrease	16	73.8 ± 16.2		16	2.0 ± 18.9	
Increase	26	55.5 ± 16.1		26	-19.1 ± 14.0	
Sunscreen use			0.478			0.005
Yes	12	65.7 ± 21.4		12	-0.4 ± 15.0	
No	30	61.2 ± 17.2		30	-15.3 ± 18.8	

Data analyzed using Student's *t*-test or §Mann-Whitney test; Values are presented as mean ± SD; 25(OH)D: 25-hydroxyvitamin D; BSA: body surface area; EAR: Estimated Average Requirement

[1]Compared to 4 month variables unless otherwise indicated; [2]%Δ body weight=(measured weight at 4 months minus measured weight at 1 month)/weight at 1 month); [3]% change TBF=(measured body fat at 4 month minus measured body fat at 1 month)/body fat at 1 month), [4]Total vitamin D = dietary and supplemental; [5]Sun exposure during synthesizing period; [6]Synthesizing period (April 1 to October 31); [7]Non-synthesizing period (November 1 to March 31); [8]Based on face, neck, hands exposed; [9]Based on equation = 3.5 hr/wk * 0.11 BSA (face, neck, hands exposed); [10]Faculatitive skin=mean of mid forehead, outer forearm, and outer lower leg (sun-exposed area)

Table 4. Predictors of Maternal 25(OH)D Concentration at 4 Months and Change in Vitamin D Status from 1 to 4 Months

	Independent variables	β coefficient	95% CI
Mother			
4 months	Intercept	59.398**	26.9, 92.0
N=41	Family income	-9.287	-19.3, 0.8
	% change TBF	-14.622**	-25.1, -4.1
	Synthesizing[1] period, weeks spent	1.159*	0.1, 2.2
	Constitutive skin phototype	0.058	-0.4, 0.5
	Diet + supplement vitamin D intake[2]	17.400**	6.7, 28.1
	R^2 = 0.46, **Root MSE =** 14.432, **p-value** <0.001		
Δ 25(OH)D	Intercept	71.787	-0.7, 144.3
N=42	Baseline 25(OH)D, nmol/L	-1.158*	-2.3, -0.1
	% change body weight	-9.406*	-17.9, -1.0
	Synthesizing[1] period, weeks spent	1.496**	0.6, 2.4
	Constitutive skin pigmentation, ^{o}ITA	-1.051	-2.6, 0.5
	Diet + supplement vitamin D intake[2]	4.766	-4.6, 14.1
	Baseline 25(OH)D * underarm skin pigmentation	0.014	0, 0
	R^2 = 0.62, **Root MSE =** 12.567, **p-value** <0.001		

25(OH)D: 25-hydroxyvitamin D; % change weight: 0= loss more than 1.9%, 1=gain or loss less than 1.9%; % change TBF: 0=loss more than 3.1%, 1=gain or loss less than 3.1%; BSA: body surface area; CI: confidence interval; Constitutive skin phototype: 0=very fair to fair, 1=medium, olive, dark, or very dark; Diet + supplement vitamin D intake: 0=(<EAR), 1=(≥EAR); MSE: mean square error; Family income: 0=(<$75,000), 1=(≥$75,000); TBF: total body fat

[1]Synthesizing period (Apr 1-Oct 31); [2]Estimated from 24 hour recall

Table 5. Factors Associated with Infant Vitamin D Status at 4 Months and Change from 1 to 4 Months of Age

Variables	4 month			From 1 to 4 months[1]		
	N=42	25(OH)D, nmol/L	p-value	N=42	Δ 25(OH)D, nmol/L	p-value
Gender			0.862			0.005§
Female	21	70.9 ± 22.2		21	17.9 ± 19.8	
Male	21	72.3 ± 28.8		21	0.7 ± 31.0	
Feeding Method			0.726ǂ			0.860ǂ
Exclusively breastfeeding	32	70.5 ± 25.1		32	8.3 ± 26.2	
Mixed feeding	2	64.2 ± 14.2		2	6.5 ± 3.0	
Exclusively formula feeding	8	77.6 ± 30.1		8	14.1 ± 35.4	
Age routine vitamin D supplementation began (days)			0.084			0.006
<14	30	75.8 ± 21.2		30	2.2 ± 23.0	
≥14	12	60.9 ± 32.4		12	27.2 ± 29.4	
Age routine vitamin D supplementation began (days)			0.023			0.012
<21	32	76.5 ± 21.6		32	3.5 ± 22.9	
≥21	10	55.8 ± 31.2		10	27.7 ± 32.5	
Ethnicity			0.291			0.057§
White	30	74.2 ± 23.6		30	15.2 ± 24.9	
Non-white	12	64.9 ± 29.6		12	-5.4 ± 28.0	
Synthesizing[2] period, weeks spent			0.266			0.046§
<4	19	66.7 ± 29.7		19	19.6 ± 30.0	
≥4	23	75.6 ± 21.1		23	0.8 ± 21.7	
Season of visit			0.109			0.127
Synthesizing[2]	18	78.8 ± 22.2		18	1.9 ± 20.2	
Non-synthesizing[3]	24	66.1 ± 26.7		24	14.9 ± 30.6	
Travel to latitude <37 °N			0.011			0.230
Yes	6	95.5 ± 25.5		6	28.9 ± 40.4	
No	36	67.6 ± 23.4		36	6.0 ± 23.5	
Constitutive[4] skin phototype			0.031			0.850
Very fair or fair	36	75.0 ± 23.7		36	9.0 ± 28.4	
Medium, olive, dark, or very dark	6	51.1 ± 27.8		6	11.3 ± 20.1	
Facultative[5] skin phototype			0.016			0.931
Very fair or fair	36	75.3 ± 23.5		36	9.5 ± 28.3	
Medium, olive, dark, or very dark	6	48.9 ± 26.4		6	8.4 ± 20.7	
Δ Forehead (ITA)			0.185			0.001
Decrease	3	52.7 ± 42.7		3	-6.0 ± 1.3	
Increase	39	70.3 ± 23.9		39	10.5 ± 27.8	
Maternal Characteristics						
Vitamin D Status (25(OH)D)			0.031			0.527
<75 nmol/L	30	66.3 ± 23.4		30	11.6 ± 20.4	
≥75 nmol/L	12	84.8 ± 26.3		12	3.7 ± 40.0	
% change TBF[6]			0.011			0.929
<0	29	78.1 ± 22.1		29	9.0 ± 30.0	
≥0	13	57.0 ± 27.1		13	9.9 ± 20.6	
Sun exposure[7]			0.626			0.018
Yes	26	73.1 ± 21.6		26	1.7 ± 23.4	
No	16	69.1 ± 31.2		16	21.7 ± 29.0	

Data analyzed using Student's t-test, ǂANOVA (between-group effect), or §Mann-Whitney test; 25(OH)D: 25-hydroxyvitamin D; BSA: body surface area; ITA: individual typology angle; SD: standard deviation ; TBF: total body fat
[1]Compared to 4 month variables unless otherwise indicated; [2]Synthesizing period (April 1 to October 31); [3]Non-synthesizing period (November 1 to March 31); [4]Constitutive skin=upper inner arm (non-exposed area); [5]Facultative skin=mean of mid forehead, outer forearm, and outer lower leg; [6]% change TBF=(measured body fat at 4 month minus measured body fat at 1 month)/body fat at 1 month); [7]Sun exposure during synthesizing period

Table 6. Predictors of Infant 25(OH)D Concentration at 4 Month and Change in Vitamin D Status from 1 to 4 Months

	Independent variables	β coefficient	95% CI
Infant			
4 months	Intercept	44.347*	5.4, 83.3
N=42	Gender	-2.589	-16.0, 10.9
	Maternal 25(OH)D at 4 months, nmol/L	14.979*	0.2, 29.7
	Travel to latitude <37 ^{o}N	-27.118**	-46.4, -7.9
	Facultative[1] skin pigmentation, ^{o}ITA	0.686*	0.1, 1.3
	Constitutive[2] skin phototype	2.509	-24.1, 29.1
	R^2 = 0.40, **Root MSE =** 20.943, **p-value** <0.002		
Δ 25(OH)D	Intercept	104.410**	57.0, 151.8
N=42	Gender	-9.592	-22.1, 3.0
	Baseline 25(OH)D, nmol/L	-0.544**	-0.8, -0.3
	Travel to latitude <37 ^{o}N	-23.452**	-40.7, -6.2
	Maternal 25(OH)D at 4 months, nmol/L	0.235	-0.1, 0.6
	Ethnicity	-14.003*	-27.3, -0.7
	R^2 = 0.59, **Root MSE =** 18.666, **p-value** <0.001		

25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; Constitutive skin phototype: 0=very fair to fair, 1=medium, olive, dark, or very dark; Ethnicity: 0=white, 1=non-white; Gender: 0=female, 1=male; MSE: mean square error; Maternal 25(OH)D at 4 months: 0=(<75 nmol/L), 1=(≥75 nmol/L); Travel to latitude <37 ^{o}N: 0=yes, 1=no

[1]Faculatitive skin=mean of mid forehead, outer forearm, and outer lower leg (sun-exposed area); [2]Constitutive skin=upper inner arm (non-exposed area)

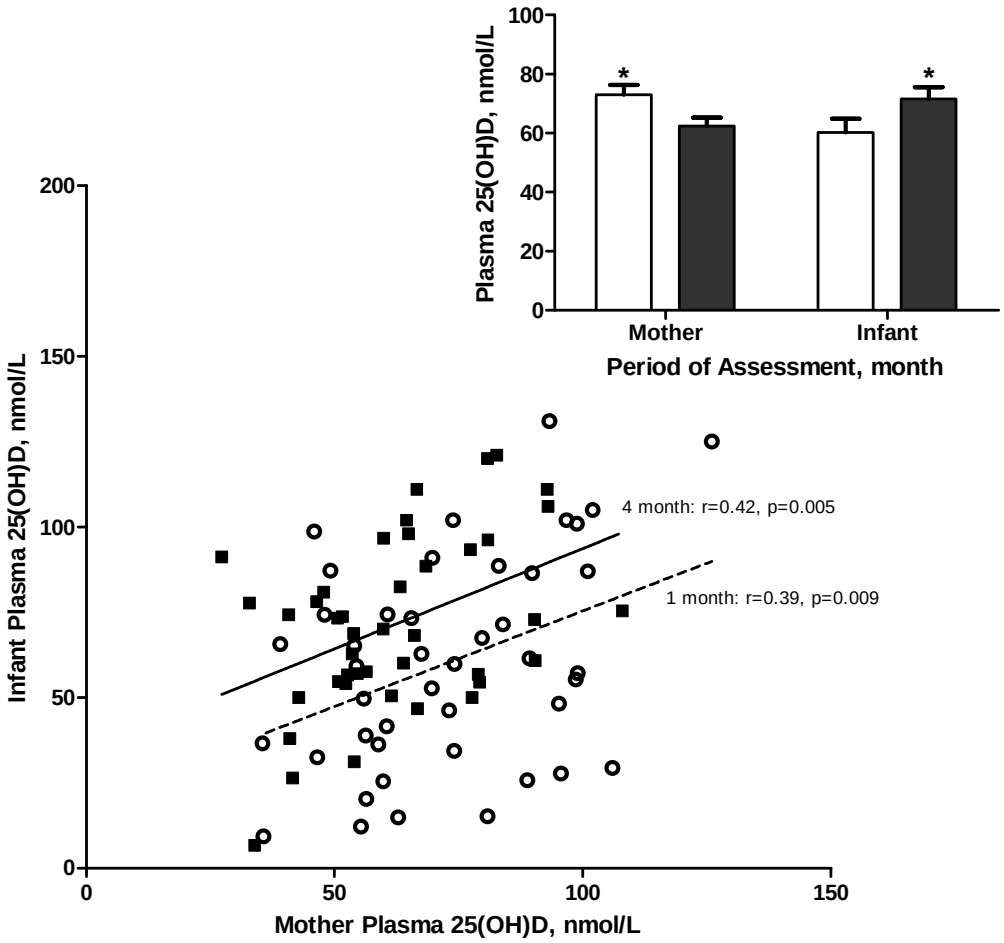

Figure 1. Relationship between maternal and infant vitamin D status at 1 month (◯) and 4 month (■). Significant correlation between maternal and infant vitamin D status at 1 month (dotted line, r=0.39, p=0.009) and 4 months (solid line, r=0.42, p=0.005) postpartum.

Insert 1. Maternal and infant vitamin D status at 1 and 4 months. Data presented as mean (SEM). Bars represent baseline (□) and follow-up visit (■). *significant differences within individuals ($p<0.05$).

Supplementary Table 1. Daily Intake of Selected Nutrients Estimated by Mean 24 hour Recalls (N=42)

Nutrients	DRI	Median	[range]
Energy (kcal)		2268	[1424, 3219]
Carbohydrates (g)	210*	268	[170, 415]
% Energy	45-65§	49.4	[28.2, 62.3]
Protein (g)	71*	91	[53, 158]
% Energy	10-35§	17.6	[11.4, 28.2]
Fat (g)		81	[40, 135]
% Energy	20-35§	34.1	[23, 43.8]
Fibre (g)	29∫	22	[13, 93]
Calcium (mg)	1000*		
Dietary		1151	[480, 2751]
Total[1]		1256	[480, 3001]
Vitamin D (IU)	400†/600*		
Dietary		237	[53, 779]
Supplemental[2]		326	[0, 2400]
Total[1]	600*	596	[99, 2476]
Folate (µg)	500*	391	[220, 1969]
Iron (mg)	9*	14	[9, 23]

Values presented as median [range] as majority of values were non-normally distributed.
AI: Adequate Intake; AMDR: Acceptable Macronutrient Distribution Range; DRI: Dietary Reference Intakes based on IOM for lactating women between 19 to 50 years; EAR: Estimated Average Requirement; FFQ2: food frequency questionnaire administered at follow-up visit; RDA: Recommended Daily Allowance; SD: standard deviation
Nutrient amount food only, unless otherwise indicated; analyses without outliers did not improve or alter results
[1]Total vitamin D/calcium = dietary and supplemental; [2]Supplemental intake documented on general health questionnaire; *(asterisk) RDA; §AMDR; ∫AI; †EAR

12. BRIDGE STATEMENT

At the time of designing this study, there was limited information on the vitamin D intake of lactating women and vitamin D status and predictors of status in lactating women and their infants in Canada. In the first manuscript, we found that the mean plasma 25(OH)D concentration in lactating women and their infants was higher than studies reporting vitamin D deficiency in this population [5, 9, 18, 25, 43, 122]. In our limited cohort, vitamin D intake was assessed using mean 24 hour recalls. However, administration of 24 hour recalls would be inefficient and increase participant burden thus resulting in decreased compliance in large cohorts and epidemiological studies. FFQs have been validated to assess vitamin D intake among non-pregnant and non-lactating women [216, 268-270] and adults [271-273]. To our knowledge, there has only been one FFQ validation study in postpartum women [274] however, whether these women were breastfeeding (exclusively or partially) was not disclosed. Due to differences in dietary intake and physiological and nutrient needs, it is necessary to validate FFQs in the population in which it will be administered. Thus, the objective of the second manuscript in this thesis was to determine the reproducibility and validity of a FFQ to assess vitamin D intake in lactating women. Larger cohorts involving lactating women are required to verify these results to embark upon epidemiological studies.

13. MANUSCRIPT 2

Reproducibility and Validity of a Food Frequency Questionnaire for the Assessment of Vitamin D Intake in Lactating Women

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13.1. Abstract

Valid dietary assessment tools to capture vitamin D intake from foods are required to facilitate research regarding the relationships among intake and vitamin D status. The objective of this study was to validate the semi-quantitative Willett FFQ, adapted for Canadians, for assessing vitamin D intake in lactating women. Healthy women (n=42) from Montreal completed a FFQ at 4 months postpartum and 24 hour dietary recalls at 1, 2.5, and 4 months postpartum. Fasted maternal venous blood sample were collected for analysis of plasma 25(OH)D concentration by competitive chemiluminescence assay (LIAISON®, DiaSorin, Stillwater, MN). A subsample (n=7) completed the FFQ at baseline for reproducibility testing. Mean vitamin D intake was similar between FFQ and mean 24 hour dietary recalls (dietary: p=0.869; total (dietary and supplemental): p=0.954). Correlation coefficients between FFQ and mean 24 hour recalls for dietary vitamin D intake was r=0.45 (p=0.003) and total vitamin D intake was r=0.80 (p<0.001). Correlation coefficients between total vitamin D intake estimated by FFQ and 25(OH)D concentration was r=0.11 (p=0.495) and between mean vitamin D intake estimated by mean 24 hour recalls and 25(OH)D concentration was r=0.24 (p=0.126). Bland-Altman analyses indicated a fair agreement between dietary methods (LoA: 359, -380 IU). After energy-adjustment, there was a satisfactory agreement between dietary methods (limit of agreement: -129, 136 IU). Based on total vitamin D intake, 69% were classified into the same tertile and weighted Kappa statistics was 0.63 between dietary methods and 45.2% were classified into the same tertile with K_W=0.14 between FFQ and 25(OH)D concentration. These findings indicate that the Willet FFQ, adapted for Canadians, is a valid tool for the assessment of vitamin D intake among lactating women, particularly with energy adjustment. However, FFQ may not be a good indicator of vitamin D status due to inability to capture endogenous vitamin D synthesis.

Key words: validation, lactating women, food frequency questionnaire, vitamin D, 25-hydroxyvitamin D

13.2. Introduction

The postpartum period is an important life transition period that may influence dietary behavior of women. Low vitamin D status in lactating women is a global issue [1, 5, 6, 9, 11, 12], including among Canadians lactating women [14, 18, 25]. Limited vitamin D stores measured during pregnancy can continue to decline post-natally [6, 13-15, 17, 25, 43, 123] up to 6 months postpartum [123]. For example, low calcium and vitamin D intakes have been documented [252] among lactating women who restrict dairy intake as a means to lose weight [275], to relieve symptoms of infant colic/gas [276, 277], or possible lactose intolerance/milk allergy [277]. The IOM increased vitamin D recommendations for lactating women to a RDA of 600 IU/d [30] from an AI of 200 IU/d emphasizing the importance of vitamin D on bone maintenance [115] and to prevent osteomalacia, osteoporosis, and bone fractures [66, 67].

Food frequency questionnaires estimate usual dietary intake and may thus be suitable for examining vitamin D intake during the last 3 months of lactation, which reflects both dietary and exogenous synthesis [38, 75, 245]. FFQs are cost-effective, have low participant burden and time, and better capture seasonal foods and foods that are not consumed daily compared to other dietary assessment methods such as food records [38, 75, 245]. The precision in quantifying intakes may vary among different populations [208] thus, it is important to validate the FFQ in the population in which the FFQ will be used. Assessment of vitamin D intake is challenged by the facts that few foods are rich in vitamin D and may not necessarily be consumed on a daily basis. Therefore, tools such as FFQs that capture usual intake over extended periods of time are required. Validation of a FFQ for vitamin D intake is difficult because both endogenous synthesis in the plasma membrane of the epidermis cells from exposure to UVB radiation and exogenous intake of supplements and food sources such as salmon and fortified milk, yogurt, orange juice, and soy beverages [77, 78, 196] contribute to vitamin D status, as measured using 25(OH)D concentration [39, 41].

Previous studies have used FFQ to estimate nutrient intakes [271] in non-pregnant [268] and pregnant women [274]; only one has estimated vitamin D

intake as part of a large range of nutrients in low-income postpartum women [274]. There has only been one vitamin D validation study which utilized both a reference method, dietary records, and biomarker, 25(OH)D concentration [271]. Wu et al. [271] concluded that the FFQ can provide reasonable estimates of vitamin D intake, classify 69% of participants into the same tertile of vitamin D intake by FFQ and food records, fair agreement between FFQ and 25(OH)D concentration using K_w, and the FFQ was related to the reference method and 25(OH)D concentration. However, the Bland-Altman method was not conducted for agreement between dietary methods [271]. The Bland-Altman is a better statistical approach for assessing agreement between two methods [278], such as vitamin D intake between the FFQ and 24 hour recalls. Two other studies validated FFQ for vitamin D intake as part of a large range of nutrients; Jacques et al. [279] only used FFQ and 25(OH)D concentration in adults including men and women whereas Brantsæter et al. [280] used both dietary records and 25(OH)D concentration in pregnant women. However, the physiological needs and dietary recommendations and intakes are different during lactation compared to pregnant women and adults [281]. Therefore the purpose of this study was to determine the reproducibility and validity of a Canadian adapted semi-quantitative FFQ in assessing vitamin D intake of lactating women using both dietary and biochemical comparators.

13.3. Subjects and Methods

13.3.1. Study Design and Subjects

Mother-infant pairs in the current manuscript were the same as those in manuscript #1. Participants were recruited as part of a clinical trial comparing supplements of vitamin D_2 and vitamin D_3 isoforms in infants (NCT01190137). Participants were recruited from May 2010 to April 2011 from CLSC Lac Saint-Louis birthing centre and Lakeshore General Hospital postnatal ward to capture seasonal differences in endogenous vitamin D synthesis. To capture seasonal differences, a range of 3-6 mother-infant pairs were recruited in each month. Spring-summer months were from April 1 to October 31 and fall-winter months were from November 1 to March 31 [26]. Upon assessment of eligibility, participants were enrolled into the study for a standardized 3 month period, baseline (1 month postpartum) and follow-up (4 months postpartum). Inclusion criteria were predominately breastfeeding (>80% of milk feeds) mothers who delivered a healthy, singleton, term (between 37-42 weeks) infant with birth weight between 3rd and 97th percentile (±3 SD) for sex according to the WHO Growth Charts [170]. Exclusion criteria were maternal diagnosis of kidney, liver, Crohn's, or celiac disease, diabetes, or other diseases or medications that may affect vitamin D metabolism [240]. Socio-demographic, pregnancy, and dietary intake information, and blood samples were collected. At both visits, frequency, dose, and brand of supplement used were documented along with administration of 24 hour recalls. Of the 44 mothers who enrolled, 2 withdrew before the follow-up visit. Forty-two (95%) were included in the validation analysis and 7 (16.7%) were included in the reproducibility analysis.

13.3.2. Administration of the FFQ

A Canadian adapted (English and French) semi-quantitative self-administered FFQ was completed to assess maternal vitamin D intake during the last 3 months of lactation (**Appendix 17.1**). The modified FFQ included the same food items as Willett FFQ but nutrient composition was based on the CNF to reflect Canadian vitamin D fortification guidelines. The FFQ contained 145 food/beverage items (not specifically rich in vitamin D sources), 12 individual

and multi-vitamin supplements, and 11 qualitative/quantitative questions regarding baking and cooking method, other foods consumed at least once a week that was not mentioned, adherence to any special diets, and changes in specific pre-determined food and beverages categories over the last ten years such as whole milk, eggs, and fish. Consumption frequencies of the following categories were assessed dairy foods (14 items); fruits (16 items); vegetables (27 items); eggs, meat and fish (22 items); cereals, breads, and starches (18 items); beverages (17 items); and sweets, baked goods, and miscellaneous (31 items). For food items, reported frequencies were allocated as follows: never=0, <once per month=0.02, 1-3 times per month=0.07, 1 time per week=0.14, 2-4 times per week=0.43, 5-6 times per week=0.80, once a day=1, 2-3 times per day=2.5, 4-5 times per day=4.5, and ≥6 per day=6.5. Serving sizes were based on household measures (tablespoon, cup) and standard units (slice, item).

Prior to completion of FFQ, women were shown food models and standard household measures to assist them in considering portion sizes. To explore reproducibility, the same FFQ was administered to the last 14 participants at the baseline visit (FFQ1) to complete off-site within 1 week of the baseline visit and returned at the follow-up visit. The FFQ1 assessed vitamin D intake during the last month of pregnancy and first month post-delivery. At the midpoint telephone call, if FFQ1 was not completed, these mothers were asked to return the blank FFQ1 at the follow-up visit. At the 4 month follow-up visit, all mothers completed FFQ on-site (FFQ2). A 3 month period between administrations of a FFQ is standard for validation studies which may be sufficient to prevent recall of previous responses [282]. Questionnaires were immediately checked for completeness and participants were consulted for clarification where necessary.

13.3.3. Three 24 hour Dietary Recall as a Reference Method

Twenty-four hour dietary recalls were collected at baseline, midpoint (2.5 months postpartum), and follow-up visits using the online National Cancer Institute Automated Self-administered 24 hour Dietary Recall (ASA24^{TM}) [244]. Each participant was provided with a unique username and password. The baseline and follow-up recalls were interview-administered on-site (primarily

scheduled on Mondays or Fridays) and midpoint recall was completed off-site by mothers (on a convenient day) to capture at least one weekend and one weekday of intake. The choice of 24 hour recall was based on the assumption that the response rate would be higher than other dietary assessment methods such as food records [38]. The ASA24^{TM} utilizes the multiple pass method [244] and to assist with portion size estimations, ASA24^{TM} included images of various food and beverage portion sizes. Participants were able to input food items missing from the database. Upon completion of the recall, the created food list was retrieved and checked for completeness. Mothers were consulted for clarification where necessary. At both visits, supplementation use including frequency, dose, and brand was documented. Among 42 participants, 78.6% (n=33) completed three 24 hour recalls and 21.4% (n=9) completed two recalls. One mother did not complete baseline recall but completed midpoint and follow-up recalls.

13.3.4. Analysis of Food Consumption Data

Each food item was based on a sample of foods from the CNF 2010b which fit specific criteria such as fat and fibre content. The frequency of consumption during the 3 month study period was multiplied by the nutrient content of the serving size to estimate daily nutrient intake. For example, for the food item low fat cheese, an average of 1) low fat cheddar or Colby cheese, 2) cheddar cheese spread, made with skim milk, processed, 3) low fat Swiss cheese, and 4) low fat Swiss cheese, processed was used for the nutrient content of low fat cheese. Nutrient analysis for 24 hour recalls were estimated using the Nutritionist Pro^{TM} Software (Axxya systems, Stafford, TX) utilizing the 2010b CNF database. For both dietary methods, a Registered Dietitian verified all vitamin D sources to reflect current market fortification. Food items with missing vitamin D content were inputted from Canadian manufacturer websites and mixed food recipes were entered based on common serving size. Nutrient content of supplements were inputted from manufacturer specifications. All nutrients were energy adjusted using the nutrient density approach per 1000 kcal [208, 245]. Supplement intake was not energy adjusted. Total vitamin D intake was calculated from foods and supplements. Macronutrients were also expressed as % from energy.

Underreporting by FFQ2 and dietary recalls were evaluated using the ratio energy intake by basal metabolic rate (EI:BMR) by Goldberg et al. [283]. Estimated BMR was calculated based on weight, height, age, and sex [284]. EI:BMR was calculated for each participant and compared with the cut-off criteria of EI:BMR <1.14, which could reflect actual EI over a given measured period [283] and has been used in pregnant women in the first trimester with moderate physical activity [285]. Analyses with and without underreporters did not alter results and were included in the final sample size. Overreporters (EI:BMR ≥2.4) were also examined [286].

13.3.5. Plasma 25(OH)D Concentration as the Biochemical Indicator

Fasted (8 hours) venous blood samples (5 ml lithium heparin) were collected at the follow-up visit. Plasma obtained by centrifugation at 3000 g, 4°C for 20 minutes and subsequently frozen at -80°C until analysis. Plasma 25(OH)D concentration was analyzed on the same assay kit in batches by "25-OH Vitamin D TOTAL" competitive chemiluminescence assay system on the automated LIAISON® analyzer (DiaSorin, Stillwater, MN). Controls were provided by the manufacturer measured in duplicates yielding values within the specified acceptable range and with an intra-assay CV of 1.1%. Intra-assay variability was also determined from samples measured in either duplicates or triplicates yielding an average CV of 3.0%. This method has 100% specificity for both $25(OH)D_2$ and $25(OH)D_3$ and detection range of 25(OH)D between 10-375 nmol/L.

13.3.6. Ethical Approval

The study was reviewed and approved by the Research Ethics Board of McGill University (**Appendix 17.2**), received a no objection letter from Health Canada, and is part of a larger Phase III clinical trial that has been registered at Clinicaltrials.gov and holds the identifier, NCT01190137. Written informed consent was received from all participants.

13.3.7. Statistical Analysis

Statistical analyses were performed using SAS 9.2 (Cary, North Carolina). No significant differences in vitamin D intake were found between those who completed 2 recalls and 3 recalls (p=0.760) thus, mean intakes from dietary

recalls were used. Continuous variables were expressed as mean ±SD or median [range]. Categorical variables were expressed as proportion (%). Statistical significance was set at $p<0.05$. All entered data were screened for accuracy, outliers (>3SD from mean), underreporters (EI:BMR <1.14, conservative value) [283, 286], and overreporters (EI:BMR ≥2.4) [286] were examined. Due to limited EI:BMR cut-off values, these cut-off criteria were used despite variability in BMR and appetite in lactating women. Use of three 24 hour recalls improved assessment of intake patterns between recalls and FFQ.

Comparison of means were calculated using Student's *t*-test or Mann-Whitney tests for normally or non-normally distributed variables, respectively, with normality being tested using qq-plots and Shapiro-Wilks statistics. Pearson's or Spearman rank correlations were calculated, with and without underreporters and outliers, depending on the distribution of the data. If removal of outliers did not alter the results, they were included in the final analyses. Validation studies have used both log-transformation of nutrients to correct for skewness [271] or non-parametric tests [216, 269, 272, 287]. Spearman may be more reliable because it uses rank order and is not sensitive to extreme values as Pearson's coefficients [216]. The Bland-Altman method, defined as the limit of agreements (LoA) (±2 SD of the mean difference) [278], assessed agreement between FFQ2 and mean dietary recalls. The mean difference (24 hour recalls-FFQ) was plotted against the mean [(24 hour recall + FFQ2)/2] for absolute and energy-adjusted dietary vitamin D intake [278]. The plot of the difference against the mean was used to investigate potential relationship between measurement error and true value [278] and how much vitamin D intake (IU/d) differed between the FFQ and 24 hour recalls. To examine relative agreement, contingency (cross) classification by tertiles [216] were performed by Chi-square and K_W was calculated for crude and energy-adjusted vitamin D. $K_W=[P_{O(W)} - P_{e(W)}]/[1- P_e]$ where P_O is the observed proportion of agreement and P_e is the expected proportion of agreement by chance [214]. K_W >0.80 indicates very good agreement, between 0.61 to 0.80 good agreement, between 0.41 to 0.60 moderate agreement, between 0.21 to 0.40 fair agreement, and <0.20 poor agreement [215].

13.4. Results

Of the 267 mothers approached to participate in the study, 223 (83.5%) were excluded due to ineligibility, unable to contact, or refused to participate, 42 (15.7%) completed the study, and 2 (0.7%) withdrew (**Appendix 17.2**). Of the 14 mothers who were administered FFQ1, compliance was low at 7 (50%). All mothers completed FFQ2 and follow-up 24 hour recalls, 41 (97.6%) completed baseline recall, and 33 (78.6%) completed midpoint recall. Thirty-three mothers completed all measures.

The mean age at delivery was 32.3 ± 3.8 years, mean gestational age was 39.6 ± 1.0 weeks, mean pre-pregnancy BMI was 23.3 ± 3.5 kg/m^2, and mean total pregnancy weight gain was 14.7 ± 4.3 kg. During pregnancy, 7.1% self-reported smoking, 38.1% self-reported regularly use of medications, and 51.4% self-reported intake of ≥1 cup/day of caffeinated coffee and/or tea. The majority of participants were living with a partner (97.6%), white (78.6%), with higher than high school education (88.1%), and house income ≥$75,000 (59.5%) based on national median. During pregnancy, all participants reported prenatal vitamin intake and 95.2% reported taking prenatal supplements ≥5 times weekly. The majority of mothers were exclusively breastfed (76.2%) at the baseline visit and their infants had a birth weight-for-age z-score between -1.23 to 2.65.

Although mothers were recruited year round, 16 (38.1%) mothers started the study during the vitamin D synthesizing period (April 1 to October 31). Baseline weight at 1 month was 67.8 ± 10.7 kg and height was 163.7 ± 6.8 cm. Final weight was 67.0 ± 11.7 kg and mothers on average spent 5.2 ± 4.9 weeks in the synthesizing period. The majority continued to take a prenatal supplement (67%), 21.4% were taking a vitamin D supplement, and 9.5% were taking a calcium supplement. Plasma 25(OH)D concentration was 62.4 ± 18.3 nmol/L.

Milk was the main dietary source of vitamin D with higher intake at 1 month than 4 month visit (375 ± 425 vs. 218 ± 363 mL/d, p=0.024). Using the FFQ2 and mean 24 hour recalls, few mothers had dietary vitamin D intake ≥EAR and ≥RDA. Between FFQ and mean dietary recalls, there were no significant differences in total vitamin D intake within the same category <EAR (p=0.377),

≥EAR (p=0.652), <RDA (p=0.812) and ≥RDA (p=0.518) (data not shown). Based on categories of EAR and RDA, estimated by 24 hour recalls, there was a significantly higher 25(OH)D concentration in total vitamin D intake ≥EAR than when intake <EAR (p=0.042) (**Figure 1**); values of 25(OH)D concentration were not different using RDA cut-offs or any of the FFQ data. Using the FFQ, total vitamin D intake <RDA was observed in 20 (47.6%) mothers with 25(OH)D concentration of 60.1 ± 20.6 nmol/L and total vitamin D intake <EAR was observed in 13 (31%) mothers with 25(OH)D concentration of 61.8 ± 23 nmol/L. Year round, 9 (21.4%) mothers had 25(OH)D concentration <50 nmol/L and 39 (71.4%) had 25(OH)D <75 nmol/L. In the non-synthesizing period, 7 (29.2%) had 25(OH)D concentration <50 nmol/L and 20 (83.4%) had 25(OH)D <75 nmol/. In the synthesizing period, 2 (11.1%) had 25(OH)D <50 nmol/L and 16 (55.5%) had 25(OH)D <75 nmol/L. Vitamin D concentration was significantly higher (73.5 ± 15.1 nmol/L) during synthesizing compared to non-synthesizing period (55.7 ± 12.2 nmol/L) with no significant differences in total vitamin D intake (**Figure 2**).

In the reproducibility analysis, mothers who completed FFQ1 were similar to the final population with the exception, younger age at delivery (p=0.010) and more weeks were spent in the synthesizing period during the study period (p=0.004). There were no significant differences in mean vitamin D intakes (dietary, supplemental, or total). Between FFQs, there was a positive correlation between supplemental (r=0.88, p<0.001) and total vitamin D intake (r=0.80, p=0.010). Adjusted vitamin D intake increased correlation coefficient (**Table 1**).

Table 2 shows the dietary intakes of selected nutrients estimated by FFQ2 and mean 24 hour recalls. There were no significant differences between absolute and energy adjusted dietary and total vitamin D intake between FFQ2 and mean 24 hour recalls. There was a better correlation between dietary methods when vitamin D supplements were included. There was a significant correlation between vitamin D intake between FFQ2 and 24 hour recalls and attenuated, energy adjustment improved correlation coefficient (r=0.48). There were no significant correlations between dietary methods and 25(OH)D concentration

(**Table 3**), adjustments for energy and season did not improve correlation coefficients (data not shown).

Figure 3 illustrates the Bland-Altman plot between FFQ2 and mean dietary recalls for crude and energy-adjusted dietary vitamin D intake. A negative mean difference was observed for crude dietary vitamin D intake and positive mean difference for energy-adjusted dietary vitamin D intake. The LoA was wider in crude dietary vitamin D intake. The removal of outliers did not alter results and were included in the final analyses. Vitamin D intake from both dietary methods and 25(OH)D concentration were divided into tertiles (same, adjacent, opposite) and cross-classification analyses were conducted (**Table 4**). Between FFQ2 and mean dietary recalls, percentage of mothers classified into the same tertile range from 50% for adjusted dietary vitamin D to 90.5 % for adjusted total vitamin D. K_W indicated fair to very good agreement. Between FFQ2 and 25(OH)D concentration, mothers classified into the same tertile range from 23.8% to 45.2% and K_W indicated poor agreement. Between dietary recalls and 25(OH)D concentration, mothers classified into the same tertile range from 35.7% to 50% and K_W indicated poor to fair agreement. Between FFQ2 and mean 24 hour recalls, mothers classified in the opposite tertile of vitamin D intake range from 0% to 28.6%. Supplemental vitamin D appears to increase with increasing tertiles of 25(OH)D concentration but was non-significant (data not shown).

There were 10 (23.8%) underreporters estimated by the FFQ2 and 3 (7.1%) estimated by mean 24 hour recalls. Underreporters had lost significantly more weight during the study period than non-underreporters (-4.5 ± 4.0 vs. -1.1 ± 4.1 %, respectively; p=0.026) with no differences observed for weight, %TBF, and % change in TBF between underreporters and non-underreporters. Underreporters had significantly higher 25(OH)D concentration than non-underreporters (74.7 ± 16.6 vs. 58.6 ± 17.3 nmol/L, respectively; p=0.014). Energy and nutrients were statistically lower for underreporters than non-underreporters except for supplemental calcium and vitamin D intake and total vitamin D intake (dietary and supplemental) (**Appendix 17.3**). After energy-adjustment, only dietary vitamin D intake was higher in underreporters and non-

underreporters (154 ± 46 vs. 118 ± 48 IU/d, respectively; p=0.041). Removal of underreporters did not alter correlation coefficients between dietary methods and 25(OH)D concentration. There were 3 (7.1%) overreporters estimated by FFQ where energy intake was 3495, 4225, and 4145 kcal and Δweight from 1 to 4 months postpartum was 2.60, 0.20, and -1.2 kg, respectively. There was one (2.4%) overreporter (energy intake 3219 kcal with Δweight of -1.2 kg) estimated by dietary recalls.

13.5. Discussion

To the best of our knowledge, this is the first study to validate a Canadian adapted semi-quantitative FFQ to assess vitamin D intake in lactating women by using both a dietary method and biochemical comparator. Previously, validated FFQs for vitamin D intake have been validated in pregnant [280, 287-289], non-pregnant and non-lactating women [216, 268-270], and general population [271-273] and few used a biochemical indicator [270, 272, 274].

Dietary and total vitamin D intakes were similar between FFQ1 and FFQ2. These correlations have been observed in several studies [268, 273]. Lower correlation coefficients have been observed for absolute (r_{avg}=0.62) [271, 274, 288] and energy-adjusted vitamin D intake (r_{avg}=0.58) [274]. To minimize correlated errors such as systematic within-person error [208], the FFQs were administered 3 months apart compared to 2 weeks [271] and 1 month [268, 274, 288]. Despite possible complications in estimating vitamin D intake during lactation due to appetite changes and desire to lose pregnancy weight gain, our results suggest that the Canadian adapted Willet FFQ may be reproducible. Although, there may be bias in the mothers who completed FFQ1, maternal characteristics were similar between mothers who completed baseline FFQ and those only completing the second FFQ.

Few validation studies estimated mean intakes of both crude and energy-adjusted dietary and total vitamin D. In our study, comparison of means revealed no significant differences in vitamin D intakes between FFQ2 and mean 24 hour dietary recalls with slightly higher agreement after energy-adjustment by the FFQ. It remains to be determined whether three 24 hour recalls is sufficient to capture vitamin D intake. FFQ tends to estimate significantly higher dietary vitamin D than the reference method in postpartum women [274], pregnant [280, 287-289], and non-pregnant and non-lactating adults. Overestimation of vitamin D intake by the FFQ could be attributed to underreporting consumption during interview-administered questionnaires or difficulty in comparing standardized FFQ portion sizes as usual portion consumed is different. This leads to inaccurate judging of the consumption frequency.

The estimates of dietary and total vitamin D from FFQ were similar to postmenopausal (dietary: 226 IU/d; total: 934 IU/d) [270] and postpartum women (diet: 169 IU/d; total: 391 IU/d) [18] and higher than pregnant (median dietary: 130 IU/d; median total: 414 IU/d) [280, 289] and non-pregnant and non-lactating adults (dietary: 139; total: 204 IU/d) [174, 268]. Higher total vitamin D intake could be attributed to intake of prenatal supplement during lactation [198]. The majority of our participants are educated, white women which are factors that have been positively associated with supplement use [253].

To our knowledge, there has been one validation study conducted in postpartum women [274]. In the present study, absolute and adjusted correlation coefficients for vitamin D intake between FFQ and dietary recalls were similar a study examining bone-related nutrients (rho= 0.55 ; rho_{adj}:0.52) [272] and validation study in adults (r=0.52) [271]. Studies examining dietary vitamin D as part as a large range of nutrients had lower crude correlation coefficients (r_{avg}= 0.27) [269, 274, 279, 287-289]. Energy-adjusted dietary and total vitamin D have been documented to increase [274, 288, 289] or decrease [272, 279, 289] correlation. In the present study, energy-adjustment did not improve the correlation. This could be related to under- and overestimation of vitamin D intake or supplement intake may not be related to energy intake [245]. Published studies and ours observed improved correlation when corrected for random within-person variation [268, 274, 288].

Few FFQ validation studies used both a reference method and a biomarker [212]. Biomarkers offer an objective and independent validation of nutrient intakes because errors associated with the determination of nutrient status by the biomarker are uncorrelated with errors associated with dietary assessment methods [208]. 25(OH)D concentration is considered the best clinical indicator of vitamin D status reflecting both endogenous and exogenous sources [38-41]. Thus, 25(OH)D concentration will not provide a perfect reflection of nutrient intake [208] and high agreement cannot be expected but better agreement might be expected in winter months or in northern climates. In white women residing in Winnipeg (latitude 49.9 ^{o}N), there was a relationship between 25(OH)D

concentration and dietary vitamin D intake using a single FFQ (r=0.26, p=0.001) but not in rural (r=0.20, p=0.350) or urban First Nation women (r=0.11, p=0.140) [17]. Larger studies ($N_{avg=}$121) observed a significant relationship between total vitamin D intake and 25(OH)D concentration (r_{avg}=0.39), independent of season [271, 279, 280]. However, sun exposure was not assessed. Although during late winter endogenous vitamin D is inhibited, tissue storage of vitamin D can last for several months to years [66] and could attenuate the decrease in 25(OH)D concentration [148]. Summer 25(OH)D concentration was positively associated with Δ25(OH)D from summer to winter over 15 month period (r=0.48, p<0.001) [148]. The non-significant correlation observed in the present study between dietary methods and 25(OH)D concentration could be attributed to limited sample size (lack of sensitivity and specificity), low variability in 25(OH)D concentration, and other predictors of maternal 25(OH)D concentration explored in manuscript 1. Energy adjusted correlation coefficient approached satisfactory (r>0.30) [208]. Correlation coefficients between 0.5 to 0.70 may seem low in comparison to highly controlled laboratory measurements however, these values are common for validity of nutrient intakes in dietary studies [208].

The objective of the present study was to compare vitamin D intake to 25(OH)D concentration as a measure of validation, not to determine the predictors of vitamin D status. There was a significant difference in 25(OH)D concentration between <EAR and ≥EAR estimated by mean 24 hour recalls. This relationship was not observed between 25(OH)D concentration and <EAR and ≥EAR or between <RDA and ≥RDA estimated by FFQ or between <RDA and ≥RDA estimated by dietary recalls. Mean 24 hour recalls gave a better estimate of vitamin D intake which is unexpected compared to FFQ which captures vitamin D intake over 3 months during lactation. The significance was not observed in RDA or any values of FFQ data which could be attributed to a possible threshold effect and combined with the limited sample size and lack of sensitivity and specificity.

The use of correlation coefficients is a controversial approach used in validation studies. Correlation ignores any systematic bias between variables and assesses association rather than agreement [213, 290]. Correlations were reported

to allow comparison with other studies and to explore the relationship between dietary methods and 25(OH)D concentration. Bland-Altman plots, cross-classification, and K_W should be used to assess agreement. Negative mean difference for crude dietary vitamin D intake indicate overreporting by the FFQ than dietary recalls and positive mean difference for energy-adjusted dietary vitamin D intake indicates underreporting by the FFQ than mean 24 hour recalls. The scatter plot for dietary intake suggests that with increasing positive mean values with decreasing differences indicate that the FFQ overestimates vitamin D intake more and more as the intake increases compared to recalls. Thus, agreement between FFQ and mean 24 hour recalls was better when corrected for energy intake. The Bland-Altman plot had comparable and better agreement than those of Hjartaker et al. (-40 IU; LoA: 280, -360 IU) [269] and Pritchard et al. (75 IU; 510, -359 IU) [270]. Agreement between FFQ and recalls was satisfactory with only 2 (5%) observations occurring outside the LoA but the wide LoA suggest some bias at higher vitamin D intake. Removal of outliers did not alter the results.

Cross-classification analyses of dietary vitamin D intake into the same quantile range was between 31 to 73% between FFQ and reference method [216, 269-272, 274, 288, 289]. The results in the present study had high agreement. Differences in agreement could be due to the duration between administration of FFQ and reference methods and the inability of 24 hour recalls to capture dietary vitamin D sources not consumed daily. Mouratidou et al. [274] found percentage of individuals classified into the extreme quintile of the distribution improved using energy-adjusted intake. This was not observed by us or Osowski et al. [272]. Overall, the FFQ can classify mothers' vitamin D intake into the same tertile estimated by FFQ and mean 24 hour recalls. In several studies, the validity of the FFQ was supported by increasing 25(OH)D concentration across increasing distributions of vitamin D intakes [279, 280, 289]. This trend was not observed possibly due to limited sample size and/or narrow range in vitamin D intakes. K_W between dietary methods and 25(OH)D concentration range from fair to very good agreement. The K_W between the dietary methods was similar to those of Wu

et al. (crude K_W=0.37) [271] and Masson et al. (adjusted K_W0.27) [216]. In the present study, K_W indicated poor to fair agreement between the dietary methods and biomarker. However, we were unable to identify a validation study that conducted K_W between dietary methods and 25(OH)D concentration.

The strengths of the present validation study were inclusion of both food and supplemental vitamin D and 25(OH)D concentration as validation criteria. Further validation of this questionnaire in a larger sample is required along with important modifications such as vitamin D fortified foods that may continue to enter the market and to separate dark fishes into separate food items as the vitamin D content greatly varies. Modifications of a FFQ have been observed to increase correlation coefficients and agreement between both FFQ and food records with serum 25(OH)D concentration [271]. The present study consisted mainly of white, higher income, and well-educated women conveniently sampled thus, is not representative of the diverse demographics and ethnic population in Canada. Mothers in the study may have different dietary patterns and provide more accurate responses, especially those who completed both FFQs, than those who would be included in an epidemiological study.

In conclusion, the Willet FFQ, adapted for Canadians, provides promising reproducible and valid evidence to estimate vitamin D intake in lactating women. The FFQ demonstrates the ability to estimate vitamin D intake and classify mothers into the same tertiles of vitamin D intake. K_W indicates moderate agreement and Bland-Altman plot indicates satisfactory agreement in energy-adjusted vitamin D intake between dietary methods. Although mean 24 hour recalls better reflect vitamin D status, the FFQ is not without benefits. Administering a large number of 24 hour recalls is impractical and increases respondent burden in epidemiological studies. The FFQ can give similar vitamin D intake patterns. The majority of lactating women were taking a supplement containing vitamin D, highlighting the importance of documenting both dietary and supplemental vitamin D intake. As vitamin D fortified foods enter the market, these foods need to be added to the FFQ to maintain accuracy in assessing vitamin D intake. Validated FFQs are required to embark upon epidemiological studies.

Table 1. Reproducibility: Vitamin D Intake (dietary, supplemental, and total) and Correlation Coefficient between FFQ1 and FFQ2 in a Subsample of the Population (n=7)

Nutrients	Crude intake[1]			Energy adjusted[1,2]		
	FFQ1	FFQ2	Correlation coefficient	FFQ1	FFQ2	Correlation coefficient
Energy (kcal)	2178 (1034-3788)	2112 (1218-4145)	0.18			
Vitamin D (IU)						
Dietary	306 (133-722)	267 (98-761)	0.71	135 (65-270)	118 (61-184)	0.81*
Supplemental[3]	517 (143-1400)	495 (29-1400)	0.88**			
Total[4]	823 (341-1577)	762 (325-1603)	0.80**	614 (236-1501)	651 (212-1511)	0.89**

FFQ1: food frequency questionnaire at baseline visit; FFQ2: food frequency questionnaire at follow-up visit; there were no significant differences between mean intakes (dietary, supplemental, total) between FFQ1 and FFQ2 for crude and energy-adjusted values

*p<0.05 (2-tailed); **p<0.01 (2-tailed); Pearson or Spearman correlations coefficient based on normality of the data; Paired t-test or Mann-Whitney test based on normality of the data; there were no outliers found

[1]Values presented as mean (range); [2]Nutrient density calculated as per 1000 kcal; [3]From general health questionnaire at 1 month postpartum for FFQ1 and 4 month visit for FFQ2; [4]Total vitamin D = dietary and supplemental

Table 2. Dietary Intakes and Correlation Coefficients between FFQ at Follow-up and Mean Dietary 24 hour Recalls (n=42)

Nutrients	Crude intake[1]			Energy adjusted[1,2]		
	FFQ2	Mean 24 hr recall	Correlation coefficient	FFQ2	Mean 24 hr recall	Correlation coefficient
Energy (kcal)	2018 (1147-4225)	2268 (1424-3219)	0.38*			
Carbohydrates (g)	259 (121-665)	268 (170-415)	0.49**	126 (83-166)	125 (74-155)	0.40**
% Energy	50 (33-66)	49 (28-62)	0.37*			
Protein (g)	89 (43-211)	91 (53-158)	0.34*	43 (27-66)	43 (27-69)	0.56**
% Energy	17 (11-26)	18 (11-28)§	0.47**			
Fat (g)	81 (44-177)	81 (40-135)	0.12	38 (25-55)	38 (26-48)	0.28
% Energy	15 (10-22)	34 (23-44)	0.32*			
Calcium (mg)						
Dietary	938 (489-2786)	1151 (480-2751)	0.54**	476 (251-773)	481 (243-1039)	0.23
Total[3]	1133 (617-2803)	1256 (480-3001)	0.60**	680 (338-1177)	709 (243-1320)	0.72**
Vitamin D (IU)						
Dietary	220 (98-761)	237 (53-779)	0.45**	112 (45-250)	101 (27-283)	0.33*
Total[3]	602 (119-2589)	596 (99-2476)	0.80**	453 (73-2562)	401 (65-2451)	0.95**
Iron (mg)	15 (7-30)	14 (9-23)	0.19	7 (4-11)	7 (4-10)	0.15
Folate (µg)	338 (184-829)	391 (220-1969)	0.20	169 (124-258)	170 (96-943)	0.30
Fibre (g)	24 (8-55)	22 (13-93)	0.42**	12 (5-19)	10 (7-45)†	0.52**

FFQ2: food frequency questionnaire at follow-up visit; hr=hour

Pearson or Spearman correlations coefficient based on normality of the data explored relationship between nutrients estimated by FFQ2 and 24 hour recalls; Student's paired *t*-test or Mann-Whitney test based on normality of the data compared nutrient intake (crude and energy adjusted) between FFQ2 and 24 hour recalls; analyses without outliers did not improve or alter results

*p<0.05 (2-tailed) indicates significant correlation coefficient between FFQ2 and 24 hour recalls; **p<0.01 (2-tailed) indicates significant correlation coefficient between FFQ2 and 24 hour recalls

† p<0.05 (2-tailed) indicates significant difference in mean intake between FFQ2 and 24 hour recalls; §p<0.01 (2-tailed) indicates significant difference in mean intake between FFQ2 and 24 hour recalls

[1]Values presented as mean (range); [2]Nutrient density calculated as percent of energy for macronutrients and nutrient density per 1000 kcal for both macro- and micronutrients; [3]Total vitamin D/calcium = dietary + supplemental

Table 3. Correlation Coefficients between FFQ and Mean 24 hour Dietary Recalls with 25(OH)D Concentration (n=42)

Vitamin D (IU)	FFQ2 vs. 25(OH)D		Mean 24 hr recall vs. 25(OH)D	
	r or rho	p-value	r or rho	p-value
Dietary				
Crude	rho= -0.24	0.126	rho= -0.02	0.923
Energy adjusted[1]	r=0.09	0.564	rho= -0.03	0.857
Total[2]				
Crude	rho=0.11	0.495	rho= 0.24	0.126
Energy adjusted[1]	rho=0.24	0.124	rho= 0.23	0.143

25(OH)D: 25-hydroxyvitamin D; FFQ2: food frequency questionnaire at follow-up visit; hr=hour; r=Pearson's correlation coefficient; rho=Spearman's rank correlation coefficient

[1]Nutrient density calculated as per 1000 kcal; [2]Total Vitamin D = dietary and supplemental

Table 4. Cross-Classification of Vitamin D Intake between Dietary Methods (FFQ, Mean 24 hour Recalls) and 25(OH)D Concentration into the Same, Adjacent, and Opposite Tertiles with Weighted Kappa Statistics (n=42)

	FFQ2 vs. 24 hr recall[1]				**FFQ2 vs. 25(OH)D**[1]				**24 hr recall vs. 25(OH)D**[1]			
	% in tertile			**K_W**	**% in tertile**			**K_W**	**% in tertile**			**K_W**
	Same	**Adjacent**	**Opposite**		**Same**	**Adjacent**	**Opposite**		**Same**	**Adjacent**	**Opposite**	
Dietary vitamin D												
Crude	61.9	28.6	9.5	0.46	23.8	47.6	28.6	-0.18	35.7	38.1	26.2	-0.02
Energy adjusted[2]	50.0	38.1	11.9	0.30	23.8	57.1	19.0	-0.07	35.7	38.1	26.2	-0.02
Total[3] vitamin D												
Crude	69.0	28.6	2.4	0.63	45.2	33.3	21.4	0.14	50.0	33.3	16.7	0.25
Energy adjusted[2]	90.5	9.5	0	0.89	40.5	42.9	16.7	0.14	42.9	38.1	19.0	0.14

25(OH)D: 25-hydroxyvitamin D; FFQ2: food frequency questionnaire at follow-up visit; hr=hour; K_W: weighted kappa statistics
For FFQ, tertiles 1 to 3 were <428, 428-668, >668 IU/d; For 24 hour recalls, tertiles 1 to 3 were <489, 489-768, >768 IU/d; For 25(OH)D, tertiles 1 to 3 were <53, 53-67, >67 nmol/L
Analyses using Pearson's chi-square (χ^2)
[1]Values expressed as n (%); [2]Nutrient density calculated per 1000 kcal; [3]total vitamin D = dietary and supplemental

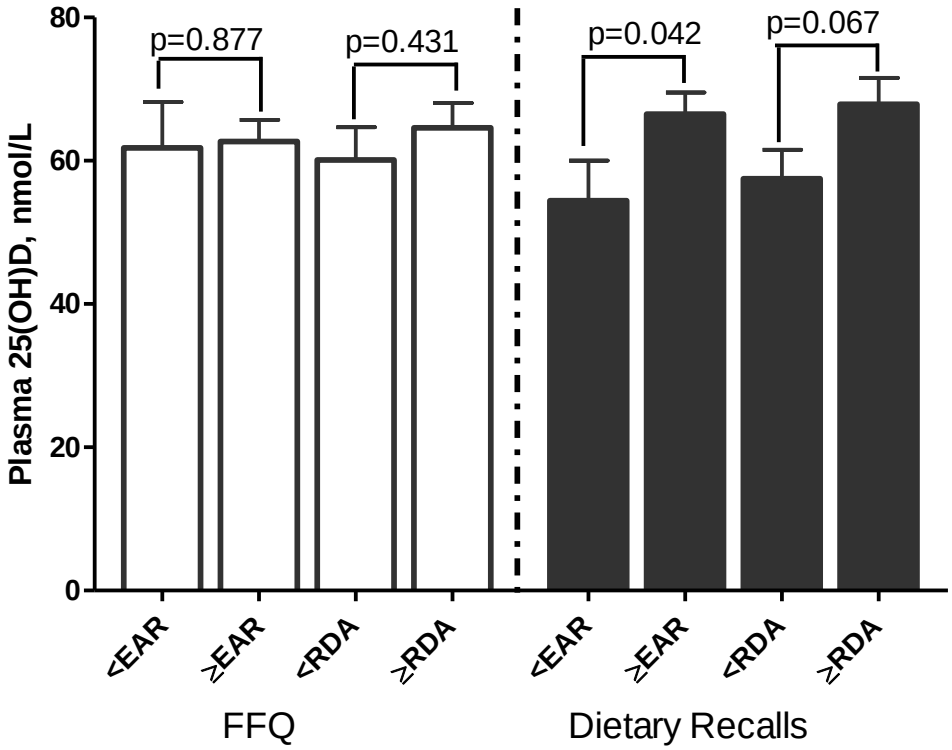

Figure 1. Plasma 25(OH)D concentration of lactating women in each vitamin D intake categorized as < or ≥ EAR and RDA as defined by the IOM [192]. Data presented as mean (SEM). Bars represent vitamin D intake by FFQ and by mean 24 hour recalls. Comparisons were conducted within same dietary methods, separated with dashed line, for EAR and RDA.

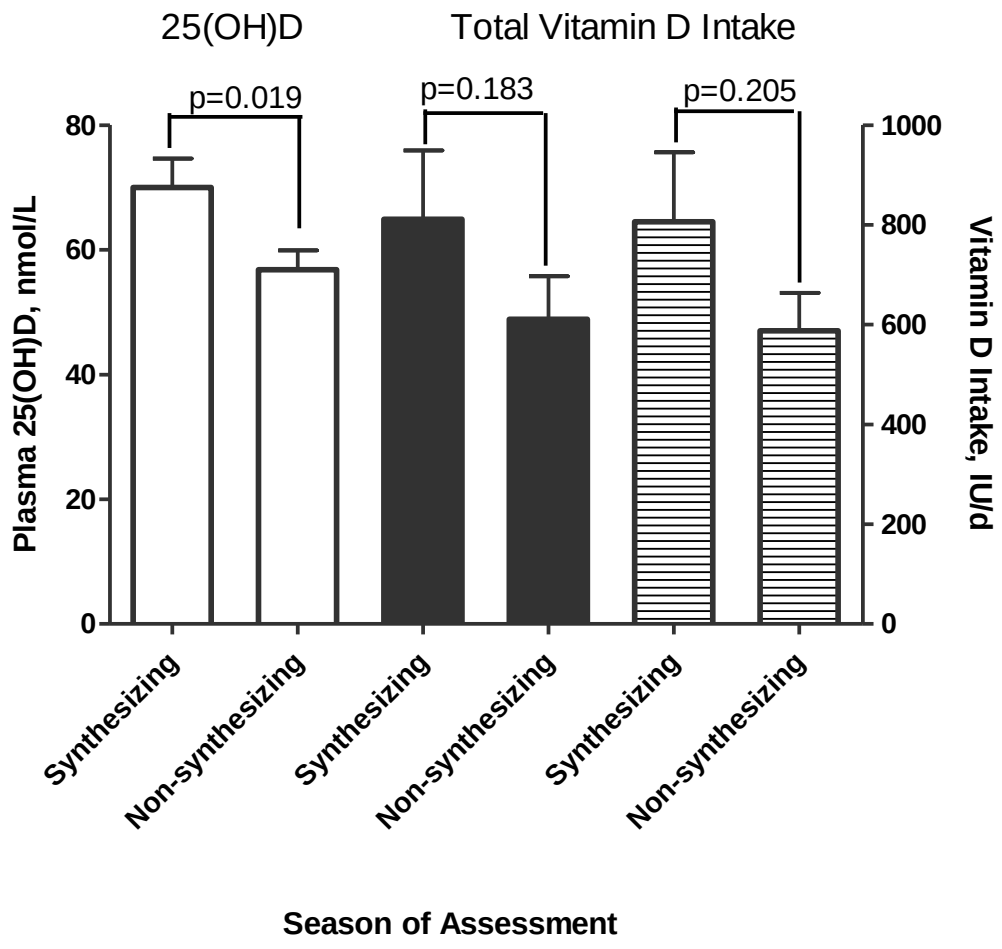

Figure 2. Maternal vitamin D status and total vitamin D intake in the synthesizing (April 1 to October 31) and non-synthesizing period (November 1 to March 31) estimated by FFQ and mean 24 hour recalls. Data presented as mean (SEM). Bars represent plasma 25(OH)D ( ), total vitamin D intake by FFQ ( ), and by 24 hour recalls ( ).

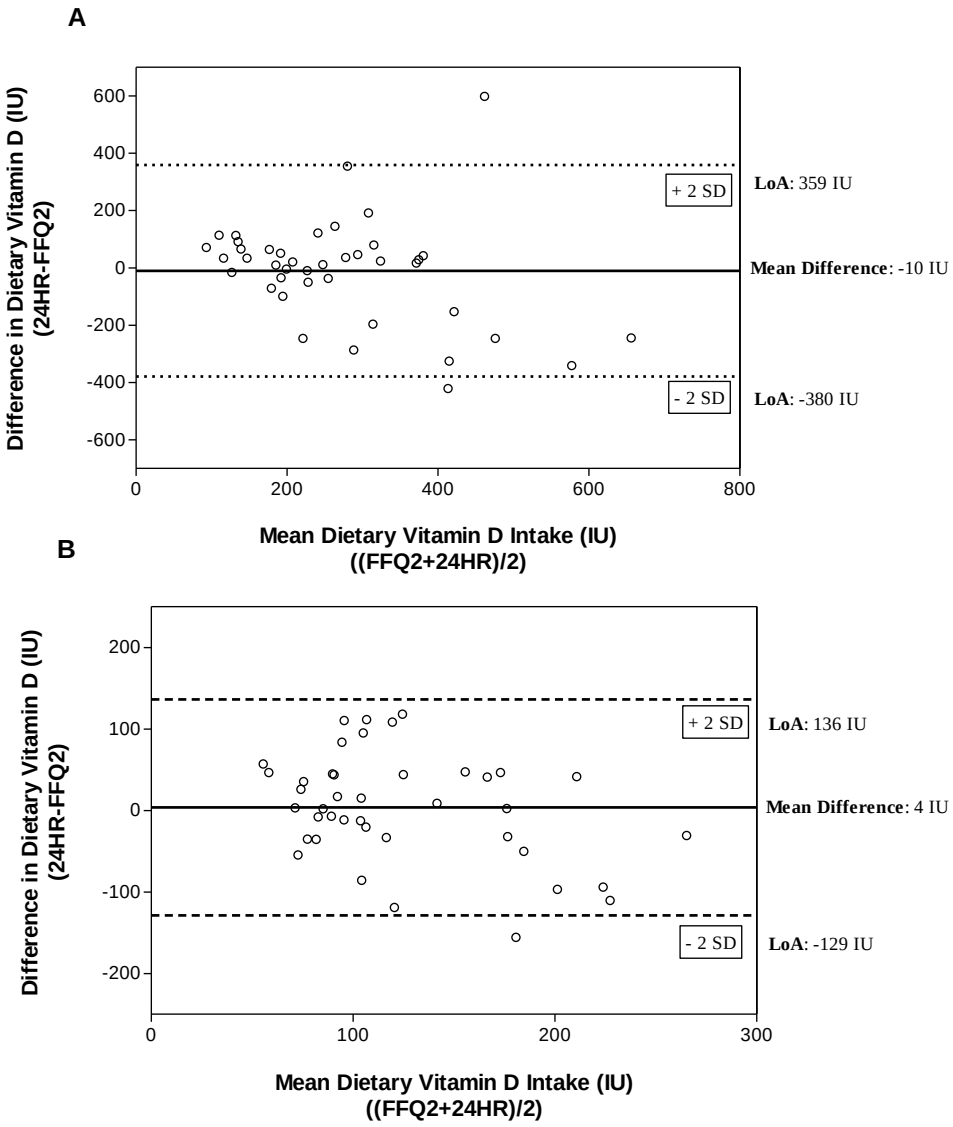

Figure 3. Bland-Altman plot showing agreement between FFQ and mean 24 hour recalls for absolute dietary vitamin D intakes (A) and energy-adjusted dietary vitamin D intakes (B). Solid line is the mean difference (A: mean difference -10 IU/d; B: mean difference 4 IU/d); dashed lines are plus or minus 2 SD (95% CI of mean for A: -68 to 48 IU/d; B: -17 to 25 IU/d); LoA is the limit of agreement (LoA for A: -380 to 359 IU/d; B: -129 to 136 IU/d). Negative mean difference indicates overall overestimation by FFQ and positive mean difference indicates overall underestimation by FFQ.

14. EXTENDED DISCUSSION

14.1. Objectives and Summary of Key Findings

The main objectives of this thesis were to determine the vitamin D status of lactating women and their infants at 1 and 4 months postpartum and changes since 1 month, identify key predictors of vitamin D status at 4 months and changes in status in lactating mother and their infants, and to validate a FFQ, adapted for Canadians, to assess vitamin D intake among lactating women. The majority of lactating women and their infants achieved the plasma 25(OH)D cut-off concentration of 50 nmol/L [30] but not the 75 nmol/L cut-off [13]. From 1 to 4 months, there was a decrease in maternal vitamin D status ($p<0.001$) and an increase in infant vitamin D status ($p=0.032$). At both visits, maternal and infant vitamin D status was higher than published studies reporting vitamin D deficiency in this population [5, 9, 18, 25, 43, 122]. Total vitamin D intake, the number of weeks spent in the synthesizing period, and % change in TBF were predictors of maternal vitamin D status at 4 months postpartum. The number of weeks spent in the synthesizing period, % change in weight, and baseline vitamin D status were predictors of maternal Δ25(OH)D concentration. Travel to a latitude <37 ^{o}N, constitutive skin pigmentation, and maternal vitamin D status were predictors of infant vitamin D status at 4 months. Baseline 25(OH)D concentration, travel to a latitude <37 ^{o}N, and ethnicity were predictors of infant Δ25(OH)D concentration since 1 month of age. Lastly, the Willett FFQ was a valid tool to assess vitamin D intake among lactating women. Despite the comprehensive assessment of potential predictors of vitamin D status in lactating women and their infants, the present study had several limitations mainly due to limited sample size. Nonetheless, this study can provide a framework for future studies.

14.2. Study Design and Assessment Tools

14.2.1. Sample Size and Population

Sample size estimation is important to reduce the likelihood that observed associations are due to chance. Small sample sizes may not have the power to detect a real effect and large sample sizes may detect a small effect as significant [291]. An objective of the study was to examine Δ25(OH)D concentration, a

continuous variable, in mother and infants; the *t*-test with paired measurements was used. Being a part of a RCT, n=44 mother-infant pairs was previously determined. In infants, with β=0.20, α (two-tailed)=0.05, n=44, and E/S=0.60, the SD of infant Δ25(OH)D concentration from 1 to 3 months of age was 25.7 nmol/L based on unpublished infant data from our group. Thus, expected effect size (E) was 15.4 nmol/L for Δ25(OH)D concentration. In mothers, β=0.20, α (two-tailed)=0.05, n=45, E/S=0.60, with E=8.3 nmol/L [35] for maternal Δ25(OH)D concentration from 1 to 4 months postpartum, the SD would be 13.8 nmol/L to detect change in status. The SD of maternal 25(OH)D concentration was double the SD of infant 25(OH)D concentration. The larger SD could be attributed to a small sample size with increased variability in maternal 25(OH)D concentration. Using the same formula but with β=0.10, α (two-tailed)=0.05, and E/S=0.50, we would need 86 mother-infant pairs, approximately twofold increase from our sample size. In the present study 5-6 predictor variables/regression equation achieved a power=0.80 and R^2=0.50 [248, 249]. The sample size in this study was unable to comprehensively explore all potential predictors of maternal and infant vitamin D status. Lastly, maternal and infant characteristics in this study were not representative of the diverse socio-demographic and ethnic Canadian population.

14.2.2. Assessment of Dietary Intake

A thorough analysis of different dietary assessment methods can be found under **section 9.9.2**. While the 24 hour recall can capture dietary vitamin D intake, the present study may have benefited from increasing the number of recalls to reflect usual intake or administering recalls in accordance with the half-life of 25(OH)D. There is no data describing the number of days needed to capture vitamin D intake in lactating women. The following equation can be applied to estimate the number of days necessary to estimate usual vitamin D intake, $n=(Z_\alpha CV_W/D_0)^2$ where n=number of 24 hour recall days needed per person, Z_α=normal deviate (% of times the measured values should be within a specified limit), $CV_W=(s\sqrt{x} * 100\%)$ is within-person coefficient of variation obtained from analysis of repeated days of dietary intake where s=within-person standard deviation and x=mean, and D_0=the specified limit (as as percentage of

long term true intake) [208]. Without data for CV_W for vitamin D, CV_w for vitamin A [208] and from the present study were used. The CV_W for vitamin A=105%, Z_α=1.645 (90%), and D_0=40% to estimate an individual's vitamin D intake to within 40% of their true mean and 90% of the time, nineteen days would be necessary. However, the number of days is lower for vitamin D intake because sources of vitamin A are scarcer. From the present study, CV_w=52.9% for vitamin D, Z_α=1.645 (90%), and D_0=40% or 25%, five or twelve, respectively, days are necessary to estimate usual vitamin D intake [208]. Administering a large number of 24 hour recalls is impractical in epidemiological studies. Vitamin D has a higher within-person variability and intake is contributed by relatively few food sources, a FFQ designed specifically to assess vitamin D intake is likely to provide the most accurate estimates of usual individual intake [208]. Lastly, examination of vitamin D food sources after nutrient analyses to ensure vitamin D content of foods reflect fortification practices in the market is a necessary step to prevent underestimation of dietary vitamin D intake.

A major strength of the study is administration of the FFQ in conjunction with dietary recalls to assess vitamin D intake. Future studies could also validate the FFQ to assess nutrient and food sources [269, 271, 288, 289] which may be of interest in epidemiological studies. Continued modification and validation of FFQ to reflect vitamin D fortified products in the market and separating food items that contain high levels of vitamin D would provide an accurate reflection of vitamin D intake. For example, separate the food item "dark meat fish" into farmed salmon, Atlantic salmon, canned salmon, mackerel, and etc. Validation of FFQ with 25(OH)D concentration during the non-synthesizing period would allow us to capture dietary vitamin D intake and possible mobilization of fat stores. This could also be administered year-round stratified by season in a large cohort.

14.2.3. Assessment of Adiposity

Additional information regarding techniques to measure adiposity can be found in **section 9.9.5**. The relationship between weight, adiposity and their changes were explored in the present study. The results suggested a threshold effect between increased 25(OH)D concentration and loss in adiposity. However,

due to the limited, homogenous sample size, we may not have the power or variance to detect the inverse relationship between adiposity and 25(OH)D concentration. Future studies need to verify our results in larger, representative populations and explore the mechanisms in which adiposity and changes in adiposity affects 25(OH)D concentration at the cellular level. The rate of re-entry is not yet understood but the release of vitamin D from fat depots is slow and proportional to the vitamin D in the adipose tissue [65, 179] and should have minimal influence to significantly elevate 25(OH)D concentration. However, there is an significant increase in 25(OH)D concentration with significant loss in weight and/or adiposity [63, 64, 180]. Low 25(OH)D concentration may be a consequence of increased PTH enhancing calcium uptake in the adipocyte which stimulates lipogenesis, inhibits lipolysis, expands adipocyte stores, and promotes weight gain [292, 293]. PTH has a negative effect on weight control [293]. Little is known about the mechanisms in which vitamin D in sequestered in human fat tissue (subcutaneous, visceral) and released into the circulation from fat tissues, especially in those undergoing rapid changes weight and TBF. Imaging techniques such as DXA or MRI are safe and can further examine the effects of fat and lean body mass on vitamin D status [233]. During lactation, there are changes in fat distribution and mobilization [158, 159, 294]; the relationship of these changes on vitamin D status remains to be explored.

14.2.4. Assessment of Sun Exposure and Skin Pigmentation

A thorough analysis of different techniques to measure skin pigmentation is described in **section 9.9.3**. In epidemiological studies, the reflectance spectrophotometer is affordable, quick, and portable equipment that requires minimal training in addition to measurement of skin colour changes due to UV radiation [224]. Reflectance spectrophotometer is being increasingly used to categorize skin pigmentation and assess UVB exposure [53, 54, 57, 58]. It is important to distinguish between skin pigmentation and photosensitive skintype. Skin pigmentation is determined by the amount of melanin in the skin [295] by classification as light or dark skinned. Photosensitive skin type is determined by the skin's potential for tanning [152, 221] by classification of Fitzpatrick scale

[221]. The spectrophotometer can assess UVB exposure by comparing differences in exposed and non-exposed body sites and changes in exposed body sites with L* and b* values and ^{o}ITA. However, the spectrophotometer is limited in capturing sun exposure based on photosensitive skintype in those who burn but do not tan and those who never tan [221]. The spectrophotometer cannot be the sole measurement for skin pigmentation and sun exposure. Photosensitive skintype may explain inverse results between skin pigmentation and vitamin D status. Very fair skinned individuals (skintype I and II) who have sun sensitive skin tend to have low vitamin D status due to sun avoidance to prevent sunburns [138, 152]. Dark skinned individuals may also, limit sun exposure to prevent deepening their skin colour [32, 264]. Others have observed that those with greater ability to tan (skin type III-IV) tend to spend more time in direct sunlight [148, 149, 152] which explains higher vitamin D status with increasing skintype. Infant skin <3 months of age may not be a good indicator of skin colour due to increased skin turnover [229] and increased skin thickness [230]. This was confirmed in the present study where there was an increase in infants classified as very fair to fair constitutive skin pigmentation from 1 to 4 months postpartum. Further analysis of infants who enrolled and completed the study during the non-synthesizing period without any sun exposure, the proportion of infants with very fair or fair constitutive skin increased. Future studies should use spectrophotometer in conjunction with a comprehensive sun exposure questionnaire to accurately capture UVB exposure.

14.2.5. Blood Sample Analysis

Information on the various assays and limitations in measuring 25(OH)D concentration can be found under **section 9.9.1**. C-3 epimers have been found in significant concentrations in infants [98, 100] and detectable concentrations in adults [100]. Since the LIAISON® used in the present study does not detect C-3 epimers, infant vitamin D status may be overestimated. From the Bland-Altman plot, adult capillary samples is ~19 nmol/L higher compared to venous samples (LoA between capillary and venous samples were -5.6 to 43 nmol/L) [205]. Whether this relationship is applicable to infant capillary samples has not been examined. The LIAISON® has 100% cross-reactivity for 25(OH)D_2 and

25(OH)D_3 but only provides a sum of the metabolites. LC-MS/MS is considered the gold standard for assessing vitamin D status and can determine the contribution of 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D to total 25(OH)D concentration [296]. This is important with food and supplemental sources contain vitamin D_2 and in studies involving infants <1 years of age.

14.3. Relevance to the Field of Research

Identifying key predictors of vitamin D status at 4 months and changes in status in lactating mothers and their infants allow health professionals to develop strategic interventions to prevent declines and improve status during this transitional period. Baseline vitamin D status predicted a decrease in both maternal and infant Δ25(OH)D concentration suggesting that those with higher baseline vitamin D status, current vitamin D intake was insufficient to maintain 25(OH)D concentration. The association between maternal and infant vitamin D status suggest that improved maternal vitamin D status may be beneficial for both mothers and infants. Furthermore, mothers participating in a randomized clinical trial supplementing their breastfed infants with vitamin D may likely be supplementing themselves and mobilizing their fat stores. Since increased sun exposure is an impractical recommendation due to concerns of skin damage, it is important to reconsider vitamin D recommendations in this population. Although the regression equations may not be transferable to other study groups, the identified predictors provide insight regarding the variables that affect vitamin D status. Therefore, the results from this study could provide a framework to prevent maternal and infant vitamin D deficiency reported worldwide; such as new RCT.

Prenatal supplementation is an important source during lactation [37]. In the present study, supplements contributed ~59% of total vitamin D intake. Total vitamin D was not correlated with plasma 25(OH)D concentration however, vitamin D intake categorized as <EAR and ≥EAR was a significant predictor of maternal 25(OH)D at 4 months. This supports the IOM recommendation for EAR [192] and suggests a threshold effect between vitamin D intake and 25(OH)D concentration. At 4 months of age, prenatal supplement use was associated with higher infant 25(OH)D concentration. Therefore, healthcare providers are

valuable educational resources and could endorse continued intake of postnatal supplements in this population.

From the results of the present study, modifiable predictors of increased vitamin D intake and loss of adiposity to prevent gestational weight retention predicted an increase in vitamin D status which is congruent with adapting a healthy lifestyle. The postpartum period provides an opportunity to promote behavioural changes that may be beneficial to both mothers and infants. These results could strengthen and promote healthy lifestyle interventions to improve vitamin D status in this population. It has been suggested that individuals with better vitamin D status may be more responsive to weight loss [293].

14.4. Future Investigation

As related to verification of the observed associations and addressing the shortcomings of the present study, the present study provides a foundation for 1) recruiting a large, representative sample of lactating women and their infants to reflect the diverse Canadian population for generalizability of the results and 2) trigger future research to understand the mechanisms of the potential predictors of vitamin D status. There is limited information regarding the mechanisms in which maternal vitamin D store transfer to infants pre- and post-natally and the storage, distribution, and release of vitamin D from body tissues during lactating and early infancy.

It would be important to examine changes in maternal and infant 25(OH)D concentrations at between 4.5 to 6 months postpartum to examine if there are further declines, increases, or plateaus in DBP and vitamin D status [123, 133, 251]. Steady state tends to be achieved ~90 days after routine supplementation [24, 34, 35, 129, 134]. In the present study, vitamin D status was examined at 1 and 4 months postpartum, it is unclear whether these mothers and infants reached a plateau in vitamin D status. We cannot confirm whether maternal or infant 25(OH)D concentration will remain unchanged with continued supplementation behavior. Several studies indicated at 4.5 and 6 months postpartum, vitamin D status or DBP were low, continued to decline, or steady state was achieved

beyond 90 days [24, 34, 35, 123, 133, 134, 251]. This would provide insight if interventions are necessary to prevent further declines in vitamin D status.

Another improvement is to validate, modify, and re-examine the FFQ based on new food sources of vitamin D. Future work should focus on addressing the missing vitamin D values and include values to reflect vitamin D fortified products in the consumer market. Future national surveys need to examine vitamin D intake (dietary and supplemental) and status in lactating women and their infants. Vitamin D deficiency in this population has long term health implications. In the present study, maternal total vitamin D intake did not adhere to the vitamin D recommendations by CPS of 2000 IU/d [13] or by Health Canada for milk [27] and fish intake [28], and postnatal supplement [29] for lactating women equates to ~1400 IU/d. The IOM increased vitamin D recommendations from RDA of 600 IU from an AI of 200 IU [192]. Although, there have been changes in vitamin D recommendations in this population, vitamin D intake has not changed. With current cross-sectional studies in lactating women in Canada [197], vitamin D intake and status are below recommendations and strategies are necessary to improve vitamin D status.

The relationship between 25(OH)D concentration with skin pigmentation, photosensitive skintype, or sun behavior require further examination. Although epidermal hyperpigmentation [256, 257] regresses post-delivery due to declines in estrogen [258], whether these changes are immediate, gradual or in the upper inner arm remain to be explored [259]. Due to the persistent racial differences in 25(OH)D concentration throughout the year, dark pigmented individuals may store less previtamin D, vitamin D, or 25(OH)D in body tissues beyond the synthesizing period [151]. There is limited information regarding vitamin D storage, distribution, and release from fat and muscle tissues into the circulation among diverse ethnic and skin pigmentation groupings. Black individuals have a relatively higher frequency of the GC*1F allele, which has the highest affinity for 25(OH)D_3 compared to the two genetic forms of DBP (CD*1S and GC*2) [88]. Black individuals also, have higher BMD and fewer bone fractures than white women [36]. The relationship between skin pigmentation and vitamin D status is

complex. Genetics, diet, and cultural variations may explain differences in 25(OH)D concentration across ethnic groups [138, 150, 152, 266] and warrants further exploration.

In the present study, baseline vitamin D status was a predictor of change in maternal and infant vitamin D status. Of the available longitudinal studies, there is an inverse relationship between increase in 25(OH)D concentration (Δ25(OH)D) and baseline 25(OH)D concentration ($r=0.56$, $p<0.001$) [264]. Those with low baseline 25(OH)D tend to have greater increase in 25(OH)D after UVB exposure [263, 264] or supplementation [175]. Black women who took ≥400 IU/d of vitamin D supplements had significantly higher 25(OH)D concentration than those who did not (60.5 ± 4.1 vs. 42.9 ± 1.2 nmol/L, respectively) [36]. On the other hand, in white women with high vitamin D status, there were no significant differences between those who took ≥400 IU/d of vitamin D than those who did not (83.7 ± 4.2 vs. 81.2 ± 1.6 nmol/L, respectively) [36]. Therefore, there may be an attenuated response to supplementation in those with higher baseline vitamin D status [297]. Baseline 25(OH)D concentration is important to capture when examining changes in vitamin D status.

In studies involving women of reproductive age, use of oral contraceptive pills are important to document. OCP users have significantly higher 25(OH)D concentration than non-OCP users [36, 107, 141, 175, 298, 299], regardless of skin pigmentation (black: weighted mean ± standard error of the mean: 50.2 ± 1.9 vs. 42.9 ± 1.1, respectively; $p<0.05$ and white: 102.2 ± 3.7 vs. 77.4 ± 1.3, respectively; $p<0.05$) [36]. The use of OCP is a predictor of Δ25(OH)D concentration over a 1-year period [141, 175]. The postulated mechanism is likely due to increasing circulating DBP as a result of high estrogen levels [300]. Increased DBP alters the relative proportion of free and protein bound 25(OH)D [300]. Increased DBP-25(OH)D complex transported to liver for hydroxylation results in higher 25(OH)D concentration [66, 175]. Therefore, hormonal contraceptives may be a strong confounding variable in vitamin D studies and should be considered when interpreting 25(OH)D values.

Future studies interested in examining the relationship between adiposity and vitamin D status specifically subcutaneous and visceral fat, loss of adiposity, distribution of fat stores, or lean muscle mass should consider the use of MRI (mothers only) or DXA. Subcutaneous and visceral fat has an inverse relationship with 25(OH)D concentration in young women [173]. Distribution of fat stores has been associated with vitamin D status in obese adolescents [62]. Fat tends to be mobilized from the trunk and thighs [158, 159] but remains unexplored. Lean body mass has been found to be associated with vitamin D status in men [267]. These measurements of adiposity remain to be explored in adults and among lactating women. In lactating women, adiposity loss due to increased exercise, mobilization of fat stores, and/or decreased caloric intake require documentation to ensure energy and protein requirements are met to support lactation [167]. Although, there is limited and inconclusive evidence regarding infant acceptance of post-exercise milk due to lactic acid which produces a sour taste [301, 302], in weight loss exercise interventions for lactating women, duration and intensity of exercise, breast milk production and quality, and infant growth parameters should be documented.

Therefore, the results of the present study provide a framework for future studies in lactating women and their infants. There remains much to be explored through improvement in assessment methods, higher level research designs such as randomized clinical trials, and understanding relationships between 25(OH)D concentration and predictors of status at the molecular level. These considerations in future studies will move research in lactating women and their infants forward.

15. CONCLUSION

The global aim of this study was to determine the vitamin D status of lactating women and their infants and the predictors of their status at 4 months and change in vitamin D status from 1 to 4 months postpartum to contribute to the limited information in this population. The results suggest that the Canadian adapted Willett FFQ may be validated in a small sample of lactating women but its validity to estimated vitamin D intake must be verified in a larger sample to embark upon epidemiological studies. Our study explored potential predictors of vitamin D status and change in their status in a small, homogenous population. Lastly, from the results of this study, suggestions to improve the ability to explain vitamin D status include administering 24 hour recalls in accordance with the half-life of 25(OH)D, accurately documenting supplement use, using a thorough sun exposure questionnaire specifically on the last month of exposure, measuring skin pigmentation by reflectance spectrophotometer and categorizing skintype by Fitzpatrick scale [221] in mothers but not in infants <3 months of age, and assessing body composition by DXA rather than BIA so that adipose (re)distribution can be examined. The results from this study provide a foundation for larger studies in this population and for future research to examine the mechanisms in which these predictors affect vitamin D status.

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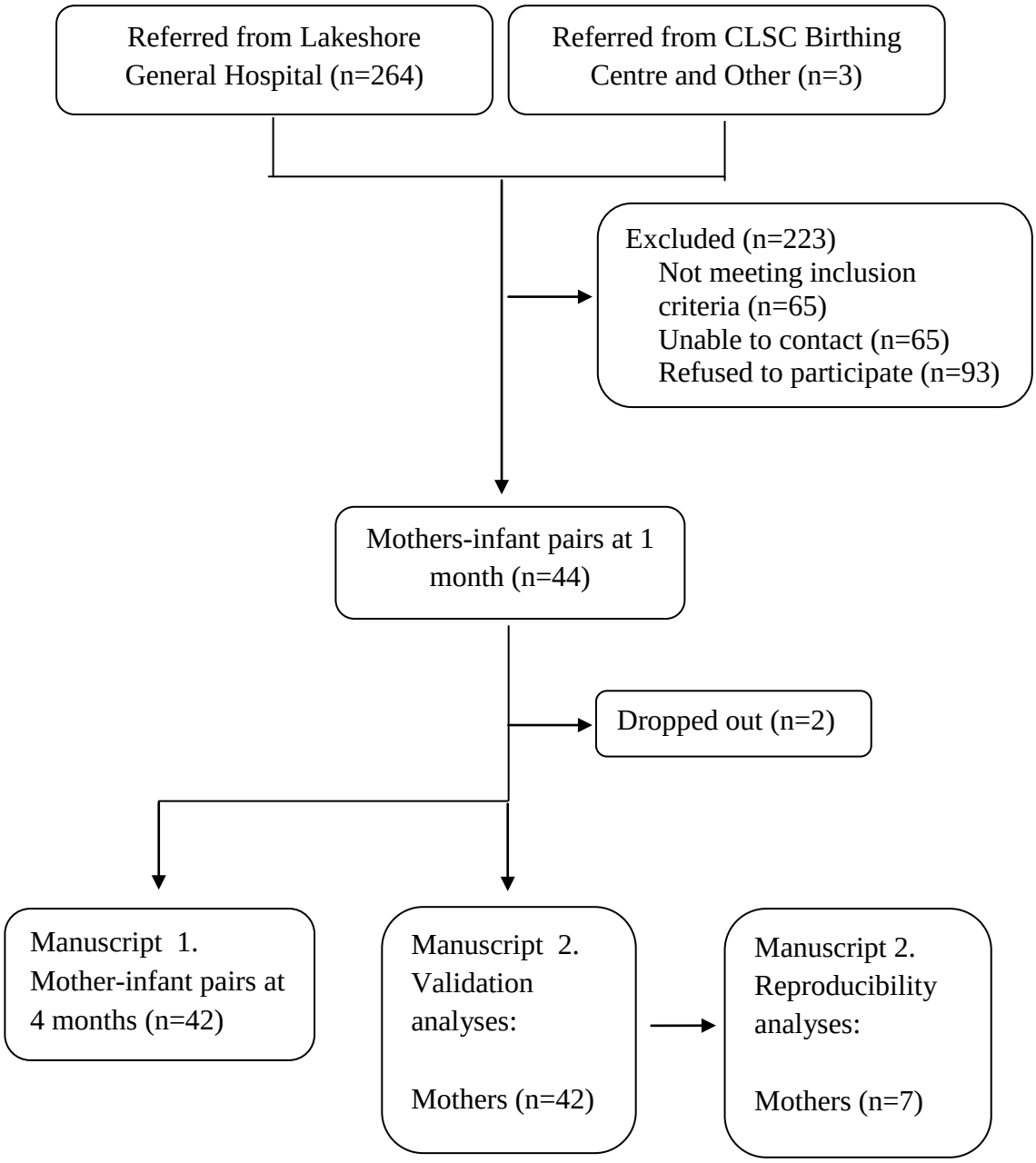
17. APPENDICES

17.1. Enrollment Flow Chart



17.3 Comparison of Underreporters and Others by FFQ with EI:BMR as a Criteria for Underreporting at the Follow-up Visit

Comparison of Under-reporters and Others by FFQ with EI:BMR (based on weight and height) <1.14 as a Criteria for Underreporting at Follow-up Visit (n=42)

Characteristics/ Nutrients	Crude intake			Energy adjusted[1]		
	Underreporters (n=10)	Others (n=32)	p-value	Underreporters (n=10)	Others (n=32)	p-value
Weight (kg)	69.2 ± 11.0	66.3 ± 11.9	0.503			
Δ Weight (%)	-4.5 ± 4.0	-1.1 ± 4.1	0.026			
BMI (kg/m^2)	25.0 ± 4.1	25.0 ± 3.9	0.978			
Body fat (%)	34.2 ± 6.1	31.9 ± 7.4	0.381			
Δ Body fat (%)	-6.3 ± 6.6	-2.1 ± 7.6	0.122			
25(OH)D (nmol/L)	74.7 ± 16.6	58.6 ± 17.3	0.014			
Energy (kcal)	1352 ± 194	2503 ± 753	<0.001§			
Carbohydrates (g)	159 ± 26	324 ± 115	<0.001§	118 ± 10	129 ± 17	0.055
Protein (g)	60 ± 8	104 ± 32	<0.001	45 ± 9	42 ± 9	0.318
Fat (g)	55 ± 13	92 ± 34	0.002§	40 ± 4	37 ± 7	0.054
Calcium (mg)						
Dietary	712 ± 161	1161 ± 486	0.001§	533 ± 131	466 ± 130	0.160
Supplemental	241 ± 144	193 ± 205	0.337§			
Total[2]	953 ± 182	1354 ± 473	0.006§	774 ± 167	659 ± 205	0.115
Vitamin D (IU)						
Dietary	205 ± 58	290 ± 141	0.084§	154 ± 46	118 ± 48	0.041
Supplemental	578 ± 752	360 ± 400	0.560§			
Total[2]	783 ± 755	650 ± 379	0.757§	732 ± 754	477 ± 393	0.295
Iron (mg)	9 ± 1	18 ± 6	<0.001§	7 ± 1	7 ± 2	0.555
Folate (µg)	254 ± 64	424 ± 149	0.003§	188 ± 41	170 ± 28	0.111
Fibre (g)	14 ± 4	29 ± 2	<0.001	10 ± 3	12 ± 3	0.162

25(OH)D: 25-hydroxyvitamin D; FFQ: food frequency questionnaire; EI: energy intake: BMR: basal metabolic rate

Data presented as mean ± SD; Student's *t*-test or §Mann-Whitney test between underreporters and non-underreporters

[1]Nutrient density calculated as per 1000 kcal; [2]Total vitamin D/calcium = dietary and supplemental

Comparison of Under-reporters and Others by FFQ with EI:BMR (based on weight and height) <1.00 as a Criteria for Underreporting at Follow-up Visit (n=42)

Characteristics/	**Crude intake**			**Energy adjusted**[1]		
Nutrients	**Underreporters (n=7)**	**Others (n=35)**	**p-value**	**Underreporters (n=7)**	**Others (n=35)**	**p-value**
Weight (kg)	71.8 ± 8.9	66.0 ± 12.0	0.236			
Δ Weight (%)	-4.8 ± 4.1	-1.4 ± 4.1	0.051			
BMI (kg/m^2)	25.0 ± 3.6	25.0 ± 4.0	0.981			
Body fat (%)	35.5 ± 5.6	31.8 ± 7.3	0.217			
Δ Body fat (%)	-4.9 ± 6.6	-2.7 ± 7.7	0.484			
25(OH)D (nmol/L)	76.2 ± 17.5	59.7 ± 17.4	0.027			
Energy (kcal)	1284 ± 120	2418 ± 775	<0.001§			
Carbohydrates (g)	157 ± 24	310 ± 119	<0.001§	122 ± 9	127 ± 17	0.409
Protein (g)	58 ± 78	100 ± 33	<0.001	46 ± 9	42 ± 9	0.305
Fat (g)	49 ± 6	90 ± 33	0.002§	38 ± 2	37 ± 7	0.495
Calcium (mg)						
Dietary	655 ± 132	1134 ± 475	0.001§	518 ± 137	474 ± 132	0.433
Supplemental	237 ± 174	198 ± 196	0.560§			
Total[2]	891 ± 175	1332 ± 458	0.005§	754 ± 192	673 ± 202	0.331
Vitamin D (IU)						
Dietary	188 ± 36	286 ± 137	0.071§	149 ± 36	122 ± 51	0.189
Supplemental	533 ± 842	387 ± 421	0.986§			
Total[2]	721 ± 848	674 ± 398	0.280§	682 ± 853	509 ± 415	0.840§
Iron (mg)	9 ± 1	17 ± 7	0.009§	7 ± 1	7 ± 2	0.389§
Folate (µg)	257 ± 48	409 ± 153	0.004§	201 ± 38	169 ± 29	0.015
Fibre (g)	15 ± 4	28 ± 11	<0.001	12 ± 3	11 ± 3	0.833

25(OH)D: 25-hydroxyvitamin D; FFQ: food frequency questionnaire; EI: energy intake: BMR: basal metabolic rate
Data presented as mean ± SD; Student's *t*-test or §Mann-Whitney test between underreporters and non-underreporters
[1]Nutrient density calculated as per 1000 kcal; [2]Total Vitamin D/Calcium = dietary and supplemental

Comparison of Under-reporters and Others by FFQ with EI:BMR (based on weight and height) <1.42 as a Criteria for Underreporting at Follow-up Visit (n=42)

Characteristics/ Nutrients	Crude intake			Energy adjusted[1]		
	Underreporters (n=20)	Others (n=22)	p-value	Underreporters (n=20)	Others (n=22)	p-value
Weight (kg)	69.9 ± 11.7	64.3 ± 11.3	0.123			
Δ Weight (%)	-2.9 ± 4.1	-1.0 ± 4.3	0.163			
BMI (kg/m^2)	25.6 ± 4.1	24.5 ± 3.7	0.356			
Body fat (%)	34.3 ± 6.2	30.8 ± 7.6	0.107			
Δ Body fat (%)	-3.7 ± 7.4	-2.5 ± 7.7	0.605			
25(OH)D (nmol/L)	68.0 ± 19.4	57.4 ± 16.1	0.058			
Energy (kcal)	1580 ± 303	2819 ± 695	<0.001			
Carbohydrates (g)	196 ± 49	364 ± 116	<0.001§	124 ± 14	129 ± 18	0.334
Protein (g)	71 ± 18	114 ± 32	<0.001	45 ± 8	41 ± 9	0.114
Fat (g)	60 ± 14	105 ± 32	<0.001§	38 ± 5	37 ± 7	0.777
Calcium (mg)						
Dietary	801 ± 241	1284 ± 514	0.003§	513 ± 136	453 ± 124	0.139
Supplemental	234 ± 198	178 ± 185	0.377			
Total[2]	1035 ± 212	1462 ± 520	0.001	747 ± 174	631 ± 211	0.060
Vitamin D (IU)						
Dietary	222 ± 87	314 ± 149	0.023§	142 ± 52	111 ± 43	0.041
Supplemental	565 ± 654	272 ± 258	0.231§			
Total[2]	787 ± 646	586 ± 256	0.753§	707 ± 647	384 ± 253	0.068§
Iron (mg)	11 ± 4	20 ± 6	<0.001§	7 ± 2	7 ± 2	0.736
Folate (µg)	280 ± 57	479 ± 149	<0.001	179 ± 32	170 ± 33	0.391
Fibre (g)	18 ± 8	32 ± 9	<0.001§	11 ± 3	12 ± 3	0.814

25(OH)D: 25-hydroxyvitamin D; FFQ: food frequency questionnaire; EI: energy intake: BMR: basal metabolic rate
Data presented as mean ± SD; Student's *t*-test or §Mann-Whitney test between underreporters and non-underreporters
[1]Nutrient density calculated as per 1000 kcal; [2]Total vitamin D/calcium = dietary and supplemental