The effects of gestational weight gain on vitamin D status of the neonate

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ABSTRACT

Introduction: Vitamin D is needed for healthy growth and development throughout life, with particular functions in musculoskeletal health and potentially metabolic programming in infancy. Vitamin D deficiency is common in pregnant women and subsequently, their offspring can be born with low status. The current Recommended Dietary Allowance (RDA) for vitamin D intake during pregnancy (600 IU/d) is likely not ideal to support the needs of both mother and fetus. Growing evidence also suggests that vitamin D requirements may be weight dependent, due to it being sequestered in adipose tissues as it is fat soluble, and/or due to volume dilution effects in larger individuals. These mechanisms can be applicable to pregnant women, as the largest constituents of gestational weight gain (GWG) are increasing fat stores and fluid accumulations. Associations between GWG and vitamin D status of neonates and their mothers has not been extensively studied.

Objectives: Primary: To test for relationships between maternal GWG and vitamin D status of their newborn infant. It is hypothesized that women who gain more weight during pregnancy than what is recommended by Health Canada, will have neonates born with lower vitamin D stores. Secondary: To explore relationships between GWG and maternal vitamin D status, pre-gravid body mass index (BMI) and vitamin D status of the neonate and mother.

Methodology: Healthy mother-infant pairs (n=59) from the greater Montreal area were recruited 24-36 hours post-partum (clinicaltrials.gov: NCT02563015). A demographic survey was administered to participants reflecting on sun exposure, physical activity, socioeconomic status, ethnicity, education and medication/supplement use. Dietary intake of vitamin D was assessed using a validated food frequency questionnaire (FFQ) to reflect pregnancy. Blood

samples were collected from mothers and their infants to assess serum 25-hydroxyvitamin D [25(OH)D] concentrations using a chemiluminescent assay, Liaison auto-analyzer (Diasorin Inc.). Infant weight, length and head circumference at birth, as well as maternal height, pre-gravid weight, and weight at birth were obtained from medical charts. Data were analyzed in three groups: mothers who had inadequate (n=17), adequate (n=18) or excessive (n=24) GWG as per Health Canada's recommendations. A mixed model ANOVA and a linear regression were performed (SAS, v9.3), with GWG and infant sex as fixed effects. Random effects included maternal age, parity, ethnicity, diet, maternal vitamin D status, education, income, infant gestational age and birth weight-for-age z-score. For the three GWG categories, $p<0.05$ after adjustment for multiple comparisons using Scheffe's test was considered statistically significant. Data are mean $\pm$ standard deviation unless otherwise noted.

Results: Maternal pre-gravid body mass index was 24.7 ± 5.1 kg/m^2, infant birth weight-for-age Z-score was 0.2 ± 0.8 and 54% were male. Infant serum 25(OH)D concentration was 40.9 ± 18.9 nmol/L, with 29% <30 nmol/L and 42% <50 nmol/L; status did not differ between sexes. Maternal serum 25(OH)D concentration was 62.7 ± 25.8 nmol/L with 7% <30 nmol/L and 35% <50 nmol/L. Maternal and infant serum 25(OH)D were significantly related ($r=0.74$, $p<0.0001$). Mean serum 25(OH)D concentrations were significantly lower between GWG groups for infants born to mothers with excessive compared to adequate GWG (ANOVA $p=0.02$). Mothers' concentrations did not differ amongst GWG categories. Serum 25(OH)D concentrations of mothers and their infants were significantly lower for non-white compared to white mothers (ANOVA $p=0.02$ and $p=0.03$, respectively); no differences in infant status by ethnicity were seen between GWG categories. In regression analysis ($R^2=0.57$), neonatal serum 25(OH)D

concentration was 15.2 nmol/L higher when born to white compared to non-white mothers and 4.2 nmol/L higher for each 10 nmol/L increment in maternal serum 25(OH)D. Significant correlations between pre-gravid BMI and serum 25(OH)D concentration were only found in neonates (r=-0.34, p=0.008; maternal model: r=0.24, p=0.06). Further investigation accounting for covariates using a mixed model ANOVA and linear regression showed no differences between BMI categories for mothers or infants.

Conclusion: These results suggest that mothers with excessive GWG are more likely to have infants born with vitamin D insufficiency, independent of being non-white or having lower vitamin D status. Additionally, a large proportion of infants (71%) were vitamin D insufficient at birth despite the fact that more than 60% of our mothers met or exceeded the RDA of 600 IU of vitamin D daily during pregnancy. Further research on the role of GWG on vitamin D status during pregnancy, as well as a revision of dietary recommendations for this population, are warranted.

RÉSUMÉ

Introduction : La vitamine D est essentielle pour la croissance et le développement de l'être humain. Elle est en plus, nécessaire pour la fonction du système musculo-squelettique et la programmation métabolique de l'enfance. Les femmes enceintes on une tendance d'avoir une carence en vitamine D, et en conséquences, leurs nouveau-nés peuvent aussi avoir des niveaux bas. La recommandation d'apport individuel pour la vitamine D pendant la grossesse (600 IU/jour) n'est probablement pas idéale pour supporter les besoins de la mère et du fœtus. Il y a de plus en plus preuve que la vitamine D est dépendante du poids d'un individu, lorsqu'il peut séquestrer dans les tissus adipeux (car il est liposoluble), ou a cause d'un effet de dilution corporelle dans les personnes ayant un surplus de poids. Ces mécanismes peuvent être applicable sur les femmes enceintes, parce ce que les plus grands constituants du gain du poids gestationnel sont : l'augmentation des réserves de gras, et l'accumulation des fluides. Les associations entre le gain du poids gestationnel et les niveaux de vitamine D des nouveau-nés et leurs mères, n'ont pas été étudiés en tant que t'elle.

Objectifs : Principale : Pour examiner les relations entre le gain du poids gestationnel et les niveaux de vitamine D des nouveau-nés. L'hypothèse propose que les femmes qui surpassent le gain du poids gestationnel recommandé par Santé Canada, auront des nouveau-nés nés avec des faibles niveaux de vitamine D. Secondaires : Observer les relations entre : le gain du poids gestationnel et les niveaux de vitamine D des mères ; l'indice de masse corporelle pré-grossesse et les niveaux de vitamine D des nouveau-nés et leurs mères.

Méthodologie : Cinquante-neuf mères en sante, de la région de Montréal et ses environs ont été recrutés (24 à 36 heures postpartum) avec leurs enfants. (clinicaltrials.gov: NCT02563015).

Les parents ont participé à un sondage démographique qui reflète : sur l'exposition a soleil ; l'activité physique ; le statut socioéconomique ; l'ethnicité ; le niveau d'éducation et l'usage des médicaments et suppléments. L'apport alimentaire de la vitamine D pendant la grossesse a été analysé en utilisant un questionnaire de fréquence alimentaire validé pour cette vitamine. Mères et enfants ont fournis des échantillons sanguins pour évaluer les concentrations de sérum 25-hydroxyvitamine D [25(OH)D] par chimiluminescence, Liaison auto-analyseur (Diasorin Inc.). L'information sur la longueur, le poids et la taille crânienne des enfants à la naissance ainsi que la taille, le poids pré-grossesse et le poids à l'accouchement des mères, ont été obtenu par les dossiers médicaux. Les données ont été analysé en trois groupes : les mères ayant un gain de poids gestationnel qui était inadéquat (n=17), adéquat (n=18) ou surpassait (n=24) les recommandations par Santé Canada. Une ANOVA de modèle mixte et une régression linéaire ont été utilisé pour les analyses (SAS, v9.3), avec le gain du poids gestationnel et sexe de l'enfant comme effets fixes. Les effets aléatoires incluent : l'âge de la mère ; la parité ; l'ethnicité ; le régime ; le statut de vitamine D de la mère ; l'éducation ; le revenu ; l'âge gestationnel et poids-pour-l'âge valeurs du z de l'enfant. Pour les trois catégories de gain du poids gestationnel, $p<0.05$ après ajustements pour des comparaisons multiples (test de Scheffé), étaient considéré statistiquement significatif. Les données présentées sont la moyenne $\pm$ écart-type sauf indication contraire.

Résultats : L'indice de masse corporelle pré-grossesse des mères était 24.7 ± 5.1 kg/m^2, poids pour l'âge a la naissance de l'enfant score Z était 0.2 ± 0.8 et 54% était mâle. Les concentrations de sérum 25(OH)D de l'enfant était 40.9 ± 18.9 nmol/L, avec 29% <30 nmol/L et 42% <50 nmol/L; le statut n'était pas différent entre les sexes. Les concentrations de sérum 25(OH)D de

la mère était 62.7 $\pm$ 25.8 nmol/L avec 7% <30 nmol/L et 35% <50 nmol/L. Les concentrations de sérum 25(OH)D de la mère et de ses enfants étaient significativement liés (r=0.74, p<0.0001). La moyenne de sérum 25(OH)D des mères était significativement plus faible entre les groupes d'enfants nés des mères qui ont surpassés les recommandations du gain de poids gestationnel, en comparaisons avec celles qui ont respectés les recommandations (ANOVA p=0.02). Les concentrations de sérum 25(OH)D de la mère n'ont pas été différent entre les trois catégories de gain de poids. Les concentrations de sérum 25(OH)D de la mère et ses enfants étaient significativement plus faibles pour les mères de peau blanche comparativement aux mères à la peau foncée (ANOVA p=0.02 et p=0.03, respectivement). Dans l'analyse de régression (R^2=0.57), Les concentrations de sérum 25(OH)D des nouveau-nés était 15.2 nmol/L plus élevé quand ils étaient nés de mères à la peau blanche, comparer a ceux qui étaient nés de mères à la peau foncée. Aussi, les niveaux de sérum 25(OH)D des nouveau-nés étaient 4.2 nmol/L plus élevés pour chaque incrément de 10 nmol/L en concentration maternelle. Des corrélations significatives entre l'indice de masse corporelle pré-grossesse et les concentrations de sérum 25(OH)D ont été seulement vue dans les nouveau-nés (r=-0.34, p=0.008 ; modèle maternelle : r=0.24, p=0.06). Des enquêtes supplémentaires (ANOVA de modèle mixte et régression linéaire) en considérant d'autres covariables ont trouvé aucune différence entre la catégorie d'indice de masse corporelle pré-grossesse pour les mères ou les enfants.

Conclusion : Ces résultats démontrent que les mères qui surpassent les recommandations du gain de poids gestationnel, sont plus enclin d'avoir des enfants nés avec l'insuffisance de vitamine D, indépendant d'être de peau blanche ou d'avoir un faible statut de vitamine D. De plus, une grande proportion des nouveau-nés (71%) avaient une insuffisance en vitamine D à la

naissance, malgré le fait que plus de 60% des mères ont consommés 600 IU ou plus de vitamine D par jour pendant leur grossesse. Des études additionnelles sur le rôle du gain de poids gestationnel sur le statut de vitamine D pendant la grossesse, ainsi qu'une révision des recommandations des apports pour cette population, sont nécessaires.

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AUTHORS' CONTRIBUTIONS

K. Mullahoo is the primary author of this thesis and participated in many aspects of the work involved in this project. K. Mullahoo, along with the Infant Study team, took part in the logistics, planning and creation of certain study documents (i.e., scripts, pamphlets, templates and standard operating procedures) for recruiting and study visits conducted at the Mary Emily Clinical Nutrition Research Unit (MECNRU). K. Mullahoo actively screened and recruited participants at the Lakeshore General Hospital for the main clinical trial this thesis is based on. This involved obtaining consent, asking demographic questionnaires, obtaining data from medical charts and bringing blood samples back to McGill University for analysis. K. Mullahoo also assisted in follow up recruitment activities such as telephoning participants, sending emails with serum 25(OH)D concentration screening results and study invitations, as well as booking study visit appointments. K. Mullahoo also actively participated in many of the baseline and follow-up study visits for the trial, which involved anthropometric measurements of infants and mothers, dual-energy x-ray absorptiometry (DXA) and its analysis of mothers and infants, bioelectrical impedance analysis (BIA), and asking questionnaires on sun exposure, supplement use and general health. K. Mullahoo assisted with data entry from screening and study visits, along with helping in the revision and data entry of the Food Frequency Questionnaire (FFQ) analysis. All elements of the literature review, manuscript, statistical analyses, discussion, tables and figures in this thesis were researched, analyzed and drafted by K. Mullahoo.

C. Vanstone was responsible for the day-to-day coordination of all study related activities, including helping with the development of many study documents (survey's, consent forms,

questionnaires, pamphlets, templates, hospital and study visit data collection forms, etc.). C. Vanstone also conducted every study visit that took place at MECNRU. Tasks involved during study visits were obtaining consent, infant anthropometrics, infant and maternal DXA scans (operating and analysis), as well as infant and maternal blood drawls.

H. Weiler is the principle investigator of this project and the MECNRU director. H. Weiler conceptualized the trial and direction of this thesis project, as well as coordinated all co-authors on this thesis. H. Weiler is also K. Mullahoo's Master's supervisor and was the primary editor of this thesis.

S. Wei contributed to the overall conception of the clinical trial this project is based on.

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ABBREVIATIONS

% BSA	Percent body surface area
1-25$(OH)_2$D	1-25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CHMS	Canadian Health Measures Survey
CPS	Canadian Paediatric Society
CSEP	Canadian Society for Exercise Physiology
CV	Coefficient of variation
DBP	Vitamin D binding protein
DEQAS	Vitamin D External Quality Assurance Scheme
DIN	Director Identification Number
DRI	Dietary Reference Intake
DXA	Dual-energy x-ray absorptiometry
EAR	Estimated Average Requirements
EER	Estimated Energy Requirements
FFM	Fat free mass
HPLC	High performance liquid chromatography
IOM	Institute of Medicine
LC-MS	Liquid chromatography-mass spectrometry

MECNRU	Mary Emily Clinical Nutrition Research Unit
MES	Canadian Maternity Experiences Survey
n	Number of participants
NAFLD	Non-alcoholic fatty liver disease
PA	Physical activity coefficient
PTH	Parathyroid hormone
RCT	Randomized control trial
RDA	Recommended Dietary Allowance
SPF	Sun protection factor
TBW	Total body water
UL	Tolerable Upper Intake Level
UV	Ultraviolet
UVB	Ultraviolet beta radiation
VDR	Vitamin D receptor
WHO	World Health Organization

UNITS

%	Percent
cm	Centimeter
d	day
hr	Hour
IU	International unit
kcal	Kilocalorie
kg	Kilogram
kHz	Kilohertz
L	Liter
m	Meter
mAmp	Milliamp
mJ	Millijoule
ml	Milliliter
nm	Nanometer
nmol	Nanomole
µg	Microgram
µSV	Micro Sievert

1.0 LITERATURE REVIEW

1. Introduction/Rationale

Within the field of nutrition, vitamin D is considered an essential nutrient for healthy growth and development. It is most recognized for its functions in bone health and in maintaining calcium balance. Vitamin D can also be a determinant in the early life origins of health and disease; a hypothesis based on the premise that unfavorable exposures *in utero* can influence chronic disease development (such as cancer, autoimmune diseases, multiple sclerosis and heart disease) later in life. [1-3]. These effects are mediated by the presence of vitamin D receptors (VDR) in the nuclei of many cells and tissues allowing or inhibiting transcription of vitamin D responsive genes [2]. Similarly, vitamin D acts on skeletal muscle tissues, which can influence the development of a leaner body composition, with potential implications in childhood obesity [4].

Vitamin D can be synthesized endogenously via ultraviolet beta (UVB) radiation from the sun, or obtained exogenously through dietary intake and supplements [2]. Sunlight exposure, and specifically UVB exposure, is limited in Canada in the winter months due to its northern latitude. Other factors that can reduce exogenous synthesis include the use of sunscreen, darker skin pigmentation, clothing and an indoor lifestyle. Foods containing this vitamin naturally are scarce, but mainly consist of fatty fish, egg yolk and irradiated mushrooms. The Government of Canada also mandates fortification of fluid milk and margarine with vitamin D [1]. Despite these fortification efforts, 32% of Canadians have blood concentrations of vitamin D – in the form of serum 25-hydroxyvitamin D (25(OH)D) – that are below a healthy target for bone health (at least 50 nmol/L) year-round, where it is estimated that 40% are below the

target during the winter months (November to March inclusive) [5]. This urges the majority of Canadians to meet their requirements via oral supplements – either in the form of a tablet, capsule, liquid or intramuscular injection – to avoid deficiencies.

A particular group where deficiency and need for supplementation is common is in pregnant and lactating women [6]. National data in Canada are lacking with regard to vitamin D status of pregnant women. The Recommended Dietary Allowance (RDA) of vitamin D for pregnant women is identical to those of adults who are not pregnant; 600 IU per day [6, 7]. The Canadian Paediatric Society recommends pregnant women to take a multivitamin containing 200 IU per day of supplemental vitamin D [5-7]. For women of reproductive age in Canada, the average intake of vitamin D from foods only is ~200 IU, thus depending on the amount of vitamin D in the supplement and maternal stores, vitamin D status may not reach the healthy target through exogenous sources as total intakes fall short of the RDA [7]. Subsequently, newborn offspring can be born with low circulating concentrations of 25(OH)D, as *in utero*, the fetus relies completely on their mothers to obtain their vitamin D via transplacental transport [6, 8]. It is estimated that about 24% of all term born infants of healthy mothers in Quebec City are born with serum 25(OH)D below 50 nmol/L [4, 9]. The Canadian Paediatric Society (CPS) recognizes that the current recommendation by Health Canada of 200 IU of supplemental vitamin D per day, and even the RDA of 600 IU/d during pregnancy is not ideal to support the vitamin D status of both mother and infant [6].

Among the modifiable factors that can affect vitamin D status, there is growing evidence that vitamin D requirements may be weight dependent, where the more someone weighs, the greater their vitamin D requirements are [6, 10]. This occurrence may be due to vitamin D being

sequestered in adipose tissues as it is fat soluble, reducing its availability [6, 11]. Another possible reason for vitamin D intakes to be based on dose by weight is due to volume dilution effects in larger individuals [12]. These mechanisms may be applicable to pregnant women, as the largest constituents of gestational weight gain – apart from the developing fetus, placenta and amniotic fluid – are increasing fat stores and fluid accumulation due to blood volume expansion and edema [13].

Upon reviewing the literature on the associations between gestational weight gain and vitamin D status of the infant, it is evident that there are insufficient data to support valid conclusions. One study conducted in Greece found no correlation between maternal weight gain and 25(OH)D status; however, it is unclear if they were referring to maternal or infant concentrations as the data were not shown [14]. Moon et al. (2016) observed that higher pregnancy weight gain (kg) from 14 to 34 weeks gestation is associated with a lower serum 25(OH)D concentration in mothers in late pregnancy (34 weeks) when compared to women with normal weight gain (regression analysis: β = -0.81; 95% CI: -1.39, -0.22; p=0.007). However, this study calculated pregnancy weight gain as the difference in weight between weights at week 34 and week 14, and this calculated weight gain was not compared against a reference value for recommended weight gain for gestational age [15].

There have been a few studies viewing relationships between pre-pregnancy body mass index (BMI) and neonatal and maternal vitamin D status. Josefson et al. 2012, determined that women who had a pre-gravid BMI that was in the obese category (≥ 30 kg/m^2), transferred less 25(OH)D to their infants compared to mothers with similar serum 25(OH)D concentrations, but with a pre-gravid BMI in the normal/healthy range (18 – 25 kg/m^2; cord 25(OH)D of 68.5 $\pm$ 4.2

nmol/L for normal BMI vs. 51.9 ± 5.8 nmol/L for obese) [16]. Similarly, Bodnar et al. (2007) demonstrated that women with a pre-pregnant BMI ≥ 30 kg/m^2 had significantly ($p < 0.05$) lower serum 25(OH)D concentrations compared to leaner women with BMI <25 kg/m^2 at 4 to 22 weeks gestation (adjusted mean of 55.9 nmol/L vs. 62.8 nmol/L respectively) and at term (adjusted mean of 60.2 nmol/L vs. 67.3 nmol/L) [17]. Both studies controlled for prenatal supplement use, but did not disclose quantities of vitamin D in the supplement or dietary intakes. To date, total weight gained during gestation – despite maternal pre-gravid BMI – and its impact on vitamin D status in both neonate and mother, has not been extensively studied. This thesis research is designed to begin to fill this knowledge gap by exploring relationships between maternal gestational weight gain (GWG) and vitamin D status.

2. Vitamin D

2.1. Nomenclature

Vitamin D, or calciferol, is a class of fat-soluble seco-sterols that function as prohormones in the human body. The two main isomers are: ergocalciferol, also known as vitamin D_2, and cholecalciferol or vitamin D_3 [7]. Vitamin D_2 is mainly found in irradiated plants such as mushrooms or yeasts and thus may be present in yeast based foods [1]. Vitamin D_3 is also found in the diet, but is derived from animal sources. This form is also synthesized endogenously through the skin via exposure to UVB radiation from sunlight. Both forms of calciferol undergo two enzymatic hydroxylation processes during metabolism. The first reaction occurs in the liver to form 25(OH)D, or calcidiol. The second takes place in the kidneys and other tissues to form 1-25-dihydroxyvitamin D (1-25$(OH)_2$D), or calcitriol, which is the

biologically active form of vitamin D capable of binding to VDR and elicitation of gene transcription [7].

For the purpose of this literature review, the term vitamin D will represent ergocalciferol and cholecalciferol, unless otherwise specified.

2.2. Sources of vitamin D

2.2.1. Endogenous synthesis

Endogenous synthesis of vitamin D_3 occurs via cutaneous exposure to solar UVB photons, with a wavelength range of 290-315 nm [2]. The precursor 7-dehydrocholesterol (or provitamin D_3), found in the plasma membranes of cells in both epidermis and dermis layers of the skin, absorbs these rays of energy to undergo thermal isomerization to convert to previtamin D_3 [2, 7]. Previtamin D_3, being thermodynamically unstable, will then transform itself to vitamin D_3 by rearrangement of its double bonds, where it is then relocated to the extracellular space, [2, 18]. Ultimately, vitamin D_3 will migrate into the circulatory system, which is mediated by its affinity to vitamin D binding protein (DBP) found in the capillary beds of the skin [2]. Overexposure to UVB rays will not cause vitamin D_3 toxicity as there are regulatory pathways in place, allowing the conversion of previtamin D_3 to several other compounds (such as lumisterol and tachysterol), as well as the ability for vitamin D_3 to convert to non-active forms [7].

There are many factors that can impede cutaneous vitamin D synthesis. Endogenous reasons include melanin and aging, whereas exogenous factors are comprised of latitude, season, clothing, sunscreen use, ozone and air pollution [18]. In research, endogenous synthesis of vitamin D can be difficult to estimate as it is usually based on subjective qualitative

questionnaires. These questionnaires often capture data regarding sunscreen use, latitude, season, time spent outdoors (minutes per day), and percent of body surface area (% BSA) exposed to direct sunlight. Sun index (not including sunscreen use) can then be calculated multiplying % BSA by time spent outdoors [19].

The skin pigmentation, melanin, can greatly reduce the synthesis of vitamin D as it absorbs UVB photons of similar wavelength as 7-dehydrocholesterol; thus in darker skinned individuals, there will be more competition for absorbance and less synthesis of vitamin D [18]. Matsuoka et al. (1991) tested this concept by exposing healthy individuals of white, East Asian, South Asian and black cultural groups, with similar baseline vitamin D status, to the same UVB wavelength, and measured vitamin D_3 synthesis in skin and status [20]. They found significant differences between racial groups in terms of serum cholecalciferol concentrations, with whites having higher values than blacks (69.9 ± 12.7 nmol/L vs. 29.7 ± 6.2 nmol/L respectively) [20]. Thus, individuals with darker skin will have a decreased ability to synthesize vitamin D_3 from the competing effect of melanin, and will require longer exposure times to generate the same amount of vitamin D as lighter skinned people (up to 2 hours versus 15 minutes) [2].

As for the aging population, the amount of cutaneous 7-dehydrocholesterol is greatly diminished [2]. It has been demonstrated that older adults (>60 years) can only produce roughly 25% of vitamin D in comparison to younger adults [2, 18]. Since this is only relevant to those >60 years, it is likely not the cause of low maternal infant status.

Skin protection from the sun is encouraged in today's society to avoid skin damage and to reduce the risk of developing skin cancer. Sunscreens, along with clothing, absorb UVB rays preventing cutaneous vitamin D synthesis [2, 18]. When applied correctly, sunscreens with sun

protection factor (SPF) of 15 can decrease endogenous synthesis of vitamin D by >98% [2]. Health Canada does not recommend the use of sunscreen on infants less than 6 months of age, and infants under one year of age should not be in direct exposure to UVB radiation from sunlight to avoid skin damage and dehydration [21].

Canada, having a latitude above the 42nd parallel, is subjected to the sun being less potent from October to April as its zenith angle increases, which reduces the amount of UVB photons penetrating the atmosphere [1, 22]. Furthermore, ozone present in air pollution can also absorb UVB photons, allowing fewer rays to reach skin surfaces [18]. This can be damaging to people living in industrialized cities, especially in northern towns, as cutaneous synthesis of vitamin D will be greatly suppressed.

2.2.2. Exogenous sources

Individuals can obtain vitamin D exogenously through their diet by consuming natural food sources, fortified foods, and dietary supplements. Foods naturally containing this vitamin consist of plant and animal sources, in the form of ergocalciferol and cholecalciferol respectively [1]. However, these sources are scarce, but mainly consist of fish, egg yolks, some mushrooms and cod liver oil [1].

As a way to help rectify the small pool of naturally occurring food sources of vitamin D, the Government of Canada mandates fortification of fluid milk, margarine and infant formula with vitamin D predominantly in the form of cholecalciferol [1]. The target fortification values of vitamin D for fluid milk ranges from 35.2 IU/100 ml to 46.9 IU/100 ml, with a goal of the consumer to obtain ~100 IU per 250 ml of fluid milk [7, 23]. The vitamin D added to milk is part of a vitamin premix, containing vitamin C and occasionally vitamin A, which comes in a liquid,

powder or bead form [23]. This mix is then diluted into a solution and added to the fluid milk prior to the pasteurization process, as it is not sterile [23]. Additionally, some food companies may choose to fortify products such as almond/rice/soy beverages and orange juice, or produce food items such as yogurt and cheese using fortified milk [1, 7].

Despite these fortification efforts, 32% of Canadians have concentrations of serum 25(OH)D that are below a healthy target for bone health (i.e., <50 nmol/L) year-round, where it is estimated that 40% are below the target during the winter months [5]. As a result of these high rates of insufficiency, the majority of Canadians will require a vitamin D supplement, or improve their dietary intake, to meet their needs and avoid deficiencies. Supplements can be found either in the form of a tablet, capsule, liquid drops or intramuscular injection, and can be formulated with either vitamin D_3 or vitamin D_2. Dose ranges from 200 IU – 1,000 IU daily for over the counter purchase, or in higher doses (i.e., 10,000 IU weekly) by prescription only [1]. They can be procured solely as a vitamin D supplement, or part of a multivitamin and mineral complex.

In Canada, the composition of commercial infant formulas is established by the Food and Drug Regulations [24]. In terms of vitamin D content, all infant formulas are fortified with enough vitamin D to meet an infant's recommended needs; to attain 400 IU per liter of formula (100 IU per 250 ml), or 40 to 80 IU per 100 kcal [1, 6, 7]. It is not recommended for infants to take an additional vitamin D supplement if they are being exclusively fed fortified infant formula, as it already contains the vitamin addition [7]. On that note, infants consuming amounts below 1 liter per day of formula, may be at risk for inadequacy of vitamin D intake.

As mentioned previously, infants who are exclusively breastfed, are recommended to take a vitamin D supplement by Health Canada [25]. These guidelines are put in place as it is not advised for young infants to be exposed to sunlight; moreover, there are low amounts of total vitamin D, including cholecalciferol and calcidiol, found in breast milk [7, 21, 26]. The antirachtic activity of human breast milk was thought to be 20-70 IU/L in the 1980's [27]. As per data from the 2010 Canadian Nutrient File, mature human breast milk contains 3 IU of vitamin D per 100 ml (i.e., 30 IU/L), or 4.1 IU/100 kcal [28]. Intake volumes of human breast milk in infants can range from around 500-1,300 ml/day based on infant-weighing [7, 26, 29]. Thus, infants would only receive anywhere from 15 to 39 IU of vitamin D daily, which is much lower than the recommended 400 IU per day. As the natural occurring amounts of vitamin D in human breast milk are low, providing an infant with a 400 IU supplement in addition to that in the milk is warranted [7].

2.3 Vitamin D status and metabolism

2.3.1 Biomarkers and assays

Serum 25(OH)D is used as the ideal biomarker for vitamin D status, as it reflects all sources of vitamin D; through endogenous synthesis, and intakes of food and supplements [1, 5, 30]. It is likewise stable, with a half-life of around 3 weeks, and its hepatic synthesis is not tightly regulated as it is mainly substrate dependent [30]. Calcitriol, or 1-25$(OH)_2$D, is not meant to be used as a biomarker of adequacy for vitamin D status [7]. Its synthesis is not dependent on vitamin D intake, and can still be within normal limits in times of deficiency due to up-regulation of the 1α-hydroxylase enzyme [7]. It also has a short half-life of a few hours, and can

be controlled by other factors such as parathyroid hormone (PTH) and fluctuates throughout the day [7].

It is important to note that 25(OH)D reflects both vitamin D_2 and vitamin D_3 intakes [7]. Therefore, 25(OH)D_2 and 25(OH)D_3 need to first be measured separately, then combined to attain a total 25(OH)D value [7]. Several assays exist in order to achieve accurate measures of total serum 25(OH)D. Historically, competitive protein-binding assays were the first methods of analyzing serum 25(OH)D in the late 1960's [7]. However, due to its laborious nature and overestimations of concentrations, this method is no longer commonly used [7]. Antibody-based 25(OH)D assays (chemiluminescent-based) were then presented in the 1980's, and are still used widely today in automated multi-well formats to increase output production (i.e., measure multiple samples at once) [7, 31]. This method, however, sometimes does not detect 100% of the 25(OH)D_2 isomer which can lead to underestimations or may detect other vitamin D metabolites, which can lead to overestimations [7]. The most accurate methods include high performance liquid chromatography, or HPLC, and liquid chromatography-mass spectrometry, or LC-MS [1, 7]. LC-MS is also recognized as the gold standard method for the analysis of vitamin D as it can quantify 25(OH)D_2 and 25(OH)D_3 with good accuracy [30]. In order to assure adequate quality assurance between different laboratories analyzing vitamin D samples, external organizations such as the Vitamin D External Quality Assurance Scheme (DEQAS) have been created [7, 32].

2.3.2 Recommendations for intake and cutoffs of adequacy

The Institute of Medicine's (IOM) expert committee has suggested cut-points of serum 25(OH)D for optimal health in relation to bone (**Table 1.1**) [7, 33]. Health Canada's

Table 1.1: Vitamin D status – cutoffs of adequacy from different organizations

Vitamin D Status Categories	Institute of Medicine [7] & Health Canada [33]	Canadian Paediatric Society [6]	Endocrine Society [34]
	All ages (infants, children, adults, pregnancy & lactation, older adults)	Infants, pregnancy & lactation	All ages (infants, children, adults, pregnancy & lactation, older adults)
At risk of deficiency	<30 nmol/L	<25 nmol/L	<50 nmol/L
Insufficient	30 – 50 nmol/L	25 – 75 nmol/L	50 – 75 nmol/L
Healthy target*	≥50 nmol/L	75 – 225 nmol/L	75 – 250 nmol/L
Potential risk for adverse effects	>125 nmol/L	>225 nmol/L	-
Potentially toxic	400 – 1250 nmol/L	>500 nmol/L	-

*Meaning adequate for bone and overall health; referred to as sufficient by the Institute of Medicine and as optimal by the Canadian Paediatric Society.

recommendations are in accordance with the suggestions from the IOM, and thus use their cutoffs as a guide [33]. The ideal concentration of serum 25(OH)D is generally described as the maximum amount needed to plateau circulating PTH, while maintaining adequate calcium absorption and bone mineral density [35, 36]. However, the range of serum 25(OH)D needed to suppress PTH circulation varies greatly; from 20 – 110 nmol/L [35]. Hence, no actual scientific consensus process has been made on the cutoffs for health as there is much debate regarding what is sufficient [33, 35].

It is commonly recognized that serum 25(OH)D concentrations below 30 nmol/L pose risk for the development of rickets in infants, as well as osteomalacia, tetany and myopathy in adults [1, 7, 33, 34]. The suggested 50 nmol/L cutoff for adequacy was set on the basis of bone health only [7]. Other experts have suggested the optimal target concentration for serum 25(OH)D should be at 75 nmol/L to account for the benefits of the non-skeletal related outcomes associated with vitamin D [6, 34-36]. Such non-skeletal outcomes include hypertension prevention, cancer prevention (ideally 75 – 110 nmol/L), and lower extremity strength/fall prevention [35]. It has been suggested that a daily intake of 1,000 IU of vitamin D could bring up about 50% of the adult populations serum 25(OH)D concentrations to 75 nmol/L [35]. However, more studies are needed to verify what intakes are required for a higher majority of the population to reach that target [35].

The Endocrine Society's Task Force came up with a similar minimum cut-point of 75 nmol/L of optimal vitamin D status for all populations (**Table 1.1**) [34]. Likewise, the CPS also suggest a similar cutoff value for optimal status for infants, pregnant and lactating women (**Table 1.1**) [6]. There lacks data on providing evidence for optimal vitamin D status of infants,

thus the <30 nmol/L, 30-49.9 nmol/L and ≥50 nmol/L ranges are used [1, 6]. Consequently, the IOM established the current AI for vitamin D for infants at 400 IU per day based on evidence determining that this amount is adequate to achieve a serum 25(OH)D concentration within the 40 to 50 nmol/L range [6].

2.3.3 Vitamin D status of Canadians

The Canadian Health Measures Survey (CHMS) (Cycle 2 data from 2009-2011) analyzed blood serum concentrations of 25(OH)D to assess the vitamin D status of Canadians [5]. The cutoffs for adequacy used were consistent with the IOM's recommendations (**Table 1.1**) [5]. To summarize their findings, 68% of all Canadians had blood concentrations of vitamin D above the cutoff, with the remaining 32% being below the cutoff; 10% of which were in the deficient category [5]. Approximately 40% of Canadians are below the cutoff in winter months [5]. The national average blood concentration was found to be 64 nmol/L, with females of all ages having higher blood concentrations than males; 67 nmol/L and 61 nmol/L, respectively [5]. If further broken down by age category, the following represent percentages ≥50 nmol/L cutoff: 89% of young children aged 3 to 5 years, 76% of children aged 6 to 11 years, 71% of children/adolescents aged 12-19 years, 59% of adults aged 20-39 years, 68% of adults aged 40 to 59 years, and 75% of older adults aged 60-79 years [5]. Young children (3 to 5 years) and older adults (60 to 79 years) had the highest blood concentrations above the vitamin D cutoff; 74 nmol/L and 70 nmol/L, respectively [5]. This may be due to these populations having greater intakes of fortified foods and supplements.

A shorter report by Statistics Canada looking at the results from the 2012 to 2013 CHMS, portrays similar findings from the previous national health survey; 65% of Canadians have

sufficient vitamin D status (≥50 nmol/L), with 25% being below this cutoff, and 10% at risk of deficiency (<30 nmol/L) [37]. It is also included in the report that racial background has an influence on vitamin D status, where non-white individuals were at higher risk for insufficiency and deficiency than white people (38% and 20%, respectively for non-white, and 21% and 6% for white) [37].

Having a high BMI has been viewed as an independent risk factor for low vitamin D status [1, 7, 38-41]. In Canada, about 26% of adults are obese and 34% are overweight [5]. Data from the 2007-2009 CHMS indicates that obese individuals had significantly lower vitamin D status than those who had a normal/healthy BMI or who were overweight; 55 nmol/L for obese vs. 61 nmol/L and 63 nmol/L for normal weight and overweight, respectively, in adults 18 to 39 years [5]. However, no significant differences were found between serum 25(OH)D status in the 2012-2013 data [37].

Pregnant and lactating women were not studied in either of the CHMS's, and therefore data are lacking on a national level in these groups. When looking at the aforementioned national data for adults aged 20 to 39 years of age (i.e., during reproductive ages), that group represented the lowest vitamin D status amongst the populations studied. Albeit, the females in that age category have higher serum 25(OH)D concentrations than males; 66 nmol/L vs. 55 nmol/L, respectively [5].

Several studies identifying vitamin D status of pregnant women were performed in different provinces across Canada. Weiler et al. conducted a study in Winnipeg in 2005 (n = 50) and demonstrated that around 30% of white and 75% of non-white pregnant women had serum 25(OH)D concentrations below 37.4 nmol/L (described as deficiency in the report) [1,

42]. Additionally, 78% of these women were taking a prenatal supplement [42]. Using extrapolated data from the controls (n = 1,975; 33% with BMI ≥25 kg/m^2) of a nested case control study from two Canadian cohorts located in Halifax and Quebec City, it was shown that 46% of pregnant women had serum 25(OH)D concentrations below 50 nmol/L [43]. Another study conducted in Vancouver in 2011 looking at vitamin D status in multiethnic pregnant women (n = 336; 26% with BMI ≥25 kg/m^2) taking prenatal supplements containing vitamin D, revealed that 24% of those women had plasma 25(OH)D concentrations below 50 nmol/L [44]. In Toronto, Kramer et al. (2016) observed a cohort of 467 pregnant women and determined 31.5% of them had serum 25(OH)D below 50 nmol/L. Of these women with low status, 63% were white, 74.2% percent were taking some form of vitamin D supplement, and mean pre-gravid BMI was 25.6 kg/m^2 [45]. Lastly, Aghajafari et al. (2016) looked at vitamin D status of 92 multiethnic pregnant women in Edmonton and Calgary, where only 4% had 25(OH)D concentrations below 50 nmol/L [46]. All the women in the study were taking vitamin D supplements (mean 1,290 ± 923 IU/day; range 170 to 4,400 IU/day). These studies that span from the East to West coasts of Canada, could demonstrate a rough idea of what national data could look like. If looking at the weighted average of proportions of these studies, it is estimated that around 29% of pregnant women in Canada have vitamin D status below 50 nmol/L [43-46].

2.3.4 Vitamin D metabolism

Vitamin D is a fat soluble vitamin and when ingested, it gets absorbed along with other dietary fats into the enterocytes of the small intestine [7]. Within these cells, chylomicrons will form, containing lipoproteins, vitamin D, cholesterol, triglycerides, and other lipids [7]. The

chylomicrons are then transported through the lymphatic system prior to entering the peripheral bloodstream [7]. Following cutaneous synthesis of vitamin D_3 from UVB exposure, the vitamin is then transported into the circulatory system by its affinity to DBP located in the capillaries of the skin [2].

Despite its route of synthesis – endogenous or exogenous – once vitamin D enters the circulatory system, it is transported to the liver either free, or bound to DBP [1, 7]. While speculative, vitamin D from chylomicrons could also be taken up by surrounding adipose and muscle tissues during chylomicron degradation via lipoprotein lipase in the periphery, thus leaving less available to the liver to further metabolize to 25(OH)D (**Figure 1.1**) [7]. Additionally, due to its fat soluble nature, the main storage site for vitamin D (cholecalciferol) is in adipose tissue [7]. This has been demonstrated by studies in both rats and humans using radiolabelled and stable isotope labelled vitamin D [40, 47-49].

Vitamin D being an inactive prohormone, requires activation by two hydroxylation reactions for function. The first process occurs in the liver, where the enzyme 25-hydroxylase (or CYP2R1) converts calciferol into 25-hydroxyvitamin D, or 25(OH)D [7]. Then, 25(OH)D is released back into circulation where it binds to DBP and is transported to the mitochondria of the renal cortex in the kidney for the final hydroxylation by the enzyme CYP27B1 to 1-25-dihydroxyvitamin D, or 1-25$(OH)_2$D; the active hormonal form of the vitamin [7, 50]. This process is carried out by the enzyme 1α-hydroxylase and is usually regulated by PTH when blood calcium and phosphate homeostasis is required [7, 41]. Serum 25(OH)D can also be further metabolized to 1-25$(OH)_2$D in extra-renal tissues including prostate gland, breast, colon

Figure 1.1: Potential mechanism for uptake of vitamin D in adipose tissue from exogenous sources

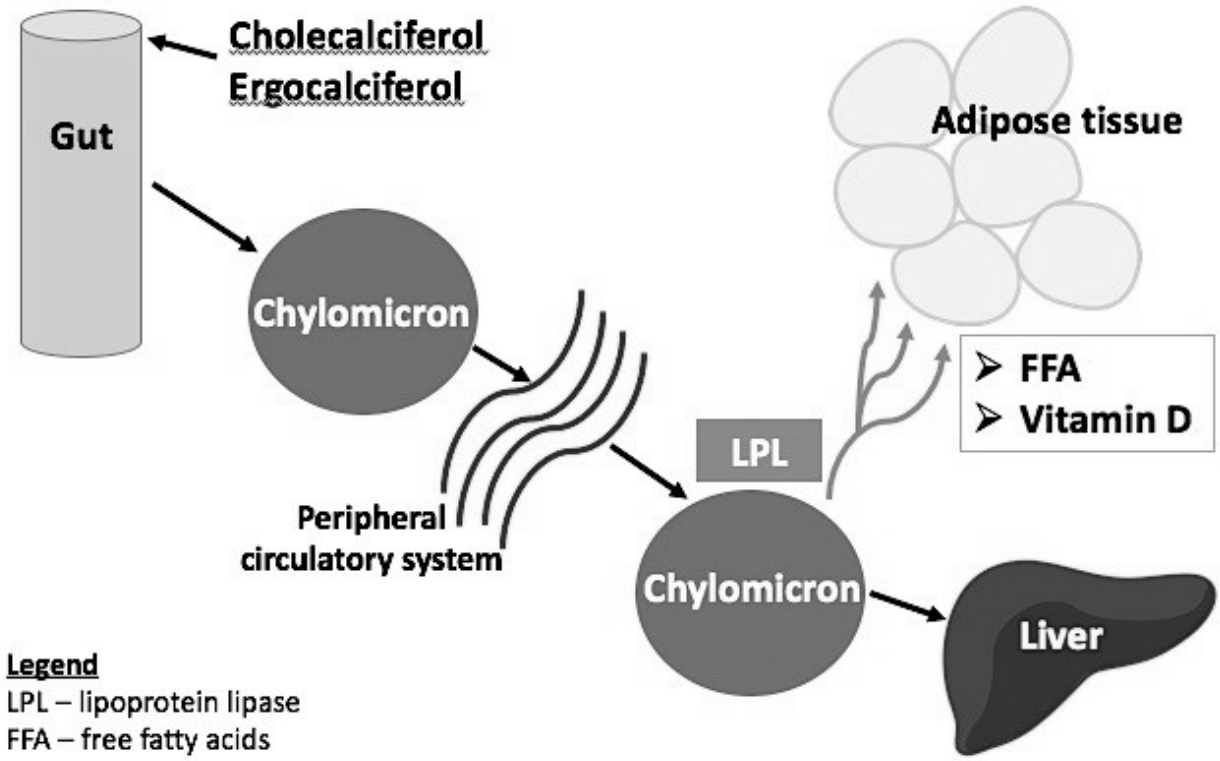

Figure 1.1 demonstrates vitamin D packaged in chylomicrons being taken up by surrounding adipose (and muscle) tissues during chylomicron degradation via lipoprotein lipase (LPL) in the periphery.

and lung [2]. In terms of excretion, vitamin D and its metabolites are eliminated mainly through feces bound to bile after degradation by the enzyme CYP24A1 [7].

Presently, no consensus has been made regarding the exact mechanism of maternal-fetal transport of vitamin D in humans [1]. However, it has been strongly suggested that the human fetus is completely dependent on the mother in attaining this essential nutrient [6, 50-53]. In a randomized control trial (RCT) done by Delvin et al. (1982), it was found that maternal concentrations of serum 25(OH)D are higher than fetal concentrations, and that cord serum 25(OH)D concentrations are directly correlated with maternal serum concentrations ($r=0.90$, $p<0.005$) [51]. This suggests that transport of vitamin D from the mother to her fetus likely occurs via a passive route, from high to low concentration [1]. Similarly, in a study done in Egypt (2013), maternal and cord serum 25(OH)D concentrations were collected in the third trimester of pregnancy of 92 healthy pregnant women. They established that mothers with vitamin D insufficiency (<50 nmol/L) compared to mothers with sufficient status (≥50 nmol/L), gave birth to term-born offspring with insufficient serum 25(OH)D concentrations (33.4 ± 18.3 nmol/L vs. 55.4 ± 17.4 nmol/L, respectively, $p=0.01$) [54]. Significant correlations between newborns and maternal 3rd trimester serum 25(OH)D_3 concentrations was also found ($r=0.89$, $p=0.01$).

Circulating concentrations of 1-25$(OH)_2$D are increased during pregnancy due to a combination of increased synthesis in the kidneys and placental decidua cells [50, 55]. What drives this increased production is unclear, but it is theorized that it may be due to pregnancy-induced hyperparathyroidism, coupled with reduced clearance of this metabolite [50, 55]. It is postulated that the placental synthesis of 1-25$(OH)_2$D is necessary for a successful pregnancy [53]. In early pregnancy, it acts by its immunological properties to help with trophoblast

invasion and implantation, as well as fetal-placental development [53]. Throughout the gestational period, and especially during fetal skeletal development, 1-25$(OH)_2$D acts to maintain calcium homeostasis for bone mineralization and growth [53]. Nonetheless, it is hypothesized that increased synthesis of 1-25$(OH)_2$D is dependent on 25(OH)D concentrations; with maximal 1-25$(OH)_2$D output attained when serum 25(OH)D concentrations are at least 100 nmol/L [52, 56, 57]. Therefore sufficient vitamin D status is needed during pregnancy to account for this.

It has been observed that individuals with greater amounts of adipose tissue have lower serum 25(OH)D concentrations [6, 7, 11, 39, 58]. A groundbreaking experimental study was conducted by Wortsman et al. (2000), to assess if obesity had any effects on both cutaneous synthesis of vitamin D_3 and on intestinal absorption of vitamin D_2. Obese (BMI $\geq$ 30 kg/m^2) white men were compared to white males with BMI <25 kg/m^2, where both groups had no differences in baseline concentrations of serum 25(OH)D. The study was conducted in the winter months, and consisted of two study visits. The first visit was aiming at testing the capacity of cutaneous synthesis by exposing the participants (n = 13 per group) to UVB radiation (1 dose 27-mJ/cm^2, wavelength 290-320 nm) [38]. Serum 25(OH)D concentrations were measured prior to exposure, and 24-h post exposure, as this is when cutaneous synthesis of vitamin D_3 peaks in the bloodstream [38]. The second visit consisted of providing each subject (n = 11 per group) with a single high dose of vitamin D_2 of 50,000 IU, and measuring serum 25(OH)D concentrations at 6, 10 and 24 hours post ingestion. Their main findings showed significant increases in serum 25(OH)D concentrations in both groups after UVB exposure and oral intake, where there were significant differences (up to 57%) in the response

between the groups – i.e., there was a significantly lower response in serum 25(OH)D concentrations in the obese group, despite their being given the same treatment as the non-obese individuals [38].

Gallagher et al. (2013) also conducted two dose-response RCT's in both thin and obese vitamin D insufficient women, with an age range of 57-90 years (n = 163 for the first trial of 1 year, and n = 488 for the second trial of 3 years duration). They were provided an array of oral vitamin D supplements from 400 IU to 4,800 IU daily. One of the main findings of this study was that women with normal BMI's had higher responses in serum 25(OH)D concentrations than the obese women when given the same dose of vitamin D [11]. Similarly, Arunabh et al. (2003) found a statistically significant inverse relationship between serum 25(OH)D concentrations and percent body fat in their RCT of 410 healthy women aged 20-80 years, after adjusting for age, race, seasonal variations, and oral intake [58].

Inadequate exposure to sunshine is also a potential cause for low vitamin D status. Overweight/obese individuals may avoid going outside (due to reduced mobility, and/or social impacts), or cover more of their skin, and some pregnant women are put on bed rest [52, 58]. It is crucial then that these individuals acquire their vitamin D via diet and/or supplementation. However, supplementation doses/requirements do not account for higher BMI's, and dietary intake of vitamin D may not be adequate for everyone.

2.4 Functional roles of vitamin D

The active metabolite 1-25$(OH)_2$D is required for physiological function in the human body [1]. Its classical role revolves around calcium and phosphorous homeostasis and for optimal bone health [7]. More evidence is now showing it has other biological functions as

VDRs are present in many tissues of the body [2, 7]. VDRs can be found in both small and large intestines, heart, brain, kidney, reproductive organs, adipocytes, osteoblasts, immune cells, and beta islet cells of the pancreas to name a few [2]. The action of vitamin D on these tissues are at the cellular level, where 1-25$(OH)_2$D (transported by DBP) binds to the VDR in the nucleus [2, 7]. Once bound to VDR, it dimerizes with the retinoic acid X receptor, followed by binding with the vitamin D-responsive element (a DNA sequence) [2]. Once this complex has formed, cofactors can bind to it, leading to the expression or repression of vitamin D-responsive genes [2]. Because vitamin D has a great influence on the human genome – where almost 5% of it has been shown to be regulated by calcitriol in gene array studies – it is possible it also has pleiotropic properties [1, 7].

2.4.1 Classical functions: calcium homeostasis and bone health

Calcitriol is needed to maintain adequate plasma calcium and phosphorous concentrations in circulation for physiological functions involved with nerve transmission, vasodilation, muscle contractility, hormone secretion, as well as bone mineralization [7]. It has action on three main sites in the body: the intestines (predominantly the duodenum and jejunum), bone, and kidneys [7]. When blood ionized calcium concentrations drop below 2.12 mmol/L, PTH is released to activate 25(OH)D to 1-25$(OH)_2$D [1, 7]. Calcitriol stimulates the absorption of both calcium and phosphorus in the intestine and, with the help of PTH, mobilizes calcium from bones by stimulating osteoclast activity [7]. Lastly, calcitriol and PTH signal to the kidneys to reabsorb calcium in the distal tubules [7]. Once optimal concentrations of calcium are achieved, PTH concentrations decrease by ways of negative feedback [2].

The elevation in blood calcium and phosphorous concentrations, mainly by intestinal and renal absorption, are necessary to achieve the endpoint of vitamin D action: mineralization of bone [59]. As bone is a living dynamic tissue, it is important to maintain a sufficient vitamin D status, including adequate calcium intake, to ensure proper bone modeling and remodeling [7].

2.4.2 Other functions of vitamin D

There is growing evidence concerning an increase in vitamin D intake/status and lower chronic disease risk [2, 3, 35, 60]. Cardiovascular health, predominantly blood pressure, is thought to be influenced by vitamin D through its possible vasoactive properties and by suppressing renin production, as seen in animal models [2, 35]. Serum 25(OH)D concentrations between 75 to 100 nmol/L have shown to be optimal for protection against hypertension and mortality risk in human epidemiological studies [35]. Other chronic diseases that may have reduced incidence with adequate vitamin D status include the development of type 1 diabetes (as it stimulates insulin production in the islet cells of the pancreas), rheumatoid arthritis, and Crohn's disease [2]. Furthermore, the prevalence and incidence of cancers such as colorectal, prostate and breast, as well as multiple sclerosis, have been associated with individuals living in higher latitudes [2].

Vitamin D has great implications on immune health as VDR are found in cytotoxic T cells, macrophages, monocytes, activated T and B cells, and epidermal keratinocytes [2, 7]. In macrophages and monocytes, calcitriol stimulates the production of cathelicidin, an antimicrobial peptide which helps fight bacterial infections [7]. Gingivitis and periodontal disease have been associated with vitamin D inadequacy, which are thought to be linked to vitamin D's anti-inflammatory effects. [7, 35]. Due to its anti-proliferative activity and action in

epidermal keratinocytes, topical application of 1-25$(OH)_2D_3$ (and analogues developed accordingly) is a clinically effective treatment for psoriasis globally [2]. High doses of vitamin D_3 also has antitumor properties in leukemia patients and can suppress autoimmune diseases, but with hypercalcemia as a main repercussion [2, 7]. Active calcitriol is thought to reduce cancer risk due its ability to increase apoptosis and cell differentiation in tumor cells, and reduce their proliferation [35].

2.4.3 Consequences of deficiency

The most notable consequences of vitamin D deficiency revolve around bone abnormalities due to imbalances in calcium and phosphorous metabolism [2, 34]. Poor vitamin D status will allow for decreased intestinal absorption of dietary calcium; 10-15% vs. 30% absorption in a vitamin D replete individual [2]. This will drive an increase in PTH concentrations to maintain circulating calcium homeostasis by mobilizing calcium from the skeleton [34]. Additionally, the kidneys will excrete more phosphorous in the urine, causing hypophosphatemia [34]. This, when coupled with increased calcium mobilization, will cause inadequate mineralization of bones [34]. In infants, demineralized bones can cause rickets, or the weakening and softening of bones, as well as impair linear growth [2, 34]. In adults, osteomalacia, or softening of bones, can develop, with osteoporosis being a more severe consequence, and increased fracture risk is also associated with vitamin D deficiency [2, 34].

Non-skeletal outcomes include muscle weakness and myopathy, as well as possible programming for chronic health diseases and mortality, as mentioned in the previous section [2, 3, 35]. Poor vitamin D status in pregnancy has been associated with premature births, gestational diabetes, preeclampsia, low neonatal vitamin D status, as well as innate and

adaptive immune responses [52, 53, 61]. Maternal status can also influence early life *in utero* by ways of metabolic or fetal programming; defined as an early life adaptation to a stress that can change the metabolic function of the organism, since the adapted response will continue to be expressed even when the stress is no longer present [60]. An example of this is the growing evidence that low intrauterine 25(OH)D status is associated with reduced infant muscle mass and increased childhood adiposity [4, 62, 63]. Therefore, it is of high importance to attain sufficient serum 25(OH)D concentrations during the antenatal period to not only deter from short term consequences, but potential longer term metabolic outcomes.

2.4.4 Toxicity

Hypervitaminosis D, or vitamin D toxicity, can only occur with high intakes of vitamin D and not by excessive sun exposure [2, 7, 64]. Prolonged exposure to UVB rays leads to the isomerization of the subcutaneous previtmin D_3 to other vitamin D metabolites, which have no metabolic activity [2]. Since the development of synthetic vitamin D in the 1930's, toxicity cases began to arise as unregulated fortification practices became increasingly popular [7, 64]. Even in the present day, the main causes of intoxication are related to over the counter products containing higher doses of vitamin D than what is marked on the label, as its content is not regulated [65]. The CPS advocates serum 25(OH)D concentrations above 225 nmol/L pose risk for hypercalcemia and calcium deposition in soft tissues, with concentrations >500 nmol/L are considered toxic [6].

The onset of symptoms of an overdose would present in the forms of gastrointestinal disruptions (nausea, vomiting, diarrhea, constipation, anorexia), drowsiness, bone pain, decreased appetite, headaches, joint and muscle pains, polyuria, dehydration, and kidney

stones [64, 65]. The main metabolic consequence of hypervitaminosis D is hypercalcemia, which is driven by an increase in serum 25(OH)D, as the hydroxylation reaction in the liver is substrate dependent [65]. This could then lead to calcification of soft tissues and organs [7, 64, 66]. These extreme consequences have only been seen and documented in cases where adult individuals have chronically consumed 40,000 IU daily or greater [67]. Infants and young children were only observed to have hypercalcemia when single intakes of 240,000 IU or greater of vitamin D were taken [65]. Additionally, infants who were treated with single high doses (600,000 IU) of vitamin D to prevent rickets in the 1930's, were found to have compromised linear growth [68]. It is not surprising that large bolus doses could cause such metabolic disturbances, given the vitamins lipophilic nature and ability to store in adipose tissues for up to two month [65].

2.5 Current recommendations

The Government of Canada follows recommendations for vitamin D based on the IOMs latest Dietary Reference Intakes (DRIs), updated in 2011 [33]. The DRIs for vitamin D were established assuming little sun exposure and focused on adequacy for skeletal health since other data on outcomes were not consistent, or too few studies to draw a conclusion upon (**Table 1.2**) [7, 33]. It is recommended for individuals to have daily intakes that meet their RDA, and to avoid intakes greater than the UL, to avoid potential adverse health effects [7, 33]. Current recommendations for additional supplementation practices, on top of intake from foods, have only been advised in breastfed infants, pregnant and lactating women, as well as all adults over the age of 50 years [7]. Older adults are recommended to take a daily vitamin

Table 1.2: Daily Dietary Reference Intake's for vitamin D

Population	Adequate Intake (AI)	Estimated Average Requirement (EAR)	Recommended Dietary Allowance (RDA)	Tolerable Upper Intake Level (UL)
Infants 0-6 months	400 IU (10 μg)	-	-	1,000 IU (25 μg)
Infants 7-12 months	400 IU (10 μg)	-	-	1,500 IU (38 μg)
Children 1-3 years	-	400 IU (10 μg)	600 IU (15 μg)	2,500 IU (63 μg)
Children 4-8 years	-	400 IU (10 μg)	600 IU (15 μg)	3,000 IU (75 μg)
Children and Adults 9-70 years	-	400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)
Pregnancy and Lactation	-	400 IU (10 μg)	600 IU (15 μg)	4,000 IU (100 μg)
Adults >70 years	-	400 IU (10 μg)	800 IU (20 μg)	4,000 IU (100 μg)

Adapted from: Health Canada. (2012). Vitamin D and Calcium: Updated Dietary Reference Intakes.

D supplement to reduce the risk of osteoporosis, as endogenous synthesis and gastrointestinal absorption decline with age [5, 7].

Pregnant and lactating women are encouraged to take a daily multivitamin supplement containing at least 200 IU of vitamin D. These supplements generally contain less than the RDA of 600 IU per capsule/tablet, which as previously mentioned, may lead to some women not meeting their requirements as good sources of vitamin D are limited to a few foods (**Table 1.3**) [5-7]. As previously stated, it is recommended for all exclusively breastfed infants to be given daily vitamin D supplements (400 IU) to meet the Adequate Intake (AI) set to avoid rickets, as breast milk naturally contains low amounts of vitamin D [5, 7, 26]. Currently, there are no concrete recommendations for vitamin D requirements for individuals with a higher body weight or BMI. There could be cause for future research in setting the DRI for vitamin D, given the growing evidence demonstrating that this population likely has higher requirements [6].

2.5.1. Supplementation practices

As per the Cycle 2 data of the CHMS, only 34% of Canadians were taking a supplement containing vitamin D, where young children (age 3 to 5 years) and older adults (age 40 to 79 years) had the highest consumption [5]. Young adults, aged 12 to 39 years, had the lowest intakes across the population [5]. Pregnant and lactating women were not subcategorized in this survey and it has been established that Canadian data on intakes of vitamin D via supplements and diet are lacking in this population [1]. In a study conducted in 121 postpartum women in northern Canada (mix of Caucasian, Inuit and Native Indians), approximately 24% of them were taking a supplement containing vitamin D during pregnancy [69]. A smaller study conducted in 50 postpartum women in Winnipeg Manitoba, demonstrated that 78% of them

Table 1.3: Vitamin D content of some commercially available prenatal supplements

Commercial Supplement	Serving (daily)	Vitamin D Content (IU/serving)	Vitamin D Content (IU/pill)
Baby & Me (DIN 80045889) MegaFoods	4 tablets	600	150
Basic Prenatal Cap (DIN 80062175) Thorne Research Inc.	3 capsules	1,000	333
Centrum Prenatal ± DHA (DIN 80045822) Pfizer Canada Inc.	1 tablet	600	600
Doctor's Choice Prenatal Formula (DIN 02231700) Enzymatic Therapy (Canada) Inc.	4 tablets	200	50
Multi Expecting Prenatal Vitamins (DIN 80009461) SiSu	2 capsules	500	250
MultiSure Prenatal (DIN 80048588) Webber Naturals Pharmaceuticals Ltd.	1 softgel	200	200
MultiVitamins Prenatal Formula (DIN 80027300) Progressive	3 capsules	400	133
Nestle Materna (DIN 80001842) or Generic: Personelle, Life, Option, Equate, Kirkland.	1 tablet	400	400
Pregvit (DIN 02451573) Duchesnay Inc.	2 tablets	250 or 600	125 or 300
Pregvit 5 (DIN 02451581) Duchesnay Inc.	2 tablets	250 or 600	125 or 300
Prenatal Complete with DHA (DIN 80060521) Jamieson	1 capsule	400	400
Women's Prenatal (DIN 80068318) Pure Food Whole Earth & Sea	2 tablets	1,000	500

Data obtained from label claims.
DIN: Drug Identification Number

were taking a maternal supplement during pregnancy; however, the authors do not disclose the amounts of vitamin D found in the supplements [42].

Several studies have looked at providing higher dose supplementation of vitamin D to lactating mothers, as a means to increase breast milk concentrations, and consequently, raise serum 25(OH)D concentrations of their infants [1, 27, 70-72]. A Canadian study by March et al. in 2015, conducted in British Columbia, provided white and non-white women (n = 226, mean pre-gravid BMI of 23.2 ± 3.7 kg/m^2) with three doses of vitamin D_3 (400 IU, 1,000 IU or 2,000 IU daily) that was part of a multivitamin supplement, starting from their second trimester until eight weeks post-partum. No additional vitamin D was given to the breastfed infants, and serum 25(OH)D concentrations in both mother and infant were measured. Women taking the highest dose of 2,000 IU had the most benefit, where 98% of infants were protected from deficiency (<30 nmol/L) and 87% from insufficiency (<50 nmol/L) [71]. Hollis et al. (2015), conducted a study in the United States of America comparing three doses of maternal vitamin D_3 supplementation during lactation of 400 IU, 2,400 IU or 6,400 IU daily, to paired infant supplementation doses of 400 IU, 0 IU (placebo) and 0 IU, respectively [70]. Participants (n = 344) were white, black and Hispanic, were recruited at four to six weeks postpartum, and had to be exclusively breastfeeding with singleton pregnancies. The authors' main objectives were to compare serum 25(OH)D concentrations of the infant from infant-mother pairs who received the 6,400 IU/day dose, vs. the infant-mother pair receiving 400 IU/day, at four and seven months postpartum. There were no significant differences in baseline values of circulating 25(OH)D in any of the groups. Mean maternal BMI for the 400 IU dose group was 27.8 ± 5.5 kg/m^2, and 27.4 ± 4.3 for the 6,400 IU group, but it is unclear if this represents BMI during

pregnancy or pre-gravida. Their findings showed that maternal supplementation of 6,400 IU daily significantly increased infant serum 25(OH)D in the same manner as providing the infant with 400 IU daily at the four month mark, which remained elevated at seven months [70]. These outcomes suggest that high dose vitamin D supplementation of 6,400 IU daily to mothers only, will allow for sufficient serum 25(OH)D concentrations in both mothers and infants (≥50 nmol/L), as that dose is sufficient to allow enough vitamin D to pass through breast milk to attain infant adequacy.

It would have been interesting to see the dose response as Hollis et al. (2015) used the UL of vitamin D in pregnant and lactating women, 4,000 IU daily, as a treatment group. Luckily in 2004, Hollis and Wagner conducted a similar vitamin D dose response study in the United States using two treatment groups (n = 9 per group) in lactating mothers [27]. The treatments were 2,000 IU (1,600 IU of vitamin D_2 + 400 IU of vitamin D_3) and 4,000 IU (3,600 IU of vitamin D_2 + 400 IU of vitamin D_3) of vitamin D daily. Total antirachitic activity (i.e., therapeutically effective against rickets) of human milk was increased in both groups over time, with higher activity in the 4,000 IU group. Infants of mothers receiving 2,000 IU daily, had significant mean increases in total serum 25(OH)D concentrations, from 19.7 $\pm$ 2.7 nmol/L to 69.3 $\pm$ 9.7 nmol/L ($p<0.02$). Subsequently, mothers total circulating concentrations increased from 68.9 $\pm$ 8.2 nmol/L to 90.1 $\pm$ 6.0 nmol/L ($p<0.05$). Infants of mothers receiving 4,000 IU daily had increases in total serum 25(OH)D ranging from 33.4 $\pm$ 8.2 to 76.9 $\pm$ 12.5 nmol/L ($p<0.01$). The corresponding values for their mothers increased from 82.1 $\pm$ 6.0 nmol/L to 111.1 $\pm$ 9.7 nmol/L ($p<0.04$) [27]. Overall, the results showed total increases above the recommended cutoffs for adequacy of 50 nmol/L for both mothers and infants. This evidence supports the claims by the

CPS, who warrant daily vitamin D supplementation of 2,000 IU to pregnant and lactating women, to maintain adequacy [6].

2.6 Knowledge gap: vitamin D metabolism in obesity and gestational weight gain

There are many physiological differences in pregnant women, as well as overweight or obese individuals, when compared to non-pregnant people within a healthy BMI range. A common denominator between these groups includes a higher than normal total body weight, which translates to them having higher blood volumes and larger adipose stores [39]. The two main metabolic mechanisms of action proposed explaining the lower circulating concentration of 25(OH)D in obese/pregnant individuals will be addressed in this review. These include sequestration of vitamin D in adipose tissues, and volume dilution [7].

2.6.1 Mechanism of action: sequestration in adipose tissues

The exact mechanism(s) of action pertaining to sequestration of vitamin D into adipose tissues have not been scientifically proven. There are some suggestions, which will be mentioned shortly, but these mechanisms would require further research studies to determine their validity.

UVB exposure and the impacts of vitamin D synthesis and bioavailability in obese individuals has been addressed in an in vitro study by Wortsman et al. (2000). A total of four skin grafts – two from obese subjects (one old, and one young), and two from non-obese subjects (again, one young and one old) – were obtained surgically and exposed to sunlight for the same duration of time. Vitamin D_3 and its precursor, 7-dehydrocholesterol, were analyzed in all layers of the skin samples using high performance liquid chromatography. The authors found that the contents and conversion percentage of vitamin D_3 and its precursor did not

differ significantly between obese and non-obese subjects [38]. These findings, along with the aforementioned findings by Wortsman et al. (2000) (regarding serum 25(OH)D response in both obese and non-obese subjects after exposure to UVB rays), stipulate that obesity does not hinder the skin's ability to synthesize vitamin D_3; rather, it decreases its capacity to release vitamin D into circulation once formed cutaneously [38]. This is likely due to vitamin D's fat-soluble nature, allowing it to easily sequester into subcutaneous adipose tissues.

Comparably, not only will vitamin D sequester in adipose tissues after synthesis in the skin, but it may also do so when ingested and metabolized via exogenous sources. As formerly mentioned, after vitamin D is ingested and absorbed into the intestinal enterocyte, it gets packaged into chylomicrons along with lipoproteins, triglycerides and cholesterol, and travels through the lymphatic system [7]. These chylomicrons are then released into the peripheral circulatory system, where they come into contact with tissues that express lipoprotein lipase – an enzyme that hydrolyzes triglycerides to release free fatty acids into circulation [73]. During this hydrolysis process, it is possible that some of the vitamin D is released along with the free fatty acids and are taken up by adipose tissues, leaving behind less vitamin D in the chylomicron remnant upon return to the liver for further degradation and absorption (refer to **Figure 1.1**) [7]. This holds true given that adipose tissues serve as a hub for vitamin D_3 storage due to its hydrophobic structure [7, 48].

Poor vitamin D status has also been associated with non-alcoholic fatty liver disease (NAFLD), although the exact mechanism is unknown. Lee et al. (2015) demonstrated that gradual weight loss improved serum 25(OH)D concentrations in NAFLD patients [74]. However, it remains to be determined if this is due to improved liver function (as it is postulated that

increased intrahepatic fat content could hinder the liver's ability to convert cholecalciferol to 25(OH)D), or vitamin D becoming more available after being released from adipose stores as per the sequestration theory [74].

2.6.2 Mechanism of action: volume dilution effects

The other proposed mechanism by which vitamin D status is lower in people with a larger body weight is due to a volume dilution factor. In other words, higher blood volumes and higher amounts of adipose tissues dilute the concentration of vitamin D [11, 12, 39]. The study by Drincic et al. (2012) is a highly cited article arguing that volume dilution, as opposed to adipose sequestration, is the main reason for low vitamin D status in obese individuals. The authors use mathematic models to adjust for body size – specifically, a linear and a hyperbolic dilution model, to test their hypothesis that serum 25(OH)D is inversely related to body size [12].

3. Gestational Weight Gain

3.1 Current recommendations

A woman's weight, prior to and throughout gestation, is a large determinant of the health status of both the mother and fetus [75]. Potential adverse outcomes for the mother if she exceeds her weight gain recommendations include gestational diabetes, pregnancy induced hypertension and preeclampsia, labor and delivery complications, increased risk for cesarean delivery, weight retention post-partum and trouble breastfeeding [13, 75]. Possible metabolic outcomes to the fetus include macrosomia and/or large for gestational age infants, fetal programming, increased likelihood of childhood obesity with its related sequelae, increased morbidity and mortality of the neonate including preterm and stillbirth [13, 75].

Health Canada has compiled guidelines for expecting mothers to achieve a healthy weight gain throughout their gestational period. These recommendations are based largely on the IOM's 2009 revised guidelines of 'Weight Gain During Pregnancy' [75]. The total recommended weight gain, and rate of weight gain, is based on the mother's pre-pregnancy BMI, which are referenced from the World Health Organization's (WHO) international BMI categories (**Table 1.4**) [13, 76]. The Canadian guidelines use only the four main WHO BMI categories; underweight, normal weight, overweight, and obese [75]. Please refer to **Table 1.4** for the gestational weight gain recommendations for pregnant women in Canada [75].

There has been some debate regarding the WHO international BMI categories and application to different ethnic groups. Some populations may incur adverse health outcomes with a BMI below the 25 kg/m^2 cutoff due to differences in body composition/proportions [76]. The main population being Asians (countries including Thailand, Singapore, Japan, Indonesia, China, Philippines, Korea) and the major health outcomes scrutinized are type 2 diabetes and cardiovascular disease [77]. A cross-sectional study of a cohort of 490,288 adults (40 – 69 y) conducted in the United Kingdom was able to establish BMI cutoffs for obesity and diabetes risk in different ethnic populations [78]. The proposed cut-points for obese women, when compared to the 30 kg/m^2 in white populations, are as follows: 22 kg/m^2 for South Asians, 26.0 kg/m^2 for Blacks, and 24.0 kg/m^2 for Chinese [78]. Additionally, the WHO expert consultation proposed that a BMI of 23 – 27.5 kg/m^2 poses an increased risk of adverse health effects for the Asian population, and high risk is seen in BMI's >27.5 kg/m^2 [77].

Table 1.4: Canadian BMI Categories & Gestational Weight Gain Recommendations

Pre-pregnancy BMI	Recommended Weight Gain
<18.5 (underweight)	12.5 – 18 kg
18.5 – 24.9 (healthy/normal weight)	11.5 – 16 kg
25.0 – 29.9 (overweight)	7 – 11.5 kg
>30.0 (obese)	5 – 9 kg

BMI = Body Mass Index
Adapted from the Public Health Agency of Canada [79].

3.2 National trends in gestational weight gain

There has been an observed change in Canada's demographics over the years. Obesity rates are rising, the population is aging, and there is ever growing ethnic diversity with 1 out of every 5 people being foreign born [80]. As per the 2006-2007 Canadian Maternity Experiences Survey (MES) conducted by Statistics Canada, more women of childbearing age are entering pregnancy with a higher BMI than previous years [75, 81, 82]. Specifically, of the women surveyed (6421 women aged 15 years and over, with singleton live births), 21% and 13.6% of women were considered overweight or obese prior to conception, respectively [75, 83, 84]. Additionally, many of these women were unable to return to their pre-gravid BMI state postpartum; 25.6% were overweight, and 17.7% were considered obese at 5 to 14 months postpartum [83].

The average total weight gain throughout pregnancy as per the MES was 15.7 kg [75]. When looking explicitly at the BMI categories and the recommended weight gain based on preconception BMI (seen in **Table 1.4**), it is evident that most women surveyed did not follow these guidelines. The recommended weight gain for underweight women is 12.5 to 18 kg and of the women in the study, 37.2% exceeded those recommendations, with 41.1% meeting them and 21.8% falling short [75, 83]. Only 23.8% of women with a normal pre-gravid BMI met the recommended weight gain of 11.5 to 16 kg, where 52.2% of them exceeded the weight gain reference and 24.1% gained less [83]. In terms of overweight women, 26.8% gained the appropriate amount of weight during pregnancy (7 to 11.5 kg) and a staggering 67.6% of these women gained more weight than recommended, with 5.7% who gained less [83]. Finally, for obese women, whose recommended weight gain is 5 to 9 kg, at least 19.8% women met these

targets, with 47.2% exceeding them. The remaining 33% gained between 7 and 11.4 kg and could therefore fall in either of the two categories mentioned [75, 83].

The MES also sheds light on maternal age, ethnicity, and socioeconomic status, as additional determinants of gestational weight gain in Canada. Kowal et al. (2012) used secondary data from the MES to define some of these relationships. Women who were more likely to gain weight exceeding Health Canada's recommendations consisted of the following: single Canadian born mothers of low socioeconomic status; less than a high school education; lower-middle and middle income; high pre-gravid BMI; unmarried; as well as women with two or more pregnancies [84]. On the other hand, women who had inadequate weight gain throughout pregnancy consisted primarily of immigrant woman, multiparous mothers, smokers, unplanned pregnancies, mothers in the lowest and highest income groups, and those who had less than six prenatal healthcare visits [84]. Additionally, Lowell & Miller (2010) determined that younger women (15 to 19 years of age) were more likely to gain excess weight compared to older women (35 to 39 years of age); 56% vs. 35%, respectively [85]. Aboriginal women were also more likely to gain more weight during pregnancy than non-Aboriginals (55% vs 44%, respectively) [85]. When looking at differences between provinces and territories, 50% of women living in the Atlantic region of Canada, as well as 44% in the Prairies, 42% in Ontario, 41% in British Columbia, 39% in Quebec, and 37% in the territories, are gaining more than what is recommended [85]. It is apparent that a large portion of women across Canada are entering pregnancy with a high BMI, and/or are unable to meet appropriate weight gain targets throughout their pregnancies.

This poor compliance may be attributed to a lack of knowledge translation from health professional (obstetrician, midwife, family doctor or other) to client/patient. McDonald et al. (2011) conducted a cross-sectional survey in 310 pregnant women in Hamilton Ontario to determine what information regarding GWG was being told to these women. Only 28.5 % (95% CI, 23.5%-33.6%) reported receiving information from their healthcare provider about weight gain recommendations, with only 12% being counselled properly (based on the IOM guidelines) [86]. Additionally, a common misconception that women tend to follow during pregnancy is the concept of "eating for two", which can lead to overconsumption of energy and subsequently excess weight gain. Okesene-Gafa et al. (2016) surveyed a multiethnic cohort of 422 pregnant women in New Zealand, where 63.1% of women who consumed more calories during pregnancy stated the reason was because they were "eating for two" [87].

With regards to energy intake, the DRIs have been established for pregnancy, and predictive equations calculating Estimated Energy Requirements (EER) have been developed by the IOM for each trimester of pregnancy (**Table 1.5)** [13]. These guides have been set to ensure sensible energy intakes to support both mother and fetus throughout the gestational period, while avoiding excessive or inadequate weight gains [13]. These predictive equations also take into consideration physical activity levels of the individuals. Cohen et al. (2010) looked at the energy intake, physical activity, and gestational weight gain of 81 pregnant women living in Ottawa and Montreal. Their findings revealed that the average gestational weight gain was higher than Health Canada's recommendations [88]. This was due to the fact that most of the women (70.5%) were sedentary or of low activity patterns, and 57% of the women were consuming higher than their recommended EER. Cohen & Koski (2013) also investigated the

Table 1.5: Dietary Reference Intakes during Pregnancy for Adults (+ 19 years)

Trimester	Estimated Energy Requirement (EER) (kcal/day)
1st trimester	EER = 354 - (6.91 × age [years]) + PA* × [(9.36 × weight [kg]) + (726 × height [meters])] + 0
2nd trimester	EER = 354 - (6.91 × age [years]) + PA* × [(9.36 × weight [kg]) + (726 × height [meters])] + 340
3rd trimester	EER = 354 - (6.91 × age [years]) + PA* × [(9.36 × weight [kg]) + (726 × height [meters])] + 452

*PA = Physical Activity Coefficient; Sedentary = 1.0, Low Active = 1.2; Active = 1.27; Very Active = 1.45
Adapted from IOM [13].

compliance of achieving a healthy gestational weight gain by following physical activity and energy intake guidelines in 54 healthy, non-obese women from Ottawa and Montreal. Fifty-four percent of the women exceeded their EER, and 61% surpassed the gestational weight gain recommendations based on their pre-gravid BMI [89].

Looking at physical activity during pregnancy, the Canadian Society for Exercise Physiology (CSEP) provides general guidelines for healthy, low risk pregnancies. These are similar to non-pregnant adults aged 18-64 years, where one should participate in at least 150 minutes of weekly physical activity of mild to moderate intensity [90, 91]. The 150 minutes should primarily be aerobic in nature, but should include strength training as well for optimal musculoskeletal health [90]. Physical activity during pregnancy can become more challenging as pregnancy progresses due to the growing fetus, adding more weight and disturbing normal physiological movements/functions. It is always advised for pregnant women to speak to their medical doctors to ensure it is safe for them to participate in physical activity. The PARmed-X for pregnancy developed by CSEP, the Society of Obstetricians and Gynecologists of Canada and Health Canada, can also be used by health care professionals to provide physical activity prescriptions that are safe and individualized for pregnant women who were active prior to pregnancy [92]. Other general physical activity guidelines are provided in the Sensible Guide to a Healthy Pregnancy, developed by the Public Health Agency of Canada [79]. These guidelines are vaguer than the CSEP guidelines, but are encouraging for all low-risk pregnancies, regardless of physical activity prior to pregnancy.

Participating in regular physical activity during pregnancy may also be beneficial for the expecting mothers' vitamin D status. Wanner et al. (2015) looked at associations between

physical activity in adults and vitamin D using data from the United States 2003-2006 National Health and Examination Survey. Their findings showed a modest increase in serum 25(OH)D concentrations of 0.8 nmol/L in individuals who participated in 10 minutes per day of moderate to vigorous physical activity – regardless if conducted indoors or outdoors, was self-reported or measured – compared to inactive individuals [93]. This result is quite small and may not have substantial implications on status. However, Hibler et al. (2016) found a 3.8 nmol/L increase of serum 25(OH)D concentration for every hour increase of moderate to vigorous activity per week in adults when compared to sedentary individuals (95% CI 1.09–1.98; p=0.001) [94].

3.3 Body composition

3.3.1 Weight gain during pregnancy

Many physiological changes occur during pregnancy to account for fetal growth and development. Notably, a woman who becomes pregnant will produce three main 'external' entities that contribute to approximately 35% of total gestational weight gain: the fetus, the placenta, and amniotic fluid [13]. The remaining components of gestational weight gain that influence maternal body composition consist mainly of fat, protein, mineral and water; which are deposited into mammary glands, the uterus, blood, adipose tissues, as well as the fetus and placenta [13, 95]. Total body water (TBW), adipose tissues and the fetus itself, are the largest constituents of gestational weight gain, and will have the greatest effect on total weight gained [75]. TBW, comprising of both intracellular and extracellular fluids, is quite variable amongst women as it is controlled hormonally [13]. The majority of protein deposition occurs in the developing fetus in late pregnancy, with additional accumulation in the breasts, uterus, blood and placenta [13]. Fat mass accretion is inevitable in pregnancy and will mainly deposit

subcutaneously in the upper thighs, hips and dorsal areas [13]. Butte et al. (2003) measured the components (i.e., TBW, fat mass, fat free mass (FFM; representing lean mass and bone)) of gestational weight gain in 63 women of all pre-pregnancy BMI groups pre-gravida, 9 weeks, 22 weeks, 36 weeks, and post-partum [95]. The distribution of TBW, FFM and fat mass during the gestational period were similar amongst BMI groups; however, higher fat mass accretion and ultimately higher weight gains throughout pregnancy was apparent in overweight women [95].

3.3.2 Analysis of body composition

Body composition can be difficult to assess in pregnant and lactating women as there are numerous entities that can affect measurement techniques, including the invalidity of the techniques in this population. These will be outlined in this section, with focus on bioelectrical impedance analysis (BIA) and dual-energy x-ray absorptiometry (DXA).

BIA is a rapid, inexpensive, non-invasive method used to measure body composition, and is safe for use during pregnancy and postpartum. It is a two-component body composition model that has the ability to estimate an individual's FFM, fat mass, and TBW based on measurements of resistance and reactance to a weak electrical current [96]. The low amplitude (~800 mAmp), high frequency current passes through the aqueous components of the body when skin comes in contact with the conductive surfaces of the BIA machine [96]. The human body, particularly FFM, is largely made of water and ions that can conduct a current; whereas fat mass is anhydrous and will impede electrical flow [97]. The FFM compartments of the body consist of intra and extracellular fluids (together forming TBW), visceral proteins, and bone mineral content [96]. Predictive equations are then used to estimate TBW and FFM using sex, age, height, and weight as parameters. Fat mass can then be calculated as the difference

between the two (i.e., fat mass = total body mass – FFM) [97]. Taking BIA measurements does not require extensive training, and the results are available immediately. These readings are easy to reproduce with a minimal error (<1%) on repeated measurements and have minimal inter-intra reader variability [96, 97].

As the current conducts through body water and its ionic content, the hydration and electrolyte status of an individual can alter the precision of the results [98]. Thus, intakes of fluids and electrolytes, or individuals with fluid and electrolyte shifts (i.e., people with edema, ascites, or are pregnant and lactating), can impact the validity of impedance measures [96]. Additionally, there are other general guidelines suggested to obtain proper measurements including (but not limited to): fasting (no food, drink or caffeine) for at least 4 hours prior to the test; no exercise on the day of the test, no alcohol consumption for a minimum of 8 hours prior to the test; and removal of all metal items from the body [96]. As previously mentioned, maternal aspects of weight gain in pregnancy consist mainly of fluids and fat mass. As the focus of this review pertains to gestational weight gain, it is important to scrutinize how fluid shifts that occur during pregnancy (i.e., expanding blood volume, amniotic fluid, edema, mammary glands, etc.), will affect body composition analysis using BIA. These maternal physiological changes invalidate the assumptions the BIA methodology uses in terms of the hydration status of FFM [99].

Upon reviewing the literature, it seems the multi-frequency BIA, when compared to other commercially available single frequency BIA, would be most appropriate to use in pregnant and lactating populations [100-102]. The single frequency BIA uses a 50 kHz current that is transferred through surface electrodes found on foot-to-foot, hand-to-hand, or hand-to-

foot BIA models [96]. These models are not recommended for use in people with altered fluid balance as they cannot differentiate between intracellular and extracellular water, and thus measures TBW only, along with FFM and fat mass. Conversely, the multi-frequency or segmental BIA, can differentiate between both intracellular and extracellular water components as it uses a variety of alternating currents (5 to 200 kHz) to measure them, along with TBW, FFM and fat mass, by using electrodes at various sites of the body [96]. This way, it can analyze different segments of the body separately; this can be useful in postpartum and pregnant women as the trunk of the body is where most of the fluid alterations occur [103].

Despite which BIA model is used, it must not be forgotten that the predictive equations developed to determine body composition using this type of analysis were established in non-pregnant and non-lactating women, and are therefore not compatible for use in these individuals. Few studies have looked at establishing alternative/substitute predictive equations in pregnant and lactating women. Shaikh et al. (2013) developed predictive equations for postpartum women, but they pertain to Bangladeshi population only and have not been officially validated [104]. Hopkinson et al. (1997) used corrected constants for hydration in two-component BIA models to account for differences in pregnant women in Houston Texas; these constants have not been validated either [99].

DXA is a three-component model to measure body composition and is most comparable to the gold standard of body composition analysis; hydrostatic weighing [105]. When performing a whole body analysis, it measures fat mass, FFM (with the ability to differentiate lean mass and bone), bone mineral content (BMC), as well as calculating bone mineral density (BMD). This is done by comparing the attenuation of two ionizing radiation energies; where

bone attenuation is greater than soft tissues [106, 107]. This method of measuring body composition exposes the individual to low levels of radiation; around 3-6 micro Sieverts (μSV) for a whole body scan [106]. This amount is even smaller than the equivalence of one-day natural radiation exposure (general public safety limit is 100 μSV per day), therefore, it is not associated with major side effects [108]. Although the amount of radiation emitted is fairly low, patients still get exposed, which has implications in health. Using a DXA scan is not warranted in pregnant women, as radiation could cause harm to the fetus [106]. Hence, further discussion regarding DXA analysis will pertain solely to postpartum/lactating women.

DXA machinery assumes that lean mass is hydrated at a constant 73.2% in all whole body measurements [106]. Therefore, if the participant being scanned has altered hydration status, then DXA can be limiting; which can be the case for postpartum lactating women. DXA has not been validated in lactating women due to this regard. The area of the DXA scanning bed has a specific width and length (67 cm and 195.5 cm, respectively, in The Hologic Discovery A machine) [106]. Therefore, larger individuals (obese, tall, postpartum) may not fit within these limits and they will need to be placed in a non-standardized manner which reduces the accuracy of the measurements [109]. DXA analysis has a short scan time of 3 minutes, where the patient is required to remain still. This can be difficult for individuals with conditions such as intrinsic tremors, or postpartum women with stiches or who are recovering from cesarean sections. Conversely, someone who simply cannot move a part of their body to obtain proper positioning, also limits the accuracy of the results [110]. Another limitation of using DXA for measuring body composition regards inter/intra-operator variability. The operator of the DXA machine has to follow specific guidelines from the manufacturer, as well as from the institution

where the analysis is performed, in order to position, operate, and analyze the scans in a standardized way. Despite similar training, there can be variation in analyses from one operator to another, which may lead to differences in accuracy of the results [111]. The precision and least significant change of DXA analyses have many variables including: the type of machine being used, the patient population tested, the site measured, the skill of the operator, and the software used for analysis [108].

Another method for estimating body composition is by skinfolds measurements. Skinfolds are used to predict body fat levels by measuring the subcutaneous fat at various sites of the body using a skinfold caliper. Once the measurements are obtained using standardized methods, they can then be used in predictive equations to assess for body fat mass, percent body fat and fat FFM, with a standard error of only 2.6% to 3.6% if measured correctly [112, 113]. Skinfold measures are simple, safe, require little equipment, are portable, non-invasive, and are cost efficient. However, they lack the precision to demonstrate slight changes in fat mass, as one would see throughout the gestational period [13]. Additionally, the results obtained from this method are based on assumptions; where the relationship between subcutaneous fat mass and total body fat is known, and that skinfolds taken at various body sites represent the total subcutaneous adipose tissue mass [114]. The regression equations that were developed were validated for individuals with average amounts of body fat, from specific age ranges, and ethnicity (mainly white) [115, 116]. Therefore, it would be inaccurate to generalize these equations to other populations such as very lean people, athletes, obese, as well as pregnant and lactating individuals. Moreover, adequate measurements from skinfolds are difficult to reproduce, prone to human error, and requires a trained professional – the

depth of the caliper pinch, as well as the length of time the skinfold grip was held or released, also contributes to the variations in measurements [117]. Results can also vary if different equipment is used from one measurement time to another, as each caliper can differ in its calibration [113]. Taking accurate measurements is a task in itself, especially in the obese population where locating landmarks for some of the measurement sites (e.g., subscapula) can be quite difficult. Furthermore, when using skinfold predictive equations to calculate body fat mass, it leads to an underestimation of percent body fat in this population when compared with DXA [118]. These challenges could be reflected similarly if trying to measure pregnant or postpartum women.

4. Maternal weight gain and neonatal vitamin D status

As previously stated, the largest constituents of gestational weight gain are fat and fluid accumulations, and that vitamin D can likely be sequestered in adipose tissues and/or diluted in larger individuals. The current evidence regarding women exceeding gestational weight gain recommendations causes reason to believe that these women will likely have reduced bioavailability of vitamin D for non-adipose tissues, and will therefore have reduced vitamin D status on the basis of circulating 25(OH)D and that lower maternal-fetal transfer will ensue. Upon reviewing the literature on the associations between gestational weight gain and vitamin D status of the infant, it is evident that there is insufficient data to support valid conclusions. To the best of the author's knowledge, only one study conducted in Greece found no correlation between maternal weight gain and 25(OH)D status; however, it is unclear if they were referring to maternal or infant concentrations as the data was not shown [14].

Looking at associations between gestational weight gain and maternal vitamin D status, one study by Moon et al. (2016), found that higher pregnancy weight gain is associated with a lower serum 25(OH)D concentration in mothers in late pregnancy (34 weeks) when compared to women with normal weight gain (regression analysis: $\beta = -0.81$; 95% CI: -1.39, -0.22; $p=0.007$) [15]. A total of 829 pregnant mothers were enrolled in this study looking at effects of vitamin D supplementation of 1000 IU during pregnancy on serum 25(OH)D status at 34 weeks gestation. However, this study calculated pregnancy weight gain as the difference in weight between weights measured at 34 and 14 weeks gestation, and thus did not reflect total gestational weight gain. Additionally, this calculated weight gain was not compared against a reference value for recommended weight gain for gestational age (i.e., recommended weight gain velocity based on pre-pregnancy body mass index), but just as a comparison against the whole group of participants [15].

There have, however, been a few studies exploring relationships between pre-pregnancy BMI and neonatal and maternal vitamin D status. Josefson et al. (2013) conducted such a study in a cross-section of 61 mother-infant pairs in Chicago. All women participating were free of chronic illness (including gestational diabetes), had singleton term pregnancies, multiethnic (self-reported black/African American, white or other) and reported taking prenatal vitamins [16]. The dose, vitamin D content, and compliance was not recorded for the prenatal vitamins. Dietary analysis of vitamin D intake and exposure to UVB rays for estimation of endogenous synthesis was not taken into consideration in their analyses. Their findings showed that women who were in the pre-gravid obese BMI category, transferred less 25(OH)D to their infants when compared to mothers with similar serum 25(OH)D concentrations, but with a pre-

gravid BMI in the normal/healthy range (cord 25(OH)D of 68.5 $\pm$ 4.2 nmol/L for normal BMI vs. 51.9 $\pm$ 5.8 nmol/L for obese) [16].

Similarly, Bodnar et al. (2007) studied the impact of maternal pre-gravid BMI and its effect on maternal and infant vitamin D status after adjusting for ethnicity, season, gestational age, physical activity, maternal age, and prenatal vitamin use. A total of 398 maternal-infant pairs of non-Hispanic white or non-Hispanic black ethnicity from Pennsylvania were assessed. Pre-gravid BMI was obtained by using self-reported pre-pregnancy weight and measured height; serum 25(OH)D was collected at 4 to 22 weeks' gestation and pre-delivery; and cord blood was collected at delivery [17]. There were significantly more mothers of non-Hispanic black ethnicity in the obese category compared to non-Hispanic white (around 63% vs. 37%, respectively). All mothers had similar multivitamin use in the last 3 months of pregnancy, but this study also lacked data regarding the amount of vitamin D in the supplements, intake of vitamin D containing foods, and endogenous synthesis [17]. Their results revealed that women with a pre-pregnant BMI ≥30 kg/m^2 had significantly ($p<0.05$) lower serum 25(OH)D concentrations compared to leaner women with BMI <25 kg/m^2 at 4 to 22 weeks gestation (adjusted mean of 55.9 nmol/L vs. 62.8 nmol/L, respectively) and at term (adjusted mean of 60.2 nmol/L vs. 67.3 nmol/L) [17]. Additionally, obese women gave birth to neonates with significantly lower cord 25(OH)D concentrations compared to leaner mothers (adjusted mean of 49.9 nmol/L vs. 56.2 nmol/L). Vitamin D insufficiency (25(OH)D <50 nmol/L) was also found in 61% of obese mothers, and in 58.6% of offspring born to obese mothers [17].

Dror et al. (2011) observed relations between pre-pregnancy BMI (calculated using reported height and pre-pregnancy weight) and cord blood 25(OH)D concentrations in 210

mother-infant pairs in Oakland California, after adjusting for vitamin D intake from foods and supplements, skin exposure to sun, skin pigmentation, season and latitude. A total of 48.1% of women studied had a pre-gravid BMI >30 kg/m^2, both maternal and infant cord sera 25(OH)D concentrations were significantly ($p = 0.02$) associated with a higher BMI [119].

4.1 Study objectives

Total weight gained throughout gestation – despite maternal pre-gravid BMI – and its impact on vitamin D status in both neonate and mothers, has not been extensively studied. The objective of this study is to explore relationships between maternal GWG and newborn infant vitamin D status. It is hypothesized that mothers who exceed their GWG recommendations as per Health Canada's guidelines, will have infants born with low vitamin D status.

Secondary objectives include:

1) explore relationships between maternal GWG and maternal vitamin D status;

2) determine associations between pre-gravid BMI and maternal and infant vitamin D status; and

3) explore how maternal body composition can influence vitamin D status of both mothers and infants.

This study could also provide valuable insight on vitamin D status of newborn infants and postpartum mothers, as well as maternal perinatal anthropometrics, in the greater Montreal area, as data on this topic are scarce.

2.0 MANUSCRIPT

Effects of gestational weight gain on neonatal vitamin D status

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2.1 Abstract

Introduction: It is unknown if newborn vitamin D status is dependent on maternal gestational weight gain (GWG).

Objective: Explore relationships between maternal GWG and newborn vitamin D status.

Methodology: Healthy mother-infant pairs (n=59) were recruited 24-36 hours post-partum from greater Montreal area (clinicaltrials.gov: NCT02563015). Surveys captured demographics and supplement use. Vitamin D intake was assessed using a food frequency questionnaire. Infant and maternal blood samples were collected to assess serum 25-hydroxyvitamin D [25(OH)D] concentrations. Maternal perinatal and infant birth anthropometric measurements were obtained from medical records. Data were analyzed in 3 groups: mothers with adequate (n=17), inadequate (n=18) or excessive (n=24) GWG as per Health Canada's recommendations. Mixed model ANOVAs and linear regressions were performed (SAS, v9.3). Data are mean ± standard deviation unless otherwise specified.

Results: Maternal pre-gravid body mass index was 24.7 ± 5.1 kg/m^2, infant birth weight-for-age Z-score was 0.2 ± 0.8 and 54% were male. Infant serum 25(OH)D concentration was 40.9 ± 18.9 nmol/L (29% <30 nmol/L, 42% <50 nmol/L). Maternal serum 25(OH)D concentration was 62.7 ± 25.8 nmol/L (7% <30 nmol/L, 35% <50 nmol/L). Maternal-infant pairs serum 25(OH)D concentrations were correlated (r=0.74, p<0.0001). Infant 25(OH)D concentration was lower if born from mothers with excessive vs. adequate GWG (p=0.02). Mothers' status did not differ amongst GWG categories. In regression analysis (R^2=0.57), neonatal serum 25(OH)D was 15.2 nmol/L higher if born from white mothers and 4.2 nmol/L higher for each 10 nmol/L increment in maternal serum 25(OH)D.

Conclusion: These preliminary results suggest that mothers with excessive GWG are more likely to have infants with vitamin D insufficiency at birth.

2.2 Introduction

Within the field of nutrition, vitamin D is considered an essential nutrient needed for healthy growth and development. It has particular functions in musculoskeletal health and fetal programming, mediated by the presence of vitamin D receptors in the nuclei of various cells and tissues, allowing or inhibiting transcription of vitamin D responsive genes [1-3]. Apart from endogenous synthesis via ultraviolet beta (UVB) radiation of the sun, vitamin D can be obtained exogenously through dietary and/or supplemental intakes [2]. In Canada however, sun exposure is limited due to its northern latitude, and despite vitamin D fortification of fluid milk and margarine mandated by the Government of Canada, 32% of Canadians have serum 25-hydroxyvitamin D (25(OH)D) concentrations that are insufficient (below 50 nmol/L) [5]. This urges the majority of Canadians to use supplements to meet their requirements to avoid deficiencies. Serum 25(OH)D cutoffs used in population assessments in Canada and the US are ≥50 nmol/L for sufficient, 30 to 49.9 nmol/L for insufficiency and <30 nmol/L for deficiency [7].

A particular group where vitamin D inadequacy and need for supplementation is common is pregnant women [6]. Subsequently, their newborn offspring can be born with low circulating concentrations of serum 25(OH)D, as *in utero*, infants rely completely on transplacental transport to obtain vitamin D [6, 8]. Several studies identifying vitamin D status of pregnant women were performed across Canada [42-46]. The weighted average of proportions of these studies estimates that 29% of pregnant women in Canada have insufficient vitamin D status [43-46]. The Recommended Dietary Allowance (RDA) of vitamin D for pregnant women is identical to those of adults who are not pregnant; 600 IU per day, with at least 200 IU

expected to be supplied in prenatal supplements [6, 7]. These recommendations do not account for increased demands for fetal growth and development.

Among the modifiable factors that can affect vitamin D status, there is growing evidence that vitamin D requirements are weight dependent [6, 10]. This can be due to vitamin D sequestration in adipose tissues as it is fat soluble, or from volume dilution effects in larger individuals [6, 11, 12]. These mechanisms can be applied to pregnant women as some of the largest constituents of gestational weight gain are increasing fat stores and fluid accumulation [13]. Health Canada has set gestational weight gain (GWG) recommendations based on pre-pregnancy body mass index (BMI) categories (BMI <18.5 kg/m^2 – underweight; BMI 18.5–24.9 kg/m^2 – normal weight; BMI 25.0–29.9 kg/m^2 – overweight; BMI ≥30 kg/m^2 – obese) [75]. As per the 2006-2007 Canadian Maternity Experiences Survey (MES), it is evident that most women do not follow these guidelines, with over 35% of all women gaining above their GWG recommendations [81-83]. Total weight gained throughout gestation and its impact on vitamin D status in both neonate and mothers, has not been extensively studied.

The objective of this study is to explore relationships between maternal GWG and newborn infant vitamin D status. It is hypothesized that mothers who exceed their weight gain recommendations as per Health Canada's guidelines, will have infants born with low vitamin D status.

2.3 Participants and methods

The data from this cross-sectional study was derived from baseline data for a double blinded randomized control trial (NCT02563015) conducted at McGill University. The participants consisted of newborn infants, screened with consent from their mothers for vitamin D status within 24-36 hours of birth from the Lakeshore General Hospital in Pointe-Claire, QC. Collection of 0.5 ml of capillary blood obtained by heel lance for measurement of serum 25(OH)D was done at the same time as routine blood draws in hospital for newborn screening (phenylketonuria). The sample size estimation was calculated based on the primary outcome; infant vitamin D status. As no study has been conducted looking at the effect of gestational weight gain on vitamin D status, the sample size was estimated using pre-gravid BMI and its effect on vitamin D status. A sample size estimate of 25 per group was set to enable detection of clinically meaningful group differences of 16.6 ± 20.9 nmol/L of serum 25(OH)D, at a 5% significance level with 80% power. The effect size was determined based on a sample of infants born to obese and non-obese moms in Chicago [16]. The standard deviation was obtained using data from the controls of a Quebec City cohort of otherwise healthy newborn mixed group [43]. Variation may be lower due to the use of capillary vs. cord blood [120].

Inclusion criteria for vitamin D screening were healthy term-born infants of appropriate weight for gestational age, born to an otherwise healthy mother of any race. Exclusion criteria included pregnancy complications, maternal diabetes, celiac disease, inflammatory bowel disease, hypertension/preeclampsia, hepatic or renal disease. For the randomized trial, infants were eligible for the treatment group with serum 25(OH)D <50 nmol/L, and the reference group with serum concentrations ≥50 nmol/L. Mothers had to be non-smokers, with intention to

breastfeed exclusively for a minimum of 3 months, and were not taking medications that influence vitamin D metabolism (with the exception of vitamin/mineral supplements). For the healthy reference group, infants were recruited if their mothers had a pre-gravid maternal BMI between 18.5 and 27.0 kg/m^2, whereas pre-pregnancy BMI was not an exclusion criterion for infants in the treatment category.

A general demographic survey was interviewer-given at screening to mothers regarding their supplementation and medication use during pregnancy, smoking status, physical activity, sun exposure, ethnicity, race, income and education, using the same descriptors as defined by Statistics Canada. Additional obstetrical information including pre-pregnancy and delivery weight, height, parity and length of gestation was gathered by the researcher from the medical chart. GWG was determined using Health Canada's recommendations based on pre-gravid BMI (BMI <18.5 kg/m^2 – 12.5 to 18 kg; BMI 18.5–24.9 kg/m^2 – 11.5 to 16 kg; BMI 25.0–29.9 kg/m^2 – 7 to 11.5 kg; BMI ≥30 kg/m^2 – 5 to 9 kg). Participants' contact information, names and birthdates, infant birth weight, length and head circumference were also collected. Infant birth weight-for-age, weight-for-length, length-for-age and head circumference-for-age Z-scores were calculated using World Health Organization software (WHO AnthroPlus, Switzerland). Season of birth was combined as 1) summer/fall (June 20, 2016 – December 22, 2016), and 2) winter/spring (December 22, 2016 – June 19, 2017). For participants eligible for the trial or reference group, study invitations were sent along with results in an email, with additional telephone follow-ups. For parents interested in joining the study, the baseline visit was scheduled at one week to one month postpartum. Infants and their mothers came to McGill

University's Mary Emily Clinical Nutrition Research Unit in Saint-Anne-de-Bellevue, QC. A study consent form was read and signed by the mother prior to collection of data at the visit.

A 145-item semi-quantitative food frequency questionnaire (FFQ) adapted from the Harvard Food Frequency Questionnaire (validated for vitamin D intake [121]) was given to mothers at baseline visit to reflect average vitamin D intake (including supplements) throughout the gestational period. All nutrient analysis was conducted using the 2016 Canadian Nutrient File database (Health Canada). One 5 ml sample of whole blood was taken from mothers at baseline visit by a registered nurse for measurement of serum 25(OH)D. Maternal height was measured shoeless using a wall mounted stadiometer (Seca 216, Seca Medical Scales and Measuring Systems, Hamburg, Germany), and weight was obtained using a balance-beam scale (Detecto, Webb, USA). Body composition (fat mass, percent fat, total body water (TBW) and lean body mass (LBM)) of the mother was assessed using a fan-beam dual-energy x-ray absorptiometer (DXA) with whole body software (APEX version 13.3:3, Hologic 4,500A Discovery Series, Medford, MA) and bioelectrical impedance analysis (BIA) (TANITA Body Composition Analyzer, TBF-310 model, Japan).

Total serum 25(OH)D concentration of the infant capillary sample and maternal whole blood was measured using a chemiluminescent assay; Liaison auto-analyzer (Diasorin Inc.). The sensitivity of the Liaison assay was 10 nmol/L for serum 25(OH)D concentration. Quality control measures included daily use of high and low kit controls, triplicate measurement of an internal laboratory serum sample. Inter-assay and intra-assay coefficients of variation (CV) for high and low assay controls were 6.4% and 6.2%, respectively. The laboratory maintains certification with the Vitamin D External Quality Assessment Scheme (DEQAS) and NIST vitamin D standards.

Ethics. All procedures followed were in accordance with the ethical standards of St. Mary's Hospital Center, Research Review Ethics on human experimentation, which oversees research approvals of the Lakeshore General Hospital. Informed consent was obtained by parents prior to participation in screening and at initial baseline visit.

Statistics. Data were summarized and interpreted in three groups: if mothers had inadequate, adequate or excessive weight gain based on Health Canada's recommendations for GWG established from their pre-gravid BMI category. All data analysis was conducted using SAS (Version 9.3, SAS Inst.). Data were tested for normality using the Kolmogorov-Smirnov tests. Non-normal data were log transformed prior to further analysis. Infants who had serum 25(OH)D values ± 3 standard deviations from the mean were excluded from analysis (e.g., below detection limit). Regression analyses were conducted accounting for fixed (GWG, infant sex) and random (maternal age, maternal vitamin D status, ethnicity, vitamin D intake, parity, season of birth, household income, physical activity during pregnancy, infant gestational age and birth weight-for-age z-score) effects to test their relationships to infant serum 25(OH)D concentrations, as well as maternal status. Crook's D, residual and collinearity diagnostics were used for interpretation of the regression analysis. Mixed model ANOVAs were also conducted to further explore relationships between GWG and infant, as well as maternal, serum 25(OH)D concentrations considering the same covariates. Additional mixed model ANOVAs were conducted to test for relationships between pre-gravid BMI on mother and infant vitamin D status, as well as testing for ethnic influences of status. Spearman correlations and linear regressions were conducted to test relationships between mother and infant vitamin D status; as well as looking for associations between maternal pre-gravid BMI and body composition and

vitamin D status of both mother and infants. Significant differences were accepted at $p<0.05$ after post hoc testing using Scheffe's method.

2.4 Results

A total of 603 infants and their mothers were screened at the Lakeshore General Hospital 24-36 hours postpartum. Of these mother-infant pairs who were eligible for further study, 68 were recruited. Some participants had missing data (FFQ not complete); thus, only 59 mother-infant pairs were included in the analysis (see **Figure 2.1** for flow diagram). Maternal and neonatal characteristics are shown in **Table 2.1**. Average pre-gravid BMI was 24.7 ± 5.1 kg/m^2; with 3% being underweight, 59% normal weight, 24% overweight, and 14% obese as per WHO cut-points. Mean maternal serum 25(OH)D was 62.7 ± 25.8 nmol/L, where 35% of women had vitamin D insufficiency (30-49.9 nmol/L), and 7% were deficient (<30 nmol/L). Most mothers were white (n=35, 59%), non-white mothers were either Black (n=5), Hispanic (n=5), Middle Eastern/ Arab (n=6), Southeast Asian (n=2), West Asian (n=1), Chinese (n=1), Metis (n=1) or Other (n=3). The majority of mothers were university educated (80%), met or exceeded the RDA of 600 IU for vitamin D intake during pregnancy (61%), took supplements containing vitamin D prior to conception (51%), were multiparous (63%), and had a household income greater than $50,000 (68%). By design, infants were term and appropriate for gestational age (mean gestational age: 39.7 ± 0.9 weeks with a mean birth weight-for-age- Z-score: 0.2 ± 0.8). Infants were predominantly male (54%), with 46% born in the summer/fall. Average neonatal serum 25(OH)D was 40.9 ± 18.9 nmol/L, where 42% had vitamin D insufficiency, and 29% were deficient. GWG groups based on pre-gravid BMI were relatively balanced with 17 mothers having inadequate weight gain, 18 with adequate gains, and 24 exceeding recommendations. A significant correlation was found when comparing vitamin D status of maternal-infant pairs ($r=0.74$, $p<0.0001$) (**Figure 2.2**).

Linear regression models were used to explore relationships between GWG and infant and maternal serum 25(OH)D concentrations, adjusting for covariates (**Table 2.2**). In the infant model, GWG category, maternal vitamin D status and ethnicity were significantly associated with neonatal serum 25(OH)D concentrations (R^2=0.57, p<0.05). Infant serum 25(OH)D concentrations would increase by 4.2 nmol/L for every 10 nmol/L increase in maternal concentrations, and would be 7.7 nmol/L higher if born to a white mother. In terms of GWG category, infants born to mothers with excessive compared to those with adequate GWG had 5.6 nmol/L lower concentration. Vitamin D intake and ethnicity were significantly associated with maternal serum 25(OH)D concentrations (R^2=0.32, p<0.05). Maternal serum 25(OH)D concentration was higher by 10.0 nmol/L in the second vs. first tertile and in the third vs. second tertile of vitamin D intake from all sources. Serum 25(OH)D was 15.2 nmol/L greater for white mothers compared to non-white.

Mean maternal and infant serum 25(OH)D concentrations are displayed in **Figure 2.3** according to GWG category and ethnicity. Significant differences for mean serum 25(OH)D concentrations between groups were found only for infants born to mothers with excessive weight gain compared to meeting GWG recommendations (ANOVA p=0.02). When mothers in the treatment group of the RCT with BMI >27 kg/m^2 were excluded from analysis, the same results were seen (ANOVA p=0.02). No differences were found among mothers' status according to GWG category (ANOVA p=0.44). An interaction between infant sex and GWG was significant (p=0.01); however, after post-hoc testing, differences were not significant. In terms of ethnicity, significant differences were found amongst ethnic groups (white vs. non-white) on

both maternal and infant serum 25(OH)D concentrations (ANOVA p=0.02 and p=0.03, respectively); however, no differences were seen between ethnicity and GWG categories.

Simple Spearman correlations were conducted to investigate associations between pre-gravid BMI and serum 25(OH)D concentration of both mothers and neonates. Significant correlations were only found in neonates (r=-0.34, p=0.008; maternal model: r=0.24, p=0.06). Further investigation accounting for covariates (maternal model covariates include maternal age, daily vitamin D intake, ethnicity, household income, pre-gravid physical activity and education; infant model covariates include infant sex, ethnicity, maternal vitamin D concentration, maternal physical activity, season of birth and infant birth weight-for-age z-score) using a linear regression (**Table 2.3**) and a mixed model ANOVA (**Figure 2.4**) showed no significant differences between pre-gravid BMI categories for mothers or infants (regression: R^2=0.30, p=0.08 and R^2=0.52, p=0.10, respectively; ANOVA: p=0.14 and p=0.06, respectively). Only maternal vitamin D status influenced infant serum 25(OH)D concentration (R^2=0.52, p<0.001 for every 10 nmol/L increase in maternal status, infant status would increase by 4.3 nmol/L), and similar to GWG model, vitamin D intake and ethnicity were significantly associated with maternal 25(OH)D concentrations (R^2=0.30, p<0.05; 10.8 nmol/L increase in status for every increase in vitamin D intake tertile, and 16.3 nmol/L increase in status for white mothers).

In terms of maternal body composition, neither fat mass, percent fat, fat mass index, TBW, nor android and gynoid indices (total mass, fat mass, percent fat, LBM) were correlated with maternal 25(OH)D concentrations; only whole body percent fat was associated with maternal 25(OH)D concentrations in regression (R^2=0.32, p=0.04). Lean body mass and lean

mass index were correlated (r=0.28, p=0.04; r=0.34, p=0.01, respectively); however, no significance was found upon further regression analysis considering covariates.

2.5 Discussion

The results of this cross-sectional study provide considerable insight into a rather novel topic. To the best of our knowledge, this is the only study to test for associations between GWG and vitamin D status of newborn infants and their mothers. Our findings suggest that excessive total weight gain in pregnancy (seen in 41% of participating mothers), regardless of pre-gravid BMI, results in infants being born with vitamin D insufficiency or deficiency. Interestingly, the same relationship was not reflected in mother's status despite the significant correlation between maternal-infant pairs serum 25(OH)D concentrations. The mechanism(s) explaining these findings requires further study. It can be speculated from the correlations done in our postpartum mothers that the volume dilution theory may be more fitting in this population as percent fat, and not total fat mass, was associated with maternal 25(OH)D concentration. As we saw significant correlations in our mothers with higher lean body mass and serum 25(OH)D concentration, it is plausible that there may be considerable metabolism of 25(OH)D at the skeletal muscle level due to the presence of vitamin D receptors [122].

It was surprising to see the large number of infants born with serum 25(OH)D concentrations <50 nmol/L; 71% of our newborns. This is a much greater prevalence than what was seen in a study conducted in Quebec City who used the same biochemical chemiluminescent assay (Liaison auto-analyzer, Diasorin Inc.) for total serum 25(OH)D analysis; 24% <50 nmol/L (n=1027) [9]. However, that cohort measured infant cord blood vs. capillary for serum 25(OH)D concentrations. On the other hand, our findings for maternal status did compare to another Quebec City cohort study (42% <50 nmol/L compared to 46%), who also used the Liaison auto-analyzer for serum 25(OH)D analysis [43].

The correlation between our mothers and infants was not as strong as previous studies, but serum 25(OH)D from our infants were obtained via capillary instead of cord blood which is commonly used in the literature; additionally, our maternal samples were obtained up to 1-month post-partum [51, 53, 54]. Factors that could further influence these results could be from contamination of maternal blood in cord samples, skewed values due to other detectable vitamin D metabolites and/or hemolysis. Despite the fact that our mother's samples were acquired up to 1-month after infant blood was drawn (mean 2.8 ± 0.8 weeks post-partum), it is likely still reflective of mother's status at delivery given the half-life of serum 25(OH)D of around 3 weeks [1, 24].

Although maternal status is influenced by vitamin D intake, the low infant status from our sample does not seem to relate to maternal exogenous vitamin D intake or seasons of birth, as these factors were similar between infants with either sufficient or insufficient status. We did see significant differences between ethnic groups (white vs. non-white) in terms of serum 25(OH)D concentrations of both mother and neonate. The elements that influence vitamin D status based on previous diverse population studies are skin pigmentation and ethnicity; both of which were self-reported and unmeasured in the mothers of our study [123, 124]. It can also be argued that infants may not require the same 50 nmol/L serum 25(OH)D concentration cutoff for adequacy as adults do. Thus, even if the infant is born below a serum 25(OH)D concentration of 50 nmol/L, they may not be at risk of developing unwanted health consequences associated with poor vitamin D status. Unfortunately, data are lacking on providing evidence for ideal vitamin D status of infants, thus adult ranges are used by default [1, 6].

It is evident that Health Canada's GWG guidelines were not adhered to by the majority of our participants as only 30% met their recommendations based on their pre-gravid BMI. This coincides with results from the 2006-2007 MES where 33.1% of women surveyed across Canada adhered to the guidelines [81, 82]. From our data, it cannot be determined what prior knowledge or education the mothers received during their perinatal care regarding adherence to GWG guidelines. Our mothers were predominantly university educated with good socioeconomic status, but one cannot assume they were provided accurate information. McDonald et al. (2011) conducted a cross-sectional survey in 310 pregnant women in Hamilton Ontario to determine what information regarding GWG was communicated to these women. Only 28.5 % reported receiving information from their healthcare provider about weight gain recommendations, with merely 12% being counselled properly (based on the IOM guidelines) [86]. Another study investigated the compliance of achieving a healthy GWG in 54 healthy, non-obese women from Ottawa and Montreal [89]. Fifty-four percent of the women exceeded their estimated energy requirements (EER) and 61% surpassed the GWG recommendations based on their pre-gravid BMI [89]. Cohen, Plourde & Koski (2010) looked at adherence to GWG guidelines of 81 pregnant women living in Ottawa and Montreal. Seventy-nine percent of the women received advice on GWG either through books, internet, physician or other health professionals (nurse, dietitian, midwife), but it is unclear if the advice was valid/based on IOM recommendations as excess weight gain did not differ between women who received or did not receive advice [88]. Additionally, it was stated in their results that the majority of participants thought an appropriate weight gain during pregnancy was 25-35 lbs (11.5-16 kg) without reference to their pre-gravid BMI [88].

Cohen, Plourde & Koski (2010) also looked at the impact of energy intake and physical activity on GWG in these women. Their findings revealed that the average GWG was higher than Health Canada's recommendations mainly due to the fact that most of the women (70.5%) were sedentary or of low activity patterns, and 57% of the women were consuming higher than their recommended EER [88]. A common misconception that women tend to follow during pregnancy is the concept of "eating for two", which can lead to overconsumption of energy and subsequently excess weight gain. A qualitative study conducted in Pennsylvania in 29 postpartum women who were overweight or obese prior to conception, found that those who exceeded GWG (n=18) did so due to sedentary lifestyle and "eating for two" [125]. Similarly, a multiethnic cohort of 422 pregnant women in New Zealand revealed 63.1% of the women studied who consumed more energy than recommended during pregnancy stated the reason was because they were "eating for two" [87].

There was a large proportion of women who had inadequate GWG (29%) in our study. These women and their infants had mean serum 25(OH)D concentrations that were slightly below those who had adequate weight gain, although these differences were not significant. It is difficult to determine what elements contributed to the overall lower status as mean vitamin D intake was similar across all GWG groups. A cohort of 710 pregnant Sri Lankan women were studied and determined the major risks of having inadequate GWG were due to sleep deprivation, low income, standing or walking for more than 5 hours in a day during the second trimester, being multiparous, or having a male infant [126]. From our data, mothers with inadequate GWG had similar income to other groups, had relatively balanced ratio of male and female infants, and only 53% were multiparous. There were 71% of infants in the inadequate

GWG group who were born in the winter/spring. It is possible there was a decreased endogenous synthesis factor for this subgroup, as UVB skin exposure has been shown to be a significant determinant in circulating 25(OH)D concentrations during pregnancy [127]. The MAVIDOS study in the United Kingdom found this occurrence in a subgroup of pregnant women giving birth in the winter or spring and were receiving a placebo instead of 1000 IU per day of cholecalciferol. They discovered a marked decrease in plasma 25(OH)D concentrations in these mothers from the 14^{th} to 34^{th} week of gestation (winter: 14 wk 25(OH)D 53.7 ± 16.1 nmol/L vs. 34 wk 25(OH)D 30.3 ± 15.6 nmol/L; spring: 14 wk 25(OH)D 43.6 ± 15.7 nmol/L vs. 34 wk 25(OH)D 32.9 ± 19.7 nmol/L) [127].

The association between pre-gravid BMI and vitamin D status appears stronger for our mothers compared to their infants. However, our results did not correspond to what has been previously seen in the literature. Our sample of mothers did not have equally distributed pre-gravid BMIs, where majority (59%) of mothers had a normal BMI prior to conception. This could help explain our results, as the proportions of women in overweight and obese categories were inadequately powered [16, 17, 119]. One aspect to note is that the pre-pregnancy BMIs for the GWG guidelines may not be suitable for use in different ethnic groups, as some populations may experience adverse health outcomes with a BMI <25 kg/m^2 due to differences in body composition/proportions [76]. The WHO expert consultation proposed that a BMI of 23 – 27.5 kg/m^2 poses an increased risk of adverse health effects for the Asian population, with higher risk seen in BMIs >27.5 kg/m^2 [77]. With that said, using lower cutoff's for different ethnic groups in our study may have resulted in more women being classified in the overweight and obese categories, potentially leading to more women exceeding GWG guidelines. Future

studies of larger sample sizes and greater proportions of non-white women would enable a more in depth analysis of whether use of ethnic-specific cut-points is necessary.

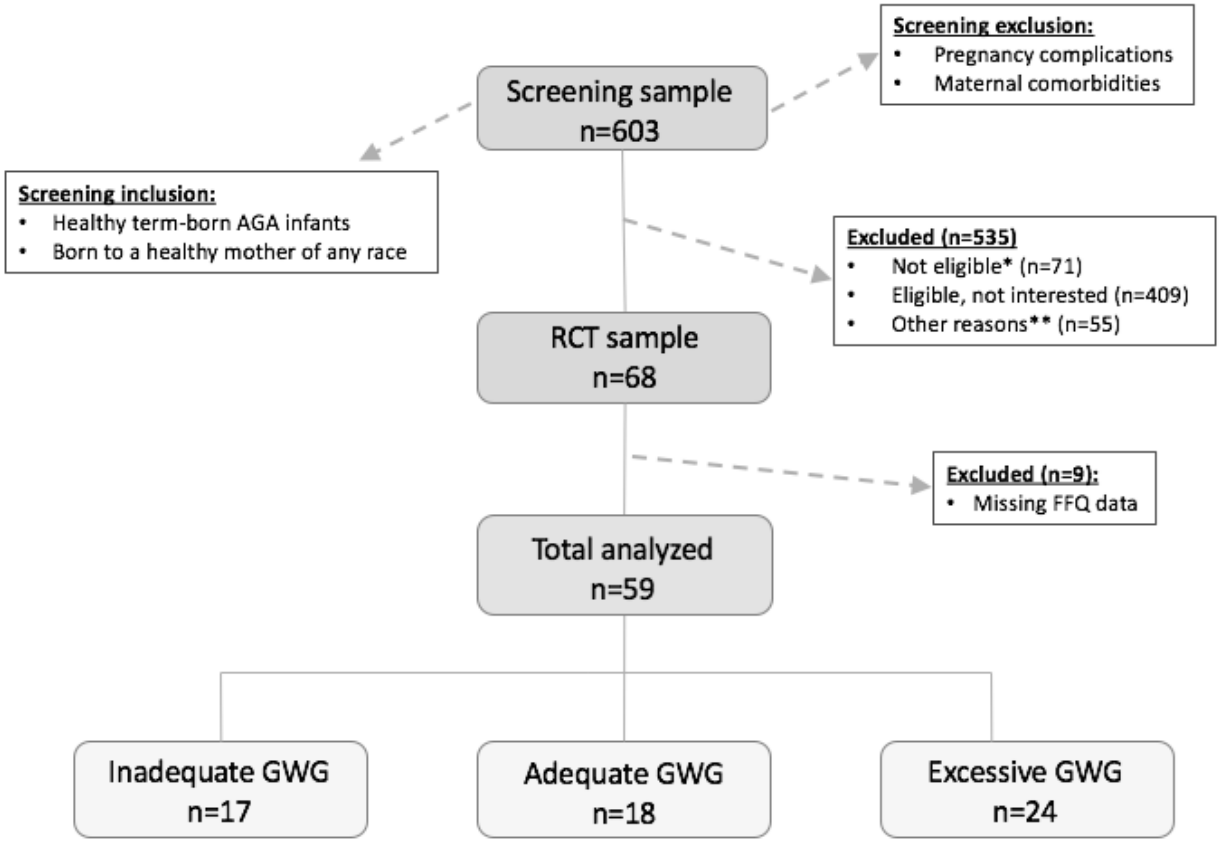
Most mothers were taking prenatal supplements containing vitamin D (93%), which may suggest our mothers have been exposed to better knowledge transfer of perinatal supplementation practices. Regardless of the high prevalence of mothers taking supplements, 42% and 71% of mothers and infants, respectively, still had serum 25(OH)D concentrations below 50 nmol/L. Given the accumulating evidence that pregnant and lactating women have higher vitamin D requirements than what is currently suggested, it would be advisable for the IOM to re-evaluate their recommendations in this population. Both fetal maturation *in utero* and increased maternal weight have been noted by the IOM as indicators that would require greater vitamin D requirements for mothers [7]. Thus, not only do pregnant women need more vitamin D based on fetal dependency and demand – especially in the third trimester when skeletal mineralization takes place – but also due to their expanding adipose stores and blood volumes throughout the gestational period [1]. These factors may not have been accounted for when the IOM set their references likely due to insufficient evidence in the literature.

As this study was based on screening data for entry into a randomized control trial, the study design was restricted, leading to some obvious limitations. For one, pre-pregnancy weight, height and weight at delivery were self-report from medical charts. It is possible, some mothers were categorized into pre-gravid BMI groups that were not reflective of their actual weight prior to conception. Vitamin D intake data was also self-report based on a food frequency questionnaire subject to recall bias and overestimation. Nonetheless, the major strength of this study is its novelty. It also provided valuable insight on the vitamin D status of

newborn infants in the greater Montreal area, suggesting that with 70% of infants with serum 25(OH)D below 50 nmol/L, that screening is not likely necessary.

Given the high prevalence of infants born with vitamin D insufficiency and mothers not meeting GWG guidelines in our study, more evidence-based guidelines and their active implementation are needed to improve the health of the maternal-infant population. Our results also shed some light on the influence of lean body mass on serum 25(OH)D concentrations, whereby higher maternal lean mass is attributed to higher 25(OH)D concentrations. This observation is consistent with prior studies and would benefit further exploration, as this relationship may have important implications in health [4, 128]. These findings are important to note since vitamin D deficiency can lead to a plethora of health related issues in the infant. Short term effects would mainly be on calcium homeostasis and the skeletal system, directly impacting bone growth and development [7]. Other metabolic aberrations – such as the development of certain autoimmune diseases, cancers, and cardiovascular disease – may only be detected in adulthood by ways of fetal programming, at which point early prevention is limited [3]. Additional research would be needed to determine if meeting or increasing the RDA with greater input on perinatal vitamin D supplementation, to account for other health outcomes aside from skeletal health, would be beneficial to the population. As for GWG, increased education strategies targeted to health care practitioners/perinatal coaches are warranted, in order to better disseminate accurate knowledge to expecting mothers on adherence to national guidelines.

Figure 2.1: Study flow diagram of participant recruitment



*smoker; not breastfeeding; taking medications that interfere with vitamin D metabolism
**Blood sample not collected; hemolysis of sample; inadequate blood volume to measure sample

Table 2.1: Baseline characteristics of mother-infant pairs

Maternal Parameters	Inadequate GWG (n=17)	Adequate GWG (n=18)	Excessive GWG (n=24)
Age at delivery (years ± SD)	32.2 ± 5.5	34.2 ± 4.0	30.6 ± 4.2
Weight gain (mean kg ± SD)	7.6 ± 3.6	12.4 ± 2.7	18.6 ± 3.8
Pre-gravid BMI (kg/m^2)			
Mean ± SD	23.4 ± 4.7	24.1 ± 4.7	26.1 ± 5.5
<18.5	1 (6%)	–	1 (4%)
18.5 – 24.9	12 (70%)	12 (67%)	11 (46%)
25.0 – 29.9	2 (12%)	4 (22%)	8 (33%)
≥30	2 (12%)	2 (11%)	4 (17%)
Body composition (mean ± SD)			
Fat mass (kg)	20.3 ± 7.4	23.4 ± 6.8	30.2 ± 9.1
% fat (%)	31.3 ± 5.6	32.2 ± 3.9	36.5 ± 4.9
LBM (kg)	41.0 ± 5.8	46.2 ± 7.2	49.2 ± 6.8
TBW (kg)	31.6 ± 2.6	33.3 ± 3.1	35.3 ± 3.3
Parity			
1st pregnancy	8 (47%)	3 (17%)	11 (46%)
2nd pregnancy	5 (29%)	7 (39%)	6 (25%)
3rd pregnancy	2 (12%)	4 (22%)	5 (21%)
4th pregnancy	2 (12%)	4 (22%)	2 (8%)
Household income (CAD)			
<$50,000	4 (24%)	3 (17%)	8 (33%)
$50,000 – 100,000	6 (35%)	6 (33%)	8 (33%)
>$100,000	6 (35%)	8 (44%)	6 (25%)
Declined answer	1 (6%)	1 (6%)	2 (9%)
Serum 25(OH)D (nmol/L)			
Mean ± SD	64.4 ± 26.5	67.7 ± 28.7	57.7 ± 23.1
Range	28.3 – 123.0	12.3 – 134.0	25.2 – 109.0
<30 nmol/L	1 (6%)	1 (6%)	2 (8%)
30-49.9 nmol/L	5 (29%)	5 (27%)	11 (46%)
≥50 nmol/L	11 (65%)	12 (67%)	11 (46%)
Vitamin D intake (mean IU ± SD)			
Tertile 1			
Total	479 ± 123	318 ± 189	395 ± 126
Supplements	245 ± 161	217 ± 214	269 ± 149
Tertile 2			
Total	686 ± 82	717 ± 79	671 ± 92
Supplements	496 ± 114	494 ± 163	465 ± 127
Tertile 3			
Total	1041 ± 119	1037 ± 202	934 ± 143
Supplements	619 ± 27	626 ± 276	665 ± 253
Vitamin D supplementation			
Pre-pregnancy	8 (47%)	11 (61%)	11 (46%)
During pregnancy	15 (88%)	16 (89%)	24 (100%)
University educated	13 (76%)	15 (83%)	19 (79%)
White	9 (53%)	13 (72%)	13 (54%)
Non-white	8 (47%)	5 (28%)	11 (46%)

Table 2.1 (con't): Baseline characteristics of mother-infant pairs

Infant Parameters	Inadequate GWG (n=17)	Adequate GWG (n=18)	Excessive GWG (n=24)
Gestational age (mean wk ± SD)	39.8 ± 1.0	39.6 ± 0.8	39.8 ± 0.9
APGAR score			
1 minute			
≥9	16 (94%)	15 (83%)	22 (92%)
<9	1 (6%)	3 (17%)	2 (8%)
5 minutes			
≥9	17 (100%)	18 (100%)	21 (88%)
<9	0	0	3 (12%)
Birth weight (mean kg ± SD)	3.2 ± 0.3	3.5 ± 0.4	3.5 ± 0.4
Birth weight-for-age Z-score	-0.2 ± 0.7	0.4 ± 0.9	0.3 ± 0.7
Sex			
Female	10 (59%)	6 (33%)	11 (46%)
Male	7 (41%)	12 (67%)	13 (54%)
Season of birth			
Summer/fall	5 (29%)	9 (50%)	13 (54%)
Winter/spring	12 (71%)	9 (50%)	11 (46%)
Serum 25(OH)D (nmol/L)			
Mean ± SD	43.2 ± 19.3	48.5 ± 22.0	33.5 ± 13.3
Range	16.5 – 75.8	15.3 – 93.0	13.3 – 60.5
<30 nmol/L	4 (24%)	3 (17%)	10 (42%)
30-49.9 nmol/L	8 (47%)	6 (33%)	11 (46%)
≥50 nmol/L	5 (29%)	9 (50%)	3 (12%)

Table 2.2: Linear regression analyses of GWG on maternal and infant serum 25(OH)D concentration

Regression Model	Parameter Estimate (95% CI)	p-value
GWG on maternal 25(OH)D – R^2: 0.32		
GWG category [1 = inadequate, 2 = adequate, 3 = excessive]	-5.09 (-12.54, 2.35)	0.18
Average daily vitamin D intake (tertiles) [1 = 100-585 IU, 2 = 586-797 IU, 3 = 816-1282 IU]	10.01 (2.14, 17.89)	0.01
Ethnicity [1 = white, 2 = non-white]	15.16 (1.75, 28.57)	0.03
GWG on infant 25(OH)D – R^2: 0.57		
GWG category [1 = inadequate, 2 = adequate, 3 = excessive]	-5.58 (-10.34, -0.83)	0.02
Ethnicity [1 = white, 2 = non-white]	7.69 (0.004, 15.37)	0.049
Mother 25(OH)D [per 10 nmol/L]	4.15 (2.57, 5.73)	<0.0001

CI = confidence interval

Table 2.3: Linear regression analyses of pre-gravid BMI on maternal and infant serum 25(OH)D concentration

Regression Model	Parameter Estimate (95% CI)	p-value
Pre-gravid BMI on maternal 25(OH)D – R^2: 0.30		
Pre-gravid BMI [1 = BMI<24.9, 2 = BMI 25.0-29.9, 3 = ≥30]	-8.28 (-17.46, 0.90)	0.08
Average daily vitamin D intake (tertiles) [1 = 100-585 IU, 2 = 586-797 IU, 3 = 816-1282 IU]	10.86 (3.02, 18.69)	0.008
Ethnicity [1 = white, 2 = non-white]	16.35 (2.58, 30.12)	0.02
Pre-gravid BMI on infant 25(OH)D – R^2: 0.52		
Pre-gravid BMI [1 = BMI<24.9, 2 = BMI 25.0-29.9, 3 = ≥30]	-4.51 (-9.90, 0.87)	0.10
Mother 25(OH)D [per 10 nmol/L]	4.31 (2.68, 5.96)	<0.0001

CI = confidence interval

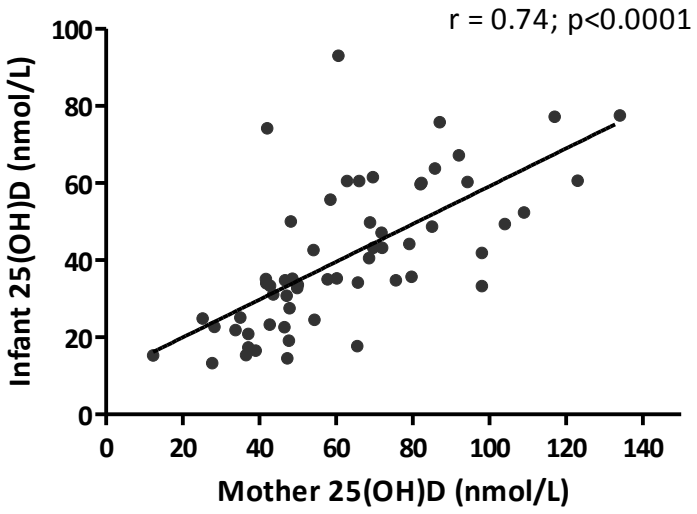


Figure 2.2. Scatter plot correlation of maternal-infant pairs serum 25(OH)D concentrations

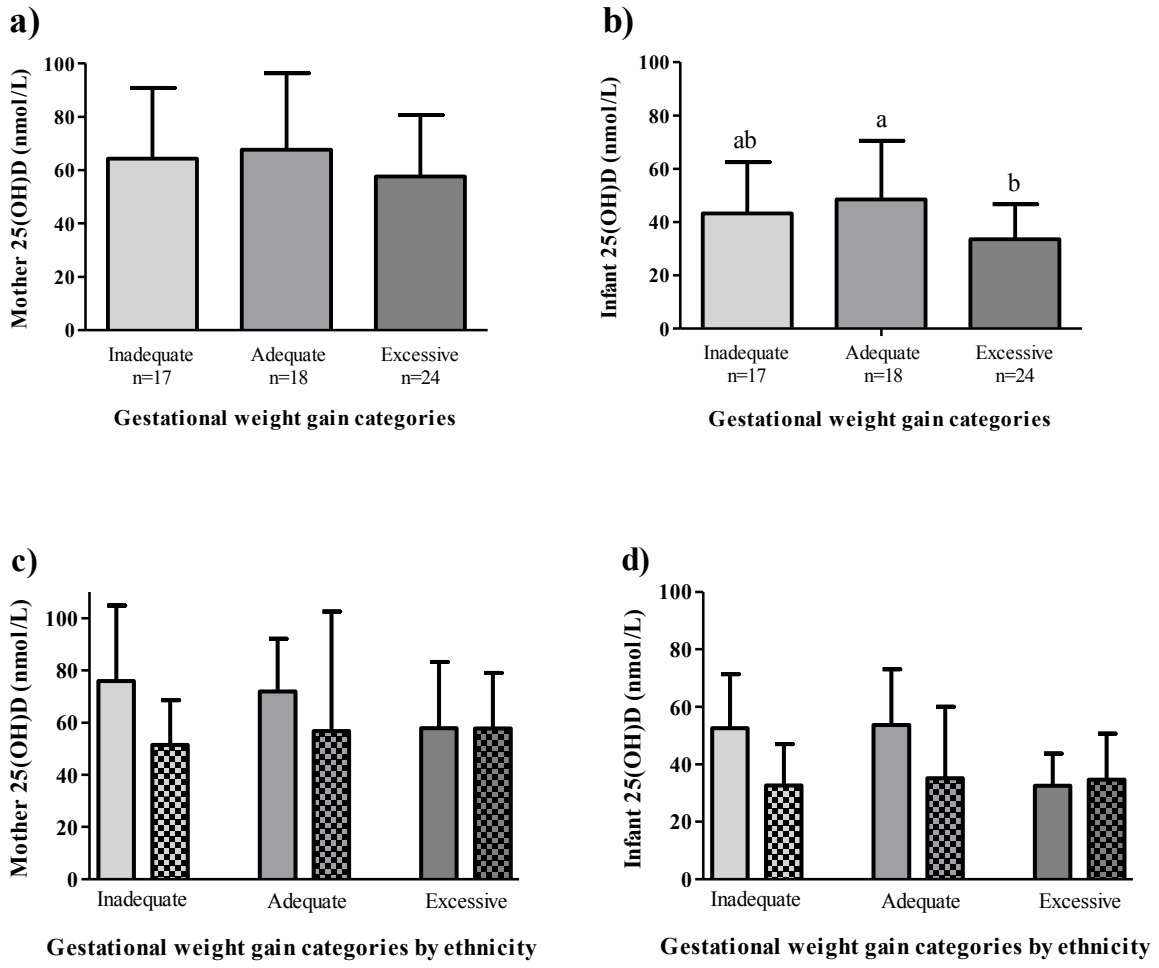


Figure 2.3. Mean serum 25(OH)D concentrations of a) mother and b) infants based on GWG category. Means ± SD from inadequate to excessive are a) 64.4 ± 24.5 nmol/L, 67.7 ± 28.7 nmol/L, 57.7 ± 23.1 nmol/L; b) 43.2 ± 19.3 nmol/L, 48.5 ± 22.0 nmol/L, 33.5 ± 13.3 nmol/L. b) Different superscripts depict significant differences ($p<0.05$) using a mixed model ANOVA adjusted for maternal age, ethnicity, vitamin D intake, season of birth, household income, birth weight-for-age Z-score, gestational age, education level and parity. Figure 2 c) and d) represent mean serum 25(OH)D concentrations based on ethnic (white (W) = solid bars, non-white (NW) = checkered bars) differences within GWG category. Mean ± SD (n) from inadequate to excessive for c) are: W: 75.9 ± 28.9 nmol/L (n=9), 71.9 ± 20.2 nmol/L (n=13), 57.8 ± 25.4 nmol/L (n=13); NW: 51.5 ± 17.1 nmol/L (n=8), 56.8 ± 45.6 nmol/L (n=5), 57.7 ± 21.3 nmol/L (n=11). Mean ± SD from inadequate to excessive for d) are: W: 52.6 ± 18.8 nmol/L, 53.7 ± 19.4 nmol/L, 32.5 ± 11.2 nmol/L; NW: 32.6 ± 14.4 nmol/L, 35.2 ± 24.8 nmol/L, 34.7 ± 15.9 nmol/L.

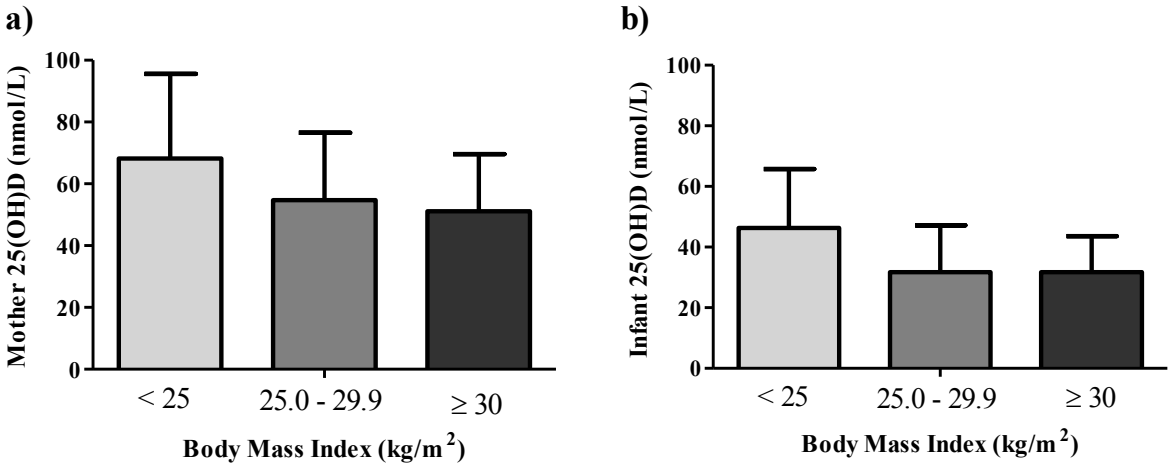


Figure 2.4. Mean serum 25(OH)D concentrations of a) mother and b) their respective infants based on maternal pre-gravid BMI. Means ± SD across BMI categories (left to right, mother-infant pairs n=37, n=14 and n=8) are a) 68.2 ± 27.4 nmol/L, 54.8 ± 21.7 nmol/L, 51.1 ± 18.5 nmol/L; b) 46.4 ± 19.4 nmol/L, 31.7 ± 15.4 nmol/L, 31.7 ± 11.9 nmol/L. No significant differences were found using a mixed model ANOVA adjusted for maternal age, ethnicity, vitamin D intake, season of birth, household income, birth weight-for-age z-score, gestational age, education level and parity.

3.0 GENERAL DISCUSSION

3.1 Findings

The aim of this thesis was to explore relationships between maternal gestational weight gain (GWG) and vitamin D status of the neonate. It was hypothesized that mothers who gain more weight than Health Canada's recommendations (based on pre-gravid BMI) would have infants born with lower serum 25-hydroxyvitamin D (25(OH)D) concentrations. The main findings of this study suggests that excessive total GWG in pregnancy (seen in 41% of participating mothers), regardless of pre-pregnancy body mass index (BMI) and ethnicity, results in infants being born with vitamin D insufficiency or deficiency. It was hypothesized earlier that this may be due to either sequestration of vitamin D in adipose tissues and/or volume dilution effects in larger individuals, allowing less maternal-fetal transfer *in utero*. After further exploration of maternal body composition on serum 25(OH)D concentrations of both mother and infant, these mechanisms did not appear to be the driving factor explaining the results reported in this thesis.

The same relationship between GWG and vitamin D concentration was not reflected in the mother despite the significant correlation between maternal-infant pairs serum 25(OH)D concentrations. With that said, it questions whether or not there is a third possible mechanism at play; a protective mechanism towards the mother, whereby she will transfer less 25(OH)D to her infant depending her needs. This theory is simply speculation and the actual mechanism(s) to help clarify these findings requires further study. However, a significant positive correlation was seen in mothers with higher lean body mass (LBM) and serum 25(OH)D concentration. It is possible that this relationship was observed due to increased metabolism of 1-25$(OH)_2$D at the

skeletal muscle level due to the presence of vitamin D receptors in this active tissue [122]. Additionally, there may be less conversion of 25(OH)D to the nonactive 24,25$(OH)_2$D by 24-hydroxylase in pregnancy as another protective mechanism [129]. Thus, mothers with higher serum 25(OH)D concentrations have greater lean mass, as in pregnancy (and early infancy), 1-25 dihydroxyvitamin D (1-25$(OH)_2$D) synthesis has been shown to be substrate dependent on 25(OH)D [57].

The high prevalence of infants born with insufficient and deficient vitamin D status was very surprising, as many of the mothers were taking prenatal supplements containing vitamin D and had sufficient status themselves. It is possible that the lower concentrations may reflect the fact that higher synthesis of 1-25$(OH)_2$D is occurring for musculoskeletal development in the fetus; though, it does not explain the large discrepancy seen when comparing to other regional studies. Chemiluminescent-based 25(OH)D assays sometimes do not detect 25$(OH)D_2$ or other vitamin D metabolites, which can lead to under/overestimations of total serum 25(OH)D concentrations [7]. Liquid chromatography-mass spectrometry (LC-MS) would have been a better method to measure vitamin D concentrations as it is recognized as the gold standard method for its analysis since it can quantify 25$(OH)D_2$ and 25$(OH)D_3$ with good accuracy [30]. Future studies using LC-MS analysis of 25(OH)D and other metabolites will offer further insight.

The correlation between the mothers and infants in this study was not as strong as previous studies, but it is important to note that the whole blood samples from these infants were obtained via capillary versus the more commonly used cord blood samples [51, 53, 54]. To the best of the author's knowledge, no studies have compared serum 25(OH)D concentrations

obtained from capillary versus cord blood samples to see if they provide similar results. Eslami et al. (2012) compared capillary, venous and cord blood samples of neonates to measure hemoglobin concentrations. Capillary samples had overall higher mean hemoglobin concentrations compared to the other two samples, but they were not significantly different [120]. With that said, it is possible that capillary and cord samples give similar results for serum 25(OH)D. Moreover, the blood samples obtained for serum 25(OH)D analysis and comparison were different between infants and mothers in this study; capillary versus venous, respectively. It is possible that measurement of 25(OH)D concentrations using Liaison can vary between these two types of samples. One study compared venous and capillary samples measured <72 hours after collection for total 25(OH)D using an enzyme linked immunoassay [130]. They found a significant correlation between the results (r=0.95) with good agreement as per Bland-Altman plots (bias: 19 nmol/L, 95% limits of agreement: -5.6 – 43 nmol/L; 95% CI: 14 – 24 nmol/L) despite venous concentrations being significantly lower when compared to capillary samples in the same individuals [130]. This could explain why the correlation between the mother-infant pairs serum 25(OH)D concentrations was significant, but not very strong. Another factor that could influence these results could be that the maternal samples were obtained up to 1-month post-partum (mean 2.8 ± 0.8 weeks post-partum). Despite this, it is likely still reflective of mother's status at delivery given the half-life of serum 25(OH)D being around 3 weeks [1, 24].

For mothers, the biggest constituents influencing their vitamin D status was ethnicity and vitamin D intake; both of which were self-reported data. These factors are consistent with the literature regarding modifiable (intake) and non-modifiable (ethnicity) factors that can influence vitamin D status [2, 7, 20, 57, 119, 131]. Sun exposure and sunscreen use during

pregnancy was asked via retrospective questionnaires at screening, from which sun index was calculated based on percent body surface area exposed. However, this data was subject to a fair amount of recall bias. Additionally, assumptions for sunscreen application were used whereby if sunscreen was applied, it was presumed to be applied properly, which may not be accurate; therefore, it was not used in the analysis. Sunlight exposure assessments are generally very imprecise and accurate information is difficult to acquire mainly due to poor memory recall and lifestyle factors (clothing, sunscreen, time spent in the shade vs. direct sun, etc.) [132]. One study used ultraviolet (UV) dosimeters to capture sun exposure in a multiethnic cohort of 502 adults in New Zealand over an 8-week period accounting for clothing [133]. Their results show that serum 25(OH)D_3 increases in a non-linear matter with UV exposure, as with prolonged exposure, concentrations will rise at a slower rate [133]. These findings do correlate with previous work investigated by the IOM which reports that overexposure to UV beta rays will not cause vitamin D_3 toxicity as there are regulatory pathways in place, allowing the conversion of previtamin D_3 to several other compounds (such as lumisterol and tachysterol), as well as the ability for vitamin D_3 to convert to non-active forms [7]. Thus, UV dosimeters may be a better alternative to capture this data; or at least to complement survey data on clothing habitus. Since serum 25(OH)D concentrations reflects both endogenous and exogenous sources of vitamin D, having maternal blood samples to obtain this measurement was thought to be adequate to compliment the self-report data in this thesis [7].

When looking at the influence of pre-gravid BMI on vitamin D status, the results did not correspond to what was previously seen in the literature [16, 17, 119]. This was most likely due to the unequally distributed pre-gravid BMIs of mothers in the study, where the majority (59%)

of them had a normal BMI prior to conception, leaving inadequately powered samples of overweight and obese women. Furthermore, the pre-pregnancy BMI categories used may not be suitable for all ethnic groups, as some populations may experience adverse health outcomes with a BMI <25 kg/m^2 due to differences in body composition/proportions [76]. The World Health Organization (WHO) expert consultation proposed that a BMI of 23 – 27.5 kg/m^2 poses an increased risk of adverse health effects for the Asian population, with higher risk seen in BMI's >27.5 kg/m^2 [77]. A cross-sectional study of a cohort of 490,288 adults (40 – 69 y) in the United Kingdom established BMI cutoffs for obesity and diabetes risk in different ethnic populations [78]. The proposed cut-points for obese women, when compared to the 30 kg/m^2 in white populations, are as follows: 22 kg/m^2 for South Asians, 26.0 kg/m^2 for Blacks, and 24.0 kg/m^2 for Chinese [78]. A sub-analysis was conducted to account for the proposed BMI cutoffs for the Asian, Black and Chinese participants of this study to see if it would alter the findings. However, all mothers in these ethnic groups had pre-gravid BMI's below the proposed obese cutoffs making this analysis unwarranted.

3.2 Strengths and limitations

The biggest strength of this study is its novelty. To the best of the author's knowledge, this is the only study aiming at determining associations between GWG and vitamin D status of newborn infants. As evidenced by these preliminary results, it is clear that there are associations between weight gain in pregnancy and vitamin D status of the neonate that require further exploration. This study also provided valuable insight on the vitamin D status of newborn infants in the greater Montreal area, which was facilitated by the acquisition of capillary blood samples 24-36 hours post-partum. These samples were conveniently obtained at

the same time as routine newborn blood screening in hospital, therefore no additional heel poke was necessary. The ease of getting these samples were in large part due to the collaboration with the local hospital staff. This demonstrates that in-hospital newborn testing for vitamin D status is achievable, which can have great implications in newborn screening for health.

Given that this study was based on screening for entry into a randomized control trial, the study design for this thesis was restricted which lead to some obvious limitations. For one, maternal blood samples were not obtained in hospital like their infants were. Even though the half-life of serum 25(OH)D is approximately 3 weeks, it would be more accurate to obtain maternal samples at the same time as infants to examine associations [1, 24]. It would have also been ideal to obtain maternal blood samples, as well as body composition analyses, prior to pregnancy and per trimester to look at changes over time during gestation. This study was fortunate to have access to dual-energy x-ray absorptiometry (DXA) technology for body composition assessment of our post-partum mothers. However, analyzing body composition over time could lead to greater insight on how this aspect can influence 25(OH)D outcomes. DXA could be conducted prior to conception, likewise during the postpartum period, but during pregnancy, analyses would have to be either by bioelectrical impedance analysis or skinfolds as DXA is not warranted in this population. Fetal size monitoring from ultrasound analysis would also be an important aspect to account for as this type of design would allow more in depth exploration of how maternal and infant body composition and size can influence vitamin D status.

This ties in to another important aspect to consider; the pattern and timing of weight gained throughout pregnancy, or per trimester. A woman could be following recommended guidelines of total weight gained for her pre-gravid BMI, but if all the weight was gained in the last trimester and not spaced out appropriately, metabolic aberrations to both the mother and fetus could arise [134]. Widen et al (2015) observed that higher than recommended weekly weight gain in the second and third trimesters are associated with increased maternal fat mass accretion, where increased fetal size is more related to higher second trimester gains [134]. Additionally, large weight gains during early pregnancy (1st trimester) has been associated with development of gestational diabetes and early childhood obesity [135, 136]. This demonstrates the importance of not only watching out for overall gestational weight gain, but when in the gestational period the weight is gained. In the present thesis, however, these concerns were likely mitigated in part by selection of only appropriate for gestational age infants at birth.

Vitamin D intake was assessed using a validated food frequency questionnaire (FFQ) for vitamin D given at baseline visit; however, supplement use during pregnancy had to be cross-matched with screening survey questionnaires as discrepancies were found between both documents in 24% of the participants. The question regarding supplement use during pregnancy in the FFQ was not well written and probably caused confusion for some mothers leading to inconsistent data. Since the FFQ was only given at the initial baseline visit, it was probably difficult for some mothers to recall what they were eating during the entire length of their pregnancy. An ideal assessment of vitamin D intake during pregnancy should be conducted during the actual pregnancy, on a trimester basis, with multiple 24-hr recalls in addition to a validated FFQ for vitamin D intake so that recall bias is decreased. This method is

also self-reported which could lead to similar over or under reporting of macronutrients and micronutrients.

Another aspect that was not assessed in this study was perinatal education regarding GWG guidelines and supplement use. It is possible that we had mothers with inadequate and excessive weight gains because they simply did not know or were not aware of how much weight they should have gained during pregnancy. The majority of our mothers were taking prenatal supplements during pregnancy, but timing of when those supplements were started was not assessed, only frequency. Health Canada has detailed information on their government websites regarding weight gain guidelines and prenatal supplement use [33, 75]. However, it is unclear how well this information is getting disseminated to the public as national data on the matter is lacking. In Quebec, public prenatal courses that include information on maternal-infant nutrition are available to expecting mothers between 20 to 25 weeks gestation; which is well into the second trimester [137]. As previously stated, early pregnancy weight gain can lead to unwarranted health outcomes [135, 136]. Thus, mothers attending these classes will have less knowledge of early pregnancy care. Ideally, prenatal education should start prior to conception; alas this is not always feasible as many pregnancies are unplanned.

3.3 Conclusion and future directions

Pregnant women and newborn infants are an under researched population, especially with regards to vitamin D status and GWG. Based on the findings of this thesis, it is evident that mothers who gain more total weight than recommended during pregnancy can have infants born with insufficient vitamin D status. This was shown irrespective of pre-gravid BMI, ethnicity or lower maternal serum 25(OH)D concentrations. Whether or not similar findings regarding

the effects of GWG on neonatal vitamin D status can be seen in other regions remains to be examined. Furthermore, adherence to GWG guidelines was quite poor in the sample of women studied. This justifies additional research to explore what type of education is being translated to pregnant women in the greater Montreal area, as well as other regions.

Vitamin D status during pregnancy, and subsequent status in newborn offspring, is influenced by endogenous and/or exogenous factors including: reduced skin exposure to sunlight, latitude, season, and skin pigmentation, as well as poor intake of vitamin D containing foods or supplements [2]. Given that many of our newborns were vitamin D insufficient and deficient, and majority of mothers were meeting or exceeding the RDA for vitamin D, it is likely that this population has higher requirements than the current DRIs for vitamin D to support both maternal and fetal health. Provided the accumulating evidence that pregnant women have higher vitamin D requirements than what is currently suggested, it would be advisable for the IOM to re-evaluate their recommendations in this population.

There may also be other mechanisms at play by which their serum 25(OH)D concentrations can falter in these women. These include sequestration of the liposoluble vitamin D into adipose tissues, dilution effect from expanding blood volumes and adipose stores, or increased metabolic demands in lean mass [12, 41]. Future research studies would be needed to determine which mechanisms are valid; as well to see if one may outweigh the other, or conversely, if they have synergistic effects on serum 25(OH)D concentrations. Further exploration of timing and quantities of weight gain throughout pregnancy could also help determine this phenomenon.

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