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Summer Season and Recommended Vitamin D Intake Support Adequate Vitamin D Status throughout Pregnancy in Healthy Canadian Women and Their Newborns

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ABSTRACT

Background: Vitamin D deficiency in pregnancy is reported as a prevalent public health problem.

Objectives: We aimed to evaluate, in pregnant Canadian women, *1*) vitamin D intake, *2*) maternal and cord serum 25-hydroxycholecalciferol [25(OH)D] and maternal 1,25-dihydroxycholecalciferol [1,25$(OH)_2$D], and *3*) factors associated with maternal serum 25(OH)D.

Methods: Women (n = 187; mean prepregnancy BMI 24.4 kg/m^2, mean age 31 y) recruited to the Be Healthy in Pregnancy study provided fasting blood samples and nutrient intake at 12–17 (early) and 36–38 (late) weeks of gestation, and cord blood. Vitamin D intakes (Nutritionist Pro™) and serum 25(OH)D and 1,25$(OH)_2$D concentrations (LC-tandem MS) were measured.

Results: Vitamin D intake was comparable in early and late pregnancy [median (IQR) = 586 (459, 859) compared with 689 (544, 974) IU/d; P = 0.83], with 71% consumed as supplements. Serum 25(OH)D was significantly higher in late pregnancy (mean ± SD: 103.1 ± 29.3 nmol/L) than in early pregnancy (82.5 ± 22.5 nmol/L; $P < 0.001$) and no vitamin D deficiency (<30 nmol/L) occurred. Serum 1,25$(OH)_2$D concentrations were significantly higher in late pregnancy (101.1 ± 26.9 pmol/L) than in early pregnancy (82.2 ± 19.2 pmol/L, $P < 0.001$, $n = 84$). Cord serum 25(OH)D concentrations averaged 55% of maternal concentrations. In adjusted multivariate analyses, maternal vitamin D status in early pregnancy was positively associated with summer season (est.β: 13.07; 95% CI: 5.46, 20.69; $P < 0.001$) and supplement intake (est.β: 0.01; 95% CI: 0.00, 0.01; $P < 0.001$); and in late pregnancy with summer season (est.β: 24.4; 95% CI: 15.6, 33.2; $P < 0.001$), nonmilk dairy intake (est.β: 0.17; 95% CI: 0.02, 0.32; $P = 0.029$), and supplement intake (est.β: 0.01; 95% CI: 0.00, 0.01; $P = 0.04$).

Conclusions: Summer season and recommended vitamin D intakes supported adequate vitamin D status throughout pregnancy and in cord blood at >50 nmol/L in healthy Canadian pregnant women. This trial was registered at clinicaltrials.gov as NCT01693510. *J Nutr* 2019;00:1–8.

Keywords: serum 25(OH)D, serum 1,25$(OH)_2$D, isomers, cord blood, human pregnancy, vitamin D intake, bone health

Introduction

Globally, vitamin D deficiency in pregnancy is purported to be a prevalent public health problem (1–3). Yet, the importance of adequate maternal vitamin D status, as measured by serum 25-hydroxycholecalciferol [25(OH)D], to pregnancy and infant health outcomes has been highlighted by recent systematic reviews and meta-analyses (4–7). Despite this the optimal target for serum 25(OH)D in pregnancy remains undefined (8) because conflicting data exist as to the additional positive impact on maternal and neonatal outcomes of maternal 25(OH)D serum concentrations above the current threshold for adequacy (≥50 nmol/L) that the Institute of Medicine (IOM) (9) has set (4). Such diversity in observations may arise because of variations across protocols in the method employed to measure 25(OH)D concentration, timing of blood sampling across pregnancy, or season (10). To our knowledge, no recent studies have profiled vitamin D metabolites using the gold-standard

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method LC tandem MS (LC-MS/MS) (11) across uncomplicated pregnancy in women of European descent living in Canada to obtain a comprehensive view of the changes in metabolism inherent to pregnancy and lifestyle.

Infant vitamin D status at birth is controlled by maternal serum concentrations of 25(OH)D during pregnancy (12), yet no reference for vitamin D adequacy exists for cord blood, with the exception that newborn serum 25(OH)D > 25–30 nmol/L will prevent nutritional rickets (8). The current recommended intakes from the IOM for pregnant women of 600 IU vitamin D/d (9) did not evaluate the intake in pregnancy that would ensure adequate vitamin D status for newborns. As recently reviewed (12), debate continues as reflected in articles that have challenged the IOM report, indicating higher vitamin D intakes are required during pregnancy to ensure adequate maternal, fetal, and neonatal health.

Trying to establish consensus on adequate vitamin D intake in pregnancy is complicated by the fact that maternal circulating vitamin D metabolites may vary by trimester owing to modulation by normal physiological changes in pregnancy. Progressive gestational weight gain in the second half of pregnancy may reduce maternal circulating 25(OH)D because body adiposity has been associated with lower serum 25(OH)D concentrations due to sequestration in metabolic fat stores, at least in the nonpregnant state (13). Further, maternal serum 1,25-dihydroxycholecalciferol [1,25$(OH)_2$D] synthesis is upregulated to facilitate increased intestinal calcium absorption and trans-placental transport to the fetus (14). This upregulation is reported to be independent of calcium metabolism (15). The rise in 1,25$(OH)_2$D is hypothesized to be dependent on the availability of the substrate 25(OH)D, or due to other factors such as increased renal and placental-decidual synthesis of 1,25$(OH)_2$D (12). Considering these pregnancy-induced adaptations, it is essential both in clinical care settings and in research settings to delineate trimester-specific vitamin D status and how this is influenced by other maternal lifestyle factors. With such information, women at risk of vitamin D deficiency/insufficiency can be targeted and offered cost-efficient treatment or supplementation (16).

The objectives of this study were to determine in a cohort of pregnant women living in southern Canada the trajectory of vitamin D metabolites across pregnancy and the factors associated with observed changes by measuring *1*) intake and sources of vitamin D, *2*) maternal and cord 25(OH)D and maternal 1,25$(OH)_2$D for ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) isomers, and *3*) factors associated with maternal serum 25(OH)D at 12–17 weeks (early pregnancy) and 36–38 weeks of gestation (late pregnancy).

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Address correspondence to SAA (e-mail: satkins@mcmaster.ca).
Abbreviations used: BHIP, Be Healthy in Pregnancy; Cyp27b1, cytochrome P450 family 27 subfamily B member 1; DEQAS, Vitamin D External Quality Assessment Scheme; EAR, Estimated Average Requirement; IOM, Institute of Medicine; LC-MS/MS, LC-tandem MS; NIST, National Institute of Standards and Technology; RCT, randomized controlled trial; UL, Tolerable Upper Intake Level; 1,25$(OH)_2$D, 1,25-dihydroxycholecalciferol; 25(OH)D, 25-hydroxycholecalciferol.

Methods

Study group

The data presented are based on 187 out of the 241 participants enrolled in the Be Healthy in Pregnancy (BHIP) study (NCT01693510), a randomized controlled trial (RCT). The full BHIP study protocol has been published (17). This observational substudy of 187 participants represents those who had detailed dietary intake and blood samples available for both early and late pregnancy time points. Cord blood collection was not feasible for all participants for practical reasons (home births, parents saving cord blood) and thus the number of dyads for which we tested all vitamin D metabolites was represented by a subsample of 41 participants who provided cord blood.

Briefly, the study protocol involved healthy pregnant women recruited from health care clinics in Hamilton, Burlington, and London, Ontario, Canada between 12 and 17 weeks of gestation according to the criteria as published (17). Ethics approval was obtained from the research ethics boards of Hamilton Health Sciences (REB Project#12-469), Western Ontario in London (HSREB 103272), and Joseph Brant Hospital in Burlington (JBH 000-018-14), all located in southern Ontario, Canada. The protocol complied with the Helsinki Declaration.

Maternal data collection

Maternal demographics, pregnancy history, and physical measurements including skinfold thickness at 4 sites were obtained from each participant upon study entry as detailed in the research design article (17). Fasted venous blood was collected at 12–17 weeks of gestation and at 36–38 weeks of gestation. Season of blood draw was classified as winter (November–April) or summer (May–October).

Dietary intake and primary food sources

Participants completed detailed diet records for 3 consecutive days, consisting of 2 weekdays and 1 weekend day, including both foods and supplements, as used previously in pregnant women (18). No diet records with implausible intakes were identified (i.e., <500 or >5000 kcal/d). Participants completed a diet record upon entry to the study (12–17 weeks of gestation) and at the end of pregnancy (36–38 weeks of gestation). All diet records were analyzed using Nutritionist Pro™ diet analysis software (version 5.2, Axxya Systems) and the Canadian Nutrient File (version 2015) to obtain daily intake of vitamin D. In addition, food and supplement sources of vitamin D were assessed manually for all participants who completed a diet record (n = 129). Food sources of vitamin D were grouped as milk [under mandatory fortification with vitamin D (100 IU/250 mL at the time of the study) in Canada (19)], nonmilk dairy products [under voluntary fortification with vitamin D in Canada (19)], fortified food (e.g., fortified orange juice and breakfast cereals), animal sources (e.g., fish, meat, and eggs), plant sources (e.g., mushrooms), and "others" when a dish combined >1 source of vitamin D.

Vitamin D status analysis

As previously reported (20), serum 25(OH)D concentrations {representing 25-hydroxy-D_2 [25$(OH)D_2$] plus 25-hydroxy-D_3 [25$(OH)D_3$] isomers} were measured by ultra-performance LC tandem MS (UPLC-MS/MS) using the Waters application note 720002748 (21) with a modified sample preparation from Hymoller that included a saponification step (22). Accuracy and precision were determined by using human serum-based quality controls and standard reference materials [National Institute of Standards and Technology (NIST) Standard Reference Material 972a] as previously described (20). Precision values for 25$(OH)D_3$ were intra-assay CV 6.9%, interassay CV 8.2%, and average mean bias −1.3%. For 25$(OH)D_2$, intra-assay CV was 10.0%, interassay CV 10.5%, and average mean bias 4.2%. These values compare with previously established vitamin D standardization program performance criteria, namely CV ≤ 10% and mean bias ≤ 5%.

Serum 1,25$(OH)_2$D concentrations {representing 1,25-dihydroxy-D_2 [1,25$(OH)_2D_2$] plus 1,25-dihydroxy-D_3 [1,25$(OH)_2D_3$] isomers} were measured by Quest Diagnostics Nichols Institute (CA, USA) using LC-MS/MS (AB Sciex LLC) in a subgroup of 81 individuals for whom a sample was available. The Mass Spect Gold® (Golden West Biologicals,

TABLE 1 Demographic characteristics of pregnant women upon study entry (12–17 wk) and newborns at birth[1]

Demographic characteristics	Values
Maternal characteristics	$n = 187$
Gestational age, wk	13.3 ± 1.7
Age, y	31 (20–42)
Prepregnancy BMI, kg/m^2	
Underweight: <18.5	3 (1)
Normal weight: 18.5–24.9	103 (55)
Overweight: 25.0–29.9	50 (27)
Obese: ≥30	31 (17)
Ethnicity	
European ancestry	164 (88)
Other	23 (12)
Household income, CAD	
<$75K/y	49 (26)
≥$75K/y	138 (74)
Education (highest degree)	
High school	3 (2)
College diploma	34 (18)
Bachelor's degree	62 (33)
Above bachelor's degree	88 (47)
Parity	
0	89 (48)
≥1	98 (52)
Newborn characteristics	$n = 41$
Gestational age, wk	39.4 ± 1.1

[1] Values are mean ± SD, mean (range), or frequency (percentage).

Inc.) ultra-sensitive human serum controls were used and the intra-assay CV generated for samples was <10%. The reporting range for this assay was 19–2400 pmol/L. Daily participant testing was performed with intra-assay controls.

Statistical analysis

The observed changes in vitamin D intake throughout pregnancy are not attributable to the intervention (unpublished data), hence data were treated as observational, and all statistical models were adjusted for factors related to the study design of the RCT. This included the randomization stratification variables [i.e., study arm, study site, and prepregnancy BMI (in kg/m^2)]. Statistical analysis was performed using JMP 9.0 (version 9.0.1, SAS Institute Inc.) and GraphPad Prism (version 7). Descriptive statistics included the means and SDs of normally distributed continuous data; medians and quartiles (IQR) for nonnormally distributed continuous data; and counts and percentages for categorical data. Regression analyses were performed to compare values between time points and significance was established at $P < 0.05$. Linear regressions were used to assess the relation between maternal and cord serum 25(OH)D concentrations. Mean values are given as mean ± SD unless stated otherwise. To determine which factors were associated with maternal vitamin D status, we conducted multivariable linear regression analyses. The variables of interest included in our multivariable regressions were decided a priori based on clinical rationale and evidence from the literature. The nonstandardized regression coefficients and their corresponding 95% CIs and P values are presented.

Results

Demographics

A total of 187 participants were enrolled near the end of the first trimester of pregnancy (Table 1). The participants were mostly of European descent, and the ethnic composition of the women

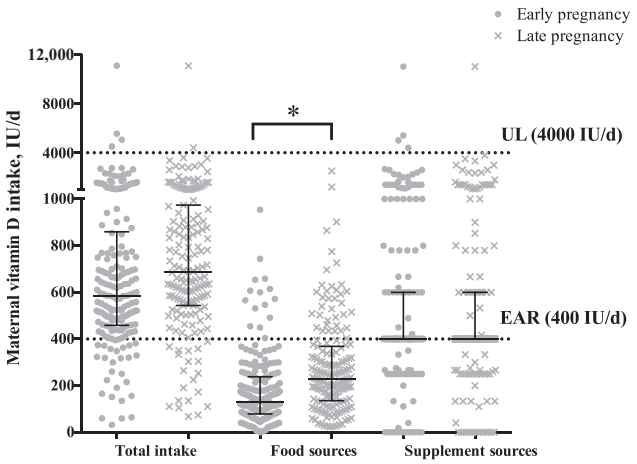

FIGURE 1 Maternal dietary intake of vitamin D, including food and supplement sources in pregnant women ($n = 187$) in early (12–17 wk) and late pregnancy (36–38 wk). Values are medians and IQRs. Dotted lines: recommendations for pregnancy by the Institute of Medicine (9). *Different between early and late pregnancy, $P < 0.001$. EAR, Estimated Average Requirement; UL, Tolerable Upper Intake Level.

grouped under "Other" included Indigenous, Asian, Arab, and South American ancestry. Participants had a mean prepregnancy BMI of 24.4 kg/m^2 (range: 17.4–39.6), and were university educated with a high percentage of high household income. About 50% of women entered pregnancy with a prepregnancy BMI classified as overweight or obese, and almost half of the participants were first-time mothers. The participants excluded ($n = 54$) in this report from the original data set of 241 participants had similar sociodemographic characteristics to the ones with a complete data set ($n = 187$) (data not shown).

Intake of total supplemental and dietary vitamin D during pregnancy

Total vitamin D intake was comparable in early and late pregnancy (Figure 1). Total intakes of vitamin D met the Estimated Average Requirement (EAR) (9) of 400 IU/d in 93% of participants in early pregnancy and in 97% in late pregnancy. Vitamin D intake from food sources alone met the EAR in only 8% of participants in early pregnancy and 19% in late pregnancy. Intake of vitamin D from food sources increased significantly ($P < 0.001$) from early (median 130 IU/d = 23% total intake) to late pregnancy (median 230 IU/d = 36%). Consumption of dairy products (i.e., milk and nonmilk dairy products) represented the source of higher vitamin D intake from food (subsample $n = 129$). Four participants exceeded the Tolerable Upper Intake Level (UL) (9) intake of 4000 IU/d in early pregnancy with the highest intake being 11,060 IU/d, and for 2 participants in late pregnancy with the highest intake being 11,050 IU/d. Exceeding the UL did not occur from food sources, but only when participants consumed single vitamin D supplements as part of their diet. Supplemental intake represented 77% (median 400 IU/d) of total vitamin D intake in early pregnancy and 64% (median 400 IU/d) in late pregnancy. Consumption of supplements containing vitamin D was high primarily because 86% of participants took a daily prenatal multivitamin in early pregnancy compared with 81% in late pregnancy. An additional 14% of participants took a single vitamin D supplement in early pregnancy, compared with 16% in late pregnancy. The range of intake of vitamin D from supplements among participants spanned 0–10,000 IU/d.

TABLE 2 Mean total, supplemental, and dietary vitamin D intake, including food sources of vitamin D, in pregnant women in early (12–17 wk) and late pregnancy (36–38 wk)[1]

	Early pregnancy		Late pregnancy		
Vitamin D intake	Food intake, IU/d	Total food intake, %	Food intake, IU/d	Total food intake, %	*P* value between time points
Milk	68	39	143	54	<0.05
Nonmilk dairy	12	7	27	10	<0.05
Fortified food	31	17	21	8	0.22
Animal sources	52	31	55	22	0.80
Plant sources	1	1	1	0	0.68
Others	9	5	16	6	<0.05
Food sources	174	100	263	100	<0.05
Supplements	400	—	400	—	0.38
Total	574	—	663	—	0.83

[1] $n = 129$.

Food sources of vitamin D intake during pregnancy

Vitamin D–fortified milk provided the greatest proportion of dietary vitamin D and rose significantly from early to late pregnancy (**Table 2**). The second most important source of dietary vitamin D was animal products (i.e., eggs, fish, and meat) (Table 2).

Maternal vitamin D status during pregnancy

The mean ± SD maternal serum 25(OH)D concentration (**Figure 2**) rose significantly in late compared with early pregnancy (103.1 ± 29.3 compared with 82.5 ± 22.5 nmol/L, $P < 0.001$, $n = 187$) and was above the cutoff for vitamin D adequacy [≥50 nmol/L as set by the IOM (9) and recommended by a global consensus recommendation for prevention of nutritional rickets (23)] at both time points. A total of 93% of participants in early pregnancy and 97% in late pregnancy met or exceeded the threshold representing a sufficient 25(OH)D concentration of 50 nmol/L. No participants were vitamin D deficient (<30 nmol/L) at either time point. In contrast, 4% of participants in early pregnancy and 22% in late pregnancy had serum 25(OH)D concentrations above the threshold of 125 nmol/L, which might be reason for concern as suggested by the IOM (9). Total vitamin D intake and circulating 25(OH)D concentration were weakly positively associated in early pregnancy ($r^2 = 0.10$, $P < 0.001$) and late pregnancy ($r^2 = 0.10$, $P < 0.01$). Most participants who consumed a dietary vitamin D amount below the EAR still had sufficient serum 25(OH)D concentrations throughout pregnancy (Figure 2). The small number of participants who consumed intakes (>4000 IU/d) of vitamin D above the upper limit set by the IOM (9) tended to have serum 25(OH)D concentrations in early and late pregnancy that might be reason for concern at >125 nmol/L (Figure 2) as set by the IOM (9).

Maternal serum 1,25(OH)$_2$D concentrations

In a subsample of 84 participants, the mean ± SD serum 1,25(OH)$_2$D concentration was significantly higher in late pregnancy (101.1 ± 26.9 pmol/L) than in early pregnancy (82.2 ± 19.2 pmol/L, $P < 0.001$) (**Figure 3**). The D_2 isomer was not detected in any serum sample at any time points. Thus,

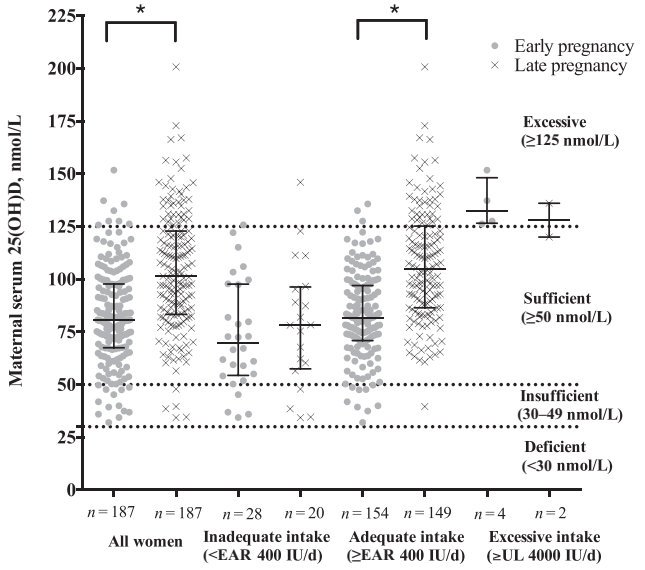

FIGURE 2 Mean ± SD maternal serum 25(OH)D concentrations grouped by adequacy of vitamin D intake in pregnant women ($n = 187$) in early (12–17 wk) and late pregnancy (36–38 wk). Dotted lines: recommendations for pregnancy by the Institute of Medicine (9). *Different between early and late pregnancy, $P < 0.001$. 25(OH)D, 25-hydroxycholecalciferol; EAR, Estimated Average Requirement; UL, Tolerable Upper Intake Level.

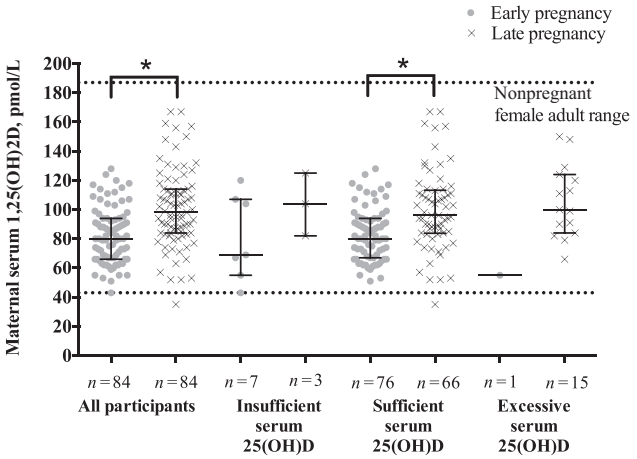

FIGURE 3 Mean ± SD maternal serum 1,25(OH)$_2$D concentrations grouped by maternal vitamin D status in pregnant women ($n = 84$) in early (12–17 wk) and late pregnancy (36–38 wk). Recommendations for pregnancy by the Institute of Medicine (9): insufficient serum 25(OH)D, 30–50 nmol/L; sufficient serum 25(OH)D, ≥50 nmol/L; excessive serum 25(OH)D, ≥125 nmol/L. Dotted lines: nonpregnant female adult range values as reported by the Mayo Clinic Laboratories (24). *Different between early and late pregnancy, $P < 0.001$. 25(OH)D, 25-hydroxycholecalciferol; 1,25(OH)$_2$D, 1,25-dihydroxycholecalciferol.

TABLE 3 Multivariable analysis of factors associated with maternal serum 25(OH)D concentrations in pregnant women in early (12–17 wk) and late pregnancy (36–38 wk)[1]

	Early pregnancy			Late pregnancy		
Variables	Estimated coefficient	95% CI	*P* value	Estimated coefficient	95% CI	*P* value
Season of blood draw (winter as reference)	13.07	5.46, 20.69	<0.001	24.35	15.55, 33.16	<0.001
Sum of skinfold	−0.14	−0.41, 0.13	0.312	0.06	−0.20, 0.32	0.654
Vitamin D intake from supplements	0.01	0.00, 0.01	<0.001	0.01	0.00, 0.01	0.001
Vitamin D intake from milk	0.01	−0.05, 0.07	0.779	0.01	−0.04, 0.05	0.843
Vitamin D intake from nonmilk dairy products	0.03	−0.16, 0.23	0.734	0.17	0.02, 0.32	0.029

[1] $n = 187$.

total 1,25(OH)$_2$D is a reflection of the D_3 isomer. No significant association was observed between serum 1,25(OH)$_2$D and 25(OH)D in early pregnancy. In late pregnancy, there was a significant weak positive association between serum 1,25(OH)$_2$D and 25(OH)D ($r^2 = 0.10$, $P = 0.04$). Participants with insufficient serum 25(OH)D (30–50 nmol/L) in late pregnancy had similar serum concentrations of 1,25(OH)$_2$D to those with values >50 nmol/L (Figure 3).

Factors associated with maternal vitamin D status in pregnancy

A multivariable analysis demonstrated that higher serum 25(OH)D concentration in early pregnancy was significantly associated with having blood drawn in the summer and with vitamin D intake from supplements (Table 3). In late pregnancy, higher maternal serum 25(OH)D concentration was significantly associated with blood drawn in the summer, vitamin D intake from supplements, and vitamin D intake from nonmilk dairy products (Table 3).

Cord serum 25(OH)D concentrations

Cord serum 25(OH)D concentrations at delivery ($n = 41$) were significantly associated with early and late pregnancy maternal serum concentrations (early pregnancy: $r^2 = 0.25$, $P < 0.001$; late pregnancy: $r^2 = 0.58$, $P < 0.001$; Figure 4). The mean ± SD cord serum 25(OH)D concentration was 56.1 ± 23.6 nmol/L. Cord serum 25(OH)D concentrations averaged 55% of maternal 25(OH)D concentrations at the end of pregnancy (range: 26–79%; Figure 5).

Discussion

Contrary to the reports of global widespread vitamin D deficiency in pregnant women including in North America (1–3), vitamin D deficiency defined as serum 25(OH)D < 30 nmol/L was not detected in this cohort of pregnant women living in southern Ontario, Canada, who were mainly of European descent and taking a daily prenatal multivitamin. Further, maternal serum 25(OH)D concentrations actually increased significantly from early to late pregnancy by 25% and serum 1,25(OH)$_2$D concentrations by 23% when measured by the gold-standard LC-MS/MS, despite the 2 metabolites being only weakly correlated in late but not early pregnancy. These results are in line with those of a systematic review of 20 observational cross-sectional studies that found no association between serum 25(OH)D and 1,25(OH)$_2$D at the end of pregnancy (25). Our vitamin D metabolite profiles expand on this body of literature by having repeated measures in early to late pregnancy and support recent findings (8, 12) suggesting that the concentration of serum 25(OH)D is not the driver of the observed rise in 1,25(OH)$_2$D from early to late pregnancy. Thus, our results challenge the hypothesis (15, 26) of a direct positive relation between the circulating substrate 25(OH)D and the active form 1,25(OH)$_2$D produced in pregnant women with adequate vitamin D status. Instead, it is postulated that other mechanisms are at play such as the increased production of 1,25(OH)$_2$D by the maternal kidneys independent of parathyroid hormone stimulation and/or the placental-decidual tissue through stimulation of Cytochrome P450 family 27 subfamily B member 1 (*Cyp27b1*) by placental hormones leading to a higher conversion rate of 1,25(OH)$_2$D (15).

The finding that most women had adequate vitamin D status despite intakes both above and below the EAR is explained by the significant positive association of maternal serum 25(OH)D with summer season, thus contributing to cutaneous de novo synthesis of vitamin D. Women with excessive vitamin D intake due to high supplement intake had serum 25(OH)D concentrations above the 125 nmol/L concentrations that might be reason for concern as recommended by the IOM (9). Results from a systematic review and meta-analysis including 12 RCTs suggested that a vitamin D supplementation of 2250 IU/d is required for pregnant women to achieve sufficient circulating

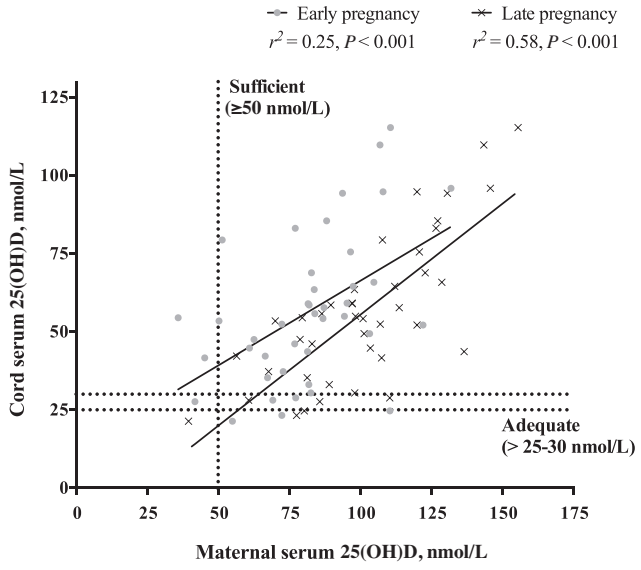

FIGURE 4 Relation between maternal and cord serum 25(OH)D concentrations (individual values displayed; $n = 41$) in mother–infant dyads in early (12–17 wk) and late pregnancy (36–38 wk). Dotted lines: recommendations for pregnancy by the Institute of Medicine (9). For cord serum, the threshold of 25–30 nmol/L is recommended to prevent nutritional rickets (8). 25(OH)D, 25-hydroxycholecalciferol.

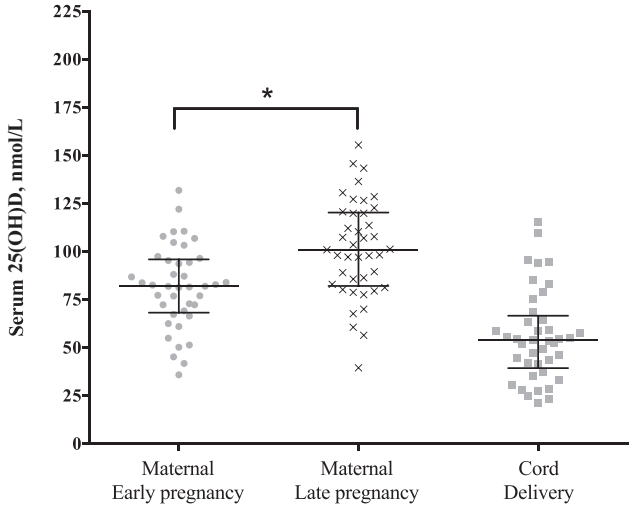

FIGURE 5 Mean ± SD maternal and cord serum 25(OH)D concentrations in mother–infant dyads ($n = 41$) in early (12–17 wk) and late pregnancy (36–38 wk), and at delivery. *Different between early and late pregnancy, $P < 0.001$. 25(OH)D, 25-hydroxycholecalciferol.

25(OH)D concentrations, in this case defined as 25(OH)D > 75 nmol/L (1). In contrast, a recent RCT from Ireland suggested a total maternal intake of 1150 IU vitamin D/d is required to maintain maternal serum 25(OH)D concentrations >50 nmol/L (8). Our results do not support either of these conclusions because 63% of women in early and 86% of women in late pregnancy with mean intakes of 647 IU/d and 713 IU/d, respectively, had serum 25(OH)D > 75 nmol/L and 94% in early and 97% in late pregnancy had serum concentrations >50 nmol/L. We hypothesize that the discrepancies between estimates of vitamin D intake are likely due to sunshine exposure and women's skin color, which directly influence vitamin D status, as noted in a systematic review that concluded, "we could not exclude the influences from sun exposure, skin characteristics, dietary intake, and vitamin D intake because these confounding factors were not assessed in the available RCT data" (1). Such inconsistency in predictions of optimal vitamin D intake in pregnancy underlies the lack of consensus on recommended dietary intake as well as serum 25(OH)D concentrations for clinical benefits in pregnant women. In another approach, it was suggested that a serum 25(OH)D concentration of 100 nmol/L is needed for optimal conversion of 25(OH)D to the active form of vitamin D by the Cyp27b1 enzyme, maximizing its potential health benefits (27, 28). We did not see such a relation in our study because we observed a weak association between serum 25(OH)D and $1,25(OH)_2D$ and only in late pregnancy.

The observed 23% rise in serum $1,25(OH)_2D$ concentration from early to late pregnancy is within the range of 10–54% reported by some (29–32) but not other (33) observational studies that have measured this metabolite across pregnancy. However, our serum $1,25(OH)_2D$ concentrations in early and late pregnancy are lower than reported in these studies, which ranged from 136.0 to 290.0 pmol/L in early pregnancy and from 212.0 to 371.8 pmol/L in late pregnancy (29–32). This is likely due to the fact that most studies used an immunoassay and not MS (29–31), yielding potentially higher circulating concentrations. Indeed, as reported by the Vitamin D External Quality Assessment Scheme (DEQAS) (34), $1,25(OH)_2D$ concentrations measured by LC/MS-MS tend to be lower than when measured by immunoassays. Unlike 25(OH)D, no reference material from the NIST (35) exists for $1,25(OH)_2D$, making it difficult to assess a "true" value. As noted by the DEQAS committee (34), large variability exists among assays, highlighting the need for measuring $1,25(OH)_2D$ by the gold-standard method LC-MS/MS. Our average mean bias for $25(OH)D_3$ using the NIST reference material measured by LC-MS/MS was −1.3%, which makes us confident in the method employed and in our results for $1,25(OH)_2D$.

In the Canadian context, detection of the D_2 isomer is not important because for both 25(OH)D and $1,25(OH)_2D$ the D_2 isomer did not contribute to the overall status. This likely is a reflection of limited food sources of vitamin D_2 in the Canadian food chain. Our results align with those from another Canadian pregnancy cohort from northern Alberta where the reported $25(OH)D_2$ concentrations [median (IQR)] measured by LC-MS/MS were 2.9 (1.7–4.4) nmol/L, contributing very little to the overall 25(OH)D status of 92.7 (79–109.4) nmol/L in 537 pregnant women in their second trimester (36). Although in Canada most food sources and supplements on the market provide vitamin D_3, new plant-based and vegetarian/vegan products supplemented with vitamin D_2 are emerging and might contribute to serum $25(OH)D_2$ concentrations in some consumers in the future. Season at time of blood draw was the factor most strongly associated with maternal 25(OH)D concentrations in early and late pregnancy, indicating the importance of sunshine exposure as reported by other Canadian (37, 38) and non-Canadian studies (32, 39, 40). But winter season did not adversely affect maternal serum 25(OH)D concentrations across pregnancy, because values were >50 nmol/L for most women all year round, likely owing to the consistent consumption of supplements containing vitamin D even at the amount of 400 IU/d.

As was expected, cord serum 25(OH)D concentrations were significantly associated with maternal concentrations, and reflected general adequacy as indicated by concentrations >30 nmol/L; only 6 samples were <30 nmol/L, aligning with results from others in Canada (38, 41, 42). Newborn serum 25(OH)D concentrations were 55% of the maternal concentrations in late pregnancy, which is within the range of some non-Canadian reports [usually 50–80% (12)] and slightly lower than reported in other Canadian cohorts [ranging between 72% (41) and 127% (38)]. The lower ratio of cord blood to maternal 25(OH)D we observed may reflect the high maternal vitamin D status in our cohort at the end of pregnancy (e.g., >100 nmol/L) compared with other cohorts (38, 41). A threshold of 25–30 nmol/L has been suggested to prevent nutritional rickets in infants (8). To our knowledge, no evidence exists that cord blood 25(OH)D beyond 50 nmol/L confers any clinical benefit to the offspring. However, excessive circulating 25(OH)D concentration (>125 nmol/L) in pregnancy was associated with infants that were smaller at 6 mo of age than infants of women with maternal status <125 nmol/L (43).

Strengths of the current study include the prospective and repeated assessment of vitamin D intake and status from early to late pregnancy and in cord blood. Vitamin D metabolites were measured using the gold-standard method (44) and included both the D_2 and D_3 isomers of 25(OH)D as well as $1,25(OH)_2D$. Our study was seasonally balanced because we continuously recruited over 4 y and included women with a wide range of prepregnancy BMI and body fat mass. Some limitations of our study also must be considered. The cohort of pregnant women was fairly homogeneous, being primarily of European descent and highly educated. This might limit

the generalizability of our findings to an ethnically diverse population as occurs in some areas of Canada. Also, the women were not vitamin D deficient and only a small percentage were vitamin D insufficient, so the results regarding factors associated with maternal status might not be extrapolated to pregnant women with low vitamin D status. Although we have recorded season, the lack of quantitative measurement of sun exposure limits the interpretation of the results, because there might be differences in individuals' exposure due to variable time spent doing outdoor activities, clothing coverage, and use of sunscreen.

In conclusion, although both vitamin D metabolites increased throughout pregnancy, they were only weakly correlated in late pregnancy. This suggests that vitamin D status per se does not drive the rise in $1,25(OH)_2D$ observed in pregnancy, but rather other factors might be at play such as upregulated synthesis in renal, placental, or decidual tissue (12). In our population, being pregnant in the summer season and achieving currently recommended vitamin D intake supported adequate maternal vitamin D status throughout pregnancy, which was linked to cord vitamin D status >50 nmol/L. Our results do not corroborate the reported widespread vitamin D deficiency in pregnant women (1, 3) and highlight the importance of considering the context of the cohort or population under study when assessing vitamin D status, because factors such as sun exposure, ethnicity, and dietary intake influence maternal status (10). Global recommendations for vitamin D supplementation in pregnancy beyond regular prenatal multivitamins may lead to excessively high serum concentrations of 25(OH)D, as we have observed. Based on emerging evidence, excessive maternal vitamin D status may also be associated with adverse outcomes (43). Future steps include investigating the association of maternal 25(OH)D concentrations with pregnancy and neonatal health outcomes, as part of the randomized controlled BHIP trial.

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