Low Serum Vitamin B-12 Concentrations Are Prevalent in a Cohort of Pregnant Canadian Women1–3

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Abstract

Background: Among Canadian women of reproductive age, 5% and 20% have serum vitamin B-12 concentrations indicative of deficiency (<148 pmol/L) and marginal status (148–220 pmol/L), respectively. Given the association between suboptimal vitamin B-12 and adverse pregnancy outcomes, an understanding of vitamin B-12 status during pregnancy, and factors that influence it, is required.

Objective: This prospective analysis from the PREFORM (PREnatal FOlic acid exposuRe on DNA Methylation in the newborn infant) study investigated 1) vitamin B-12 status in a cohort of Canadian pregnant women and their newborns, 2) the association of maternal dietary vitamin B-12 intake with maternal and cord blood concentrations of vitamin B-12 and its biomarkers, and 3) the association of fetal genetic polymorphisms with cord blood concentrations of vitamin B-12 and its biomarkers.

Methods: In pregnant Canadian women (n = 368; mean ± SD age: 32 ± 5 y), vitamin B-12 intakes were assessed in early (0–16 wk) and mid- to late (23–37 wk) pregnancy. Serum vitamin B-12 and plasma total homocysteine (tHcy) and methylmalonic acid (MMA) in maternal blood at 12–16 wk of pregnancy and at delivery (28–42 wk) and in cord blood were measured and compared by using regression analyses. The associations of 28 fetal genetic variants in vitamin B-12 metabolism and cord blood vitamin B-12, tHcy, and MMA concentrations were assessed by using regression analysis, with adjustment for multiple testing.

Results: A total of 17% and 38% of women had deficient and 35% and 43% had marginal serum vitamin B-12 concentrations at 12–16 wk of pregnancy and at delivery, respectively. Only 1.9–5.3% had elevated MMA (>271 nmol/L), and no women had elevated tHcy (>13 μmol/L). Maternal dietary vitamin B-12 intake during pregnancy was either weakly associated or not associated with maternal and cord blood vitamin B-12 (r² = 0.17–0.24, P < 0.0008), tHcy (P = NS) and MMA (r² = 0.05–0.11, P < 0.001). Fetal genetic polymorphisms were not associated with cord blood concentrations of vitamin B-12 and its biomarkers.

Conclusions: Deficient and marginal serum vitamin B-12 concentrations are prevalent in Canadian pregnant women with the use of traditional cutoffs, despite supplement use. Given the growing interest among women to adhere to a vegetarian diet that may be lower in vitamin B-12, and vitamin B-12’s importance in pregnancy, the functional ramifications of these observations need to be elucidated. This trial was registered at clinicaltrials.gov as NCT02244684. J Nutr 2016;146:1035–42.

Keywords: vitamin B-12, homocysteine, methylmalonic acid, pregnancy, maternal diet, fetal genotype

Introduction

The requirement for vitamin B-12 increases during pregnancy to facilitate the rapid expansion of maternal and fetal tissues (1). The 2007–2009 Canadian Health Measures Survey (CHMS)12 suggests that 5% and 20% of reproductive-aged women have serum vitamin B-12 in the deficient (<148 pmol/L) or marginal (148 to 220 pmol/L) range, respectively (2). However, there is a paucity of recent data concerning the vitamin B-12 status of pregnant women, a group not often represented in national surveys. A recent Canadian study reported that 23% and 35% of women in Vancouver had deficient or marginal plasma vitamin B-12 in late pregnancy, respectively (3). This high prevalence of low plasma vitamin B-12 in late pregnancy is
consistent with historical data from other developed countries (4–6).

Much remains to be understood about the factors that influence the vitamin B-12 status of women during pregnancy or that of their fetuses. Lower serum vitamin B-12 concentrations during pregnancy are influenced in part by normal physiologic changes, including hemodilution, hormonal changes, and maternal to fetal transfer of vitamin B-12 (7). They may also reflect insufficient intake to meet elevated vitamin B-12 requirements. Such may be the case among women who consume vegetarian diets that are lower in vitamin B-12 (8). On the basis of dietary recall data from the 1999–2004 NHANES, it was estimated that 7.5% of American women adhere to a vegetarian diet (9). Although women who follow a vegan diet are particularly at risk of vitamin B-12 deficiency, women who follow other types of vegetarian diets are at greater risk than are nonvegetarians (10). Furthermore, it has been suggested that the RDA for vitamin B-12 during pregnancy may be inadequate (11–14). In a recent controlled feeding study, Bae et al. (15) reported that vitamin B-12 intakes during pregnancy ~3 times the RDA increase the bioactive form of vitamin B-12 holotranscobalamin (holoTC) and may improve maternal vitamin B-12 status.

Polymorphisms in vitamin B-12 metabolism-related genes (Supplemental Figure 1) can also affect concentrations of vitamin B-12 (16), total homocysteine (tHcy) (16), and methylmalonic acid (MMA) (17). Recently Zinck et al. (16) identified associations between single nucleotide polymorphisms (SNPs) in the transcobalamin receptor (CD320) and DNA methyltransferase 2 (DNMT2) genes and vitamin B-12 status among nonpregnant women from the CHMS. These SNPs were previously associated with neural tube defects (NTDs) (18–20). Furthermore, there are a few reports of associations between fetal SNPs and the risk of spontaneous abortion (21), uteroplacental insufficiency (22), and NTDs (18), suggesting that these SNPs likely play a functional role in vitamin B-12 metabolism in utero. Regardless of etiology, maternal vitamin B-12 concentrations in the deficient range are associated with low birth weights (23) and insulin resistance and impaired cognitive development in offspring (24, 25). Marginal vitamin B-12 concentrations in early pregnancy have been shown to be an independent risk factor for NTDs (26–28), leading to a recommendation that women commence pregnancy with serum vitamin B-12 concentrations >221 pmol/L to reduce the risk of NTDs (28). The aims of the present study were as follows: 1) to assess concentrations of vitamin B-12 and its functional biomarkers, tHcy and MMA, in a cohort of Canadian women in early and late pregnancy and in their newborn infants; 2) to investigate the relation between maternal dietary vitamin B-12 intake and maternal and cord blood concentrations of serum vitamin B-12 and plasma tHcy and MMA; and 3) to examine the association of fetal genetic variants in vitamin B-12 metabolism with cord concentrations of serum vitamin B-12 and plasma tHcy and MMA.

Methods

Subjects and study design. This study focused on the analysis of serum vitamin B-12 and plasma tHcy and MMA in a cohort of pregnant women and their newborn infants participating in the PREFORM (PREnatal FOLic acid expoSure on DNA Methylation in the newborn infant) study (clinicaltrials.gov identifier: NCT02244684). Details of the PREFORM study design, collection of baseline demographic characteristics, and sample collection were reported previously (29, 30). All study procedures were reviewed and approved by St. Michael’s Hospital Research Ethics Board, and written informed consent was obtained from all women during the initial study visit. Between November 2010 and January 2012, healthy women between 12 and 16 wk of gestation, 18–45 y of age, and with an uncomplicated, singleton pregnancy were recruited from prenatal clinics at St. Michael’s Hospital (Toronto, Canada). Women were excluded if they were taking medications known to interfere with folate metabolism, planned to deliver at another hospital, or planned to bank umbilical cord blood. At birth, the infants’ anthropometric information was collected as described previously (29).

Assessment of dietary and supplemental intake. The assessment of dietary and supplemental intake of vitamin B-12 of PREFORM participants was previously published (29). Briefly, maternal dietary intakes of vitamin B-12 during early and mid- to late pregnancy were assessed by using the Block FFQ (NutritionQuest) at the first (12–16 wk of gestation) and second (33–37 wk of gestation) study visits. The vitamin B-12 food-composition values in these analyses were modified by using the Canadian Nutrient File to reflect the different fortification regulations between Canada and the United States. The FFQs were designed to capture usual intake in the previous 3 mo, so that at the first and second study visits dietary intakes between 0 and 16 wk of gestation (early pregnancy) and between 23 and 37 wk of gestation (mid- to late pregnancy), respectively, were collected. Vitamin supplement use was assessed over 3 time periods: preconception (30 d before pregnancy), early pregnancy (conception to 16 wk of gestation), and mid- to late pregnancy (23–37 wk of gestation) by using a baseline demographic questionnaire (preconception) and the Block FFQ (early and mid- to late pregnancy).

Blood sample collection and analyses. Maternal venous blood was drawn from each subject during early pregnancy (12–16 wk of gestation) and at delivery (28–42 wk of gestation). Participants were not instructed to fast before blood collection; however, the time of their last meal or snack and supplement use was recorded. At delivery, venous blood was also obtained from the umbilical cord. Blood samples were collected in evacuated tubes with and without EDTA. Serum and plasma were isolated from all whole-blood samples within 2 h of collection and stored at −80°C until further analysis.

Serum concentrations of vitamin B-12 were measured by using the Access competitive-binding immunoenzymometric assay (Beckman Coulter). To assess the accuracy and precision of these measurements, serial dilutions of 1:1 (480 pg/mL), 1:2 (240 pg/mL), and 1:8 (60 pg/mL) of the standard reference material (National Institute for Biological Standards and Control 03/178, WHO International Standard for Vitamin B-12 and Serum Folate) were prepared and serum vitamin B-12 concentrations were determined. The interassay CVs for these dilutions were 6.9%, 7.9%, and 13.9%, respectively. Plasma tHcy concentrations were measured by using a quantitative enzymatic assay with the use of the Synchron LX20 (Beckman Coulter). Intra- and interassay CVs were <5.5% and <2%, respectively. Plasma MMA concentrations were measured by LC–tandem MS (31). Intra- and interassay CVs for all runs were <5%.1

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3 Supplemental Figure 1 and Supplemental Table 1 are available from the “Online Supporting Material” link in the online posting of this article and from the same link in the online table of contents at http://jn.nutrition.org.

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5 Abbreviations used: CBS, cystathionine β-synthase; CD320, transcobalamin receptor; CHMS, Canadian Health Measures Survey; CUBN, cubulin; DNMT2, DNA methyltransferase 2; DPEP1, dipeptidase 1; FUT2, fucosyltransferase 2; holoTC, holotranscobalamin; MMA, methylmalonic acid; MMAA, methylmalonic aciduria type A protein; MTHR1, methylenetetrahydrofolate dehydrogenase; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; MUT, methylmalonyl-CoA mutase; NOX4, NADPH oxidase 4; NTD, neural tube defect; PON1, aromatic esterase 1; PREFORM, PREnatal FOLic acid expoSure on DNA Methylation in the newborn infant; SNP, single nucleotide polymorphism; TCN1, haptocorrin; TCN2, transcobalamin; tHcy, total homocysteine; TRMT1, transfer RNA (cytosine-5-) methyltransferase.
Cord blood (8 mL) mononuclear cells were extracted by using the ACCUSPIN System-Histopaque 1077 System (Sigma-Aldrich). RNAprotect Cell Reagent (200 mL; Qiagen Sciences) was then added to cord blood mononuclear cell pellets and frozen at –80°C until DNA extraction. DNA from cord blood mononuclear cells was isolated by using the DNA Isolation Kit for Mammalian Blood (Roche Diagnostics). The extracted DNA was ≥20 kb in all instances, was free of RNA contamination, and had an A260/280 ratio between 1.8 and 2.0.

Twenty-eight SNPs in 16 candidate genes involved in vitamin B-12 absorption, metabolism, and transport (Supplemental Figure 1) or associated with adverse pregnancy outcomes (18, 21, 22, 32, 33) were genotyped at the Clinical Genomics Centre (Toronto, Canada). The specific SNPs genotyped were as follows: methylenetetrahydrofolate reductase (MTHFR) rs1801133, MTHFR rs1801131, methylenetetrahydrofolate dehydrogenase (MTHFD1) rs2236225, methionine synthase (MTR) rs1805087, methionine synthase reductase (MTRR) rs1801394, cubilin (CUBN) rs11254363, CUBN rs1801222, CUBN rs1907362, haptocorrin (TCN1) rs526934, TCN1 rs34324219, transcobalamin (TCN2) rs1131603, TCN2 rs1801198, TCN2 rs575784, methylnalonyl-CoA mutase (MUT) rs1141321, MUT rs9473555, MUT rs4267943, methylnalonic aciduria type A protein (MMAA) rs2270655, NAD(P)H oxidase 4 (NOX4) rs11018628, dipeptidase 1 (DPEPI) rs1126464, cystathionine β-synthase (CBS) rs2124459, CBS rs2851391, CBS rs4920037, transfer RNA (cytosine-5-) methyltransferase (TRDMT1) rs2295809, transcobalamin receptor (CD320) rs2336573, aromatic esterase 1 (PON1) rs3917577, fucosyltransferase 2 (FUT2) rs492602, FUT2 rs601338, and FUT2 rs602662.

**Statistical analysis.** Serum vitamin B-12 and plasma functional biomarker (tHcy and MMA) concentrations at 3 time points [maternal blood during early pregnancy (12–16 wk), maternal blood at delivery, and cord blood] were compared by using least-squares equations (PROC GENMOD; SAS Institute) to take repeated measures into account. Student’s t tests (PROC TTEST) were used to compare the means of maternal and cord serum vitamin B-12 and plasma functional biomarker concentrations of supplement users with nonusers.

The associations between 1) venous cord serum vitamin B-12 and plasma functional biomarker concentrations (outcome variables) and the corresponding maternal concentrations at delivery (explanatory variables) and 2) maternal and cord serum vitamin B-12 and plasma functional biomarker concentrations (outcome variables) and maternal dietary vitamin B-12 intakes in early and mid- to late pregnancy (explanatory variables) were assessed by using least-squares regression (PROC REG). Outcome variables that were not normally distributed were log-transformed before analysis. For associations between cord blood concentrations and the corresponding maternal blood concentrations at delivery, models were adjusted for potential confounding covariates (maternal age, prepregnancy BMI, education, parity, smoking, ethnicity, infant’s sex, dietary and supplemental intake of vitamin B-12, and gestational age at delivery) after an assessment for collinearity. For the associations between maternal and cord blood concentrations and maternal dietary vitamin B-12 intakes, models were adjusted for potential confounding covariates (maternal age, prepregnancy BMI, education, parity, smoking, ethnicity, infant’s sex, total energy intake, time of last meal, vitamin B-12 supplement use, and gestational age) after an assessment for collinearity. Covariates that were significantly associated with the outcome variable (P < 0.05) or changed the effect estimate by ≥10% were retained in the final model.

An exploratory analysis was conducted to evaluate the associations of the 28 selected fetal SNPs on cord serum vitamin B-12 and plasma biomarker concentrations. Differences in cord concentrations of serum vitamin B-12 and functional biomarkers (outcome variables) across fetal genotype (explanatory variables) were assessed by using least-squares regression (PROC GLM) with a Tukey’s honestly significant difference post hoc test. The maternal blood concentration of the corresponding metabolite at delivery was included in the regression as an additional explanatory variable. Preterm infants (n = 16 with available blood samples; 28–37 wk postconceptional age) were excluded from the analysis of the association of fetal SNPs and cord blood concentrations of vitamin B-12 and functional biomarkers. All models were adjusted for the potential confounding covariate of vitamin B-12 supplement use in mid- to late pregnancy.

Data were analyzed by using Statistical Analysis Systems software (SAS version 9.3; SAS Institute). With the exception of the exploratory SNP analysis, all statistical analyses were 2-sided; an α level ≤0.05 was considered to indicate significance. The Benjamini-Yekateul method (PROC MULTTEST DEPENDENTFDR) was used to adjust the cutoff to obtain a false-discovery rate of 0.05 for the SNP analysis. All available data were used for the analysis, and sample sizes for each test were reported.

**Results**

**Subject characteristics.** Of 1315 pregnant women approached about the study, 906 met inclusion and exclusion criteria; of these, 368 women consented to participate (Figure 1). Detailed information on the PREFORM study design and characteristics of study participants have been reported in detail previously (29). Briefly, study participants were of diverse geographic origins (~45% white, 17% Asian (including Chinese, Filipino, Japanese, and Korean), 9% Latin American, 7% black, 6% South Asian, 16% other) and 54% were nulliparous, with a mean ± SD age of 32 ± 5 y. Their mean prepregnancy BMI (in kg/m²) was 24.6 ± 4.6, and they delivered at a mean gestational age of 39.3 ± 1.7 wk. A total of 23% and 62% of women had attained a college/vocational diploma and a university degree, respectively. Only 11% of families reported household incomes below the family-adjusted 2013 poverty line (34). Approximately 85% of women who completed the early pregnancy visit remained in the study at delivery. The primary reason given for not continuing in the study included transfer or delivery at another hospital or moved away (n = 24), miscarriage/termination (n = 8), not enough time or personal reasons (n = 8), uncomfortable with blood collection (n = 5), and lost contact (n = 3).

**Dietary and supplemental intakes of vitamin B-12.** Detailed information on dietary and supplemental intakes of vitamin B-12 among the PREFORM study participants was published previously (29). Briefly, maternal dietary vitamin B-12 intakes were 4.7 ± 3.1 µg/d (range: 0.9–26.1 µg/d) in early pregnancy (0–16 wk) and 4.6 ± 2.6 µg/d (range: 0.7–18.5 µg/d) in mid- to late pregnancy (23–37 wk). The RDA for vitamin B-12 for pregnant women is 2.6 µg/d (6). Eighty-eight percent of women reported taking a vitamin B-12–containing supplement ≥1 time/wk during early and mid- to late pregnancy. Median (IQR) maternal supplemental vitamin B-12 intake was 2.6 (2.6, 10.0) µg/d during early pregnancy. Supplemental vitamin use in mid- to late pregnancy was reported as a categorical variable (yes or no) only, and therefore the supplemental vitamin B-12 dose was not determined for mid- to late pregnancy.

**Maternal and cord concentrations of serum vitamin B-12 and plasma biomarkers.** During early pregnancy (12–16 wk), 16.9% and 35.0% of women had deficient (<148 pmol/L) and marginal (148–220 pmol/L) serum vitamin B-12 concentrations, respectively (Table 1). Mean maternal serum vitamin B-12 concentrations decreased by 23% during pregnancy (P < 0.005), and as a result, the percentage of women with deficient and marginal serum vitamin B-12 concentrations increased to 38.2% and 42.9%, respectively, at delivery (Table 1).

None of the women had an elevated plasma tHcy concentration (>13 µmol/L) at any time during pregnancy. In early pregnancy and at delivery, 1.9% and 5.3% of women had elevated plasma MMA concentrations (>2.71 nmol/L), respectively.
Of the 6 women with elevated plasma MMA concentrations in early pregnancy, 3 had serum vitamin B-12 concentrations in the deficient or marginal range. Of the 14 women with elevated plasma MMA concentrations at delivery, 12 had serum vitamin B-12 concentrations in the deficient or marginal range.

Maternal mean corpuscular volume was 90.2 ± 4.9 fL (range: 64.5–99.4 fL) in early pregnancy and 90.4 ± 5.7 fL (64.1–110 fL) at delivery. Mean maternal hematocrit was 0.36 ± 0.03 L/L (0.29–0.42 L/L) in early pregnancy and 0.36 ± 0.03 L/L (0.26–0.42 L/L) at delivery. Neither of the hematologic values changed significantly during pregnancy.

Cord serum vitamin B-12, plasma tHcy, and plasma MMA concentrations were 1.9, 2.3, and 0.82 times those of the corresponding maternal concentrations at delivery, respectively (P < 0.005) (Table 1). The aforementioned biomarker changes over time were similar in the subset of mother-infant dyads for whom a complete set of blood samples was available at all 3 time points (i.e., early pregnancy, delivery, and cord blood).

Comparison of concentrations of serum vitamin B-12, plasma tHcy, and MMA among vitamin B-12 supplement users and nonusers. Women who consumed vitamin B-12–containing supplements during pregnancy had significantly higher serum vitamin B-12 concentrations during early pregnancy (P < 0.0001) and at delivery (P = 0.0035) than did nonusers (Table 1). However, no significant differences in plasma tHcy and MMA concentrations were observed between vitamin B-12 supplement users and nonusers. Cord serum vitamin B-12 concentrations were also higher (P = 0.01), whereas cord plasma tHcy concentrations were lower (P = 0.04), among infants whose mothers used vitamin B-12 supplements during pregnancy.

### TABLE 1 Maternal and cord concentrations of serum vitamin B-12, plasma tHcy, and plasma MMA in a cohort of Canadian pregnant women and their newborn infants

<table>
<thead>
<tr>
<th></th>
<th>Serum vitamin B-12, pmol/L</th>
<th>Plasma tHcy, μmol/L</th>
<th>Plasma MMA, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early pregnancy (12–16 wk)</td>
<td>Delivery</td>
<td>Cord</td>
</tr>
<tr>
<td>All subjects³</td>
<td>219² (210, 229)</td>
<td>169 (162, 176)</td>
<td>321* (300, 344)</td>
</tr>
<tr>
<td>Supplement users⁴</td>
<td>227² (216, 237)</td>
<td>173 (165, 182)</td>
<td>330* (308, 355)</td>
</tr>
<tr>
<td>Non-supplement users⁵</td>
<td>172³ (151, 195)</td>
<td>142²¹⁺ (126, 161)</td>
<td>255*¹⁺ (209, 310)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>0.0035</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1 Within each row, concentrations of each nutrient at 3 time points (early pregnancy, delivery, and cord) were compared. Labeled means (in the same row for each nutrient) without a common superscript letter differ, P < 0.005. Within each column, concentrations of each nutrient were compared between supplement users and nonusers. P values at the bottom of each column were computed by using Student’s t tests to compare differences in serum and plasma concentrations between supplement users and nonusers.

2 Different from supplement users, P < 0.05. MMA, methylmalonic acid; tHcy, total homocysteine.
3 Values are arithmetic means (95% CI) for normally distributed data or geometric means (95% CI) for non-normally distributed data.
4 Sample size ranged from n = 161 to 296 due to missing values.
5 Sample size ranged from n = 22 to 38 due to missing values.

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vitamin B-12-containing supplements during mid- to late pregnancy than in those born to mothers not taking supplements. In contrast, cord plasma MMA concentrations were not significantly different between infants born to mothers who consumed vitamin B-12 supplements during mid- to late pregnancy and those born to mothers who did not.

Association of maternal delivery and cord concentrations of serum vitamin B-12 and plasma tHcy and MMA. Concentrations of maternal serum vitamin B-12 and plasma tHcy, but not plasma MMA, at delivery were significantly associated with concentrations of cord serum vitamin B-12 and plasma tHcy (P < 0.0001) (Table 2). Sensitivity analyses revealed that the aforementioned associations remained the same when women who delivered prematurely and their infants were removed from the statistical analysis.

Association of maternal dietary vitamin B-12 intake and concentrations of serum vitamin B-12 and plasma total tHcy and MMA. Even after supplemental vitamin B-12 intakes were controlled for, dietary intakes of vitamin B-12 in early pregnancy and mid- to late pregnancy were associated with maternal serum vitamin B-12 concentrations in early pregnancy and at delivery (Table 3). Likewise, maternal dietary intake of vitamin B-12 in mid- to late pregnancy was associated with cord serum vitamin B-12 concentrations (P = 0.0002). Maternal dietary intakes of vitamin B-12 in early and mid- to late pregnancy were either not associated or were very weakly associated with plasma tHcy and MMA concentrations in maternal blood during early pregnancy, at delivery, and in cord blood. Sensitivity analyses showed that the aforementioned associations remained the same when women who delivered prematurely were removed from the analysis.

Association of fetal genetic variants on cord concentrations of serum vitamin B-12 and plasma tHcy and MMA. An exploratory analysis was conducted to evaluate the association of 28 fetal SNPs on cord serum vitamin B-12 and plasma tHcy and MMA concentrations. All of the SNPs displayed Hardy-Weinberg distribution in the sample population (P > 0.05), with the exception of PON1 rs3917577 and FUT2 rs602662. All of the SNPs had a minor allele frequency >10%, except for rs11018628 (9.4%), and rs1131603 (minor allele frequency = 3.3%). CUBN rs1907362 (6%), MUT rs2336573 (7.4%), rs1131603 (6%), and rs11018628 (9.4%), and CD320 rs2336573 (7.4%). The associations between fetal genotypes (explanatory variables) and cord concentrations of serum vitamin B-12 and plasma tHcy and MMA (outcome variables) are summarized in Supplemental Table 1. A mutation in MUT (rs4267943), which encodes the mitochondrial enzyme methylmalonyl-CoA mutase, was associated with cord plasma MMA concentrations (P = 0.04). A mutation in TCN1 (rs34324219), which encodes the vitamin B-12 transporter protein haptocorrin, was associated with cord serum vitamin B-12 concentrations (P = 0.02). After applying Tukey’s honestly significant difference post hoc test to adjust for multiple comparisons, these findings were no longer significant. None of the other SNPs were significantly associated with cord concentrations of serum vitamin B-12 or plasma tHcy or MMA.

Discussion

Although ~90% women in our study were consuming vitamin B-12-containing supplements and most had dietary vitamin B-12 intakes well above the RDA, 16.9% and 35.0% of women had deficient and marginal serum vitamin B-12 concentrations during early pregnancy, respectively. Normal physiologic factors, such as hemodilution, hormonal changes, and vitamin B-12 transfer to the fetus, likely contributed to these observations (7). However, it is important to also acknowledge that because the cutoff for marginal serum vitamin B-12 aligns with that established by Molloy et al. (28) at 15 wk of gestation to be maximally protective against an NTD (221 pmol/L), fully 50% of women in our sample were at risk of a vitamin B-12-dependent NTD. NTDs occur as a result of a failure of the neural tube to close properly during the first month of pregnancy, often before a woman is aware she is pregnant. Data from the 2007–2009 CHMS suggest that ~25% of women of reproductive age have vitamin B-12 concentrations that are not maximally protective against an NTD (2). In a large population-based case-control study from the province of Ontario, post-mandatory folic acid fortification of the food supply in Canada, Ray et al. (35) reported a tripling of the OR of NTD among women in the lowest quartile of vitamin B-12 status, measured by holoTC (the biologically active portion of plasma vitamin B-12). Consistent with other studies (7, 36, 37), maternal serum vitamin B-12 concentrations in the present study decreased by 23% during pregnancy, which increased the prevalence of deficient and marginal vitamin B-12 status to 38.2% and 42.9% at delivery, respectively. This decrease is unrelated solely to hemodilution because mean ± SD maternal hematocrit did not significantly change between early pregnancy (0.36 ± 0.03 L/L; range: 0.29–0.42 L/L) and delivery (0.36 ± 0.03 L/L; 0.26–0.42 L/L). Deficient and marginal vitamin B-12 status is associated with adverse birth and offspring health outcomes (23–28). The prevalence of deficient and marginal vitamin B-12 status in our study cohort in early and late pregnancy is quite comparable to the recent study from Vancouver, Canada (3). Contrary to our findings, a recent study from Alberta reported that <1% of pregnant women are vitamin B-12 deficient (defined as plasma holoTC concentrations <35 pmol/L) (38). However, holoTC concentrations measured with the AxSYM Active B-12 assay (Abbott), as was done in the Alberta study, are higher after processing with EDTA (39). Therefore, the holoTC concentrations reported in this study may be falsely elevated and the low prevalence of low holoTC may not reflect a true prevalence of vitamin B-12 deficiency in pregnancy.

No woman had a plasma tHcy concentration >13 μmol/L, a widely accepted cutoff for hyperhomocysteinemia (40); and
1.9% and 5.3% of women had elevated plasma MMA concentrations >271 nmol/L during early pregnancy and at delivery, respectively. Several studies concluded that plasma tHcy and MMA concentrations during pregnancy may not reflect vitamin B-12 status due to pregnancy-induced physiologic changes, including hemodilution, increased remethylation of tHcy (41), and increased renal excretion due to elevated glomerular filtration rate (42). In the present study, of the 14 women with elevated plasma MMA concentrations at delivery, 12 had serum vitamin B-12 concentrations in the deficient or marginal range. However, many more women, even in the deficient range, did not exhibit elevated plasma MMA concentrations. It has been shown that a greater amount of vitamin B-12 is partitioned toward the biologically active holoTC during pregnancy (15) to ensure that the vitamin B-12 requirements for maternal tissue are met. This observation offers an additional explanation for why tHcy and MMA are not elevated during pregnancy despite the reduction in serum vitamin B-12 concentrations.

Some vitamin B-12 and plasma MMA concentrations were significantly higher, whereas plasma tHcy concentrations were significantly lower, in cord blood than in maternal blood at delivery. These results are consistent with previous findings (7, 43–45). Elevated cord serum vitamin B-12 concentrations are believed to reflect the fetus’ high demand for vitamin B-12 (46–49). Most fetal vitamin B-12 is retained in the fetal circulation, as opposed to being stored in the liver, where it is readily available to participate in biochemical reactions (50). It is not clear whether MMA concentrations in cord blood reflect vitamin B-12 deficiency in infants. We did not observe an association between serum vitamin B-12 and plasma MMA in cord blood. Lower tHcy concentrations in cord blood may be related to rapid remethylation of tHcy to methionine for the provision of tetrahydrofolate for DNA synthesis and S-adenosylmethionine for biological methylation (43). Therefore, cord plasma MMA and tHcy concentrations do not likely reflect fetal vitamin B-12 status.

Consistent with other studies (45–49), cord serum vitamin B-12 and plasma tHcy concentrations were strongly associated with corresponding maternal concentrations at delivery, which shows the importance of maternal vitamin B-12 status on that of the fetus. In contrast to a previous study (43), we did not observe an association between maternal delivery and cord plasma MMA concentrations. Others observed an association between maternal delivery and cord plasma MMA concentrations only among women with holoTC concentrations below the preconceptional median (7).

Maternal vitamin B-12 supplement use during pregnancy resulted in higher maternal and cord serum vitamin B-12 concentrations and lower cord plasma total homocysteine concentrations, consistent with the findings from a vitamin B-12 supplementation clinical trial (51). In contrast, maternal dietary vitamin B-12 intake during pregnancy was only weakly associated with maternal serum vitamin B-12 concentrations during early pregnancy and at delivery as well as with cord serum vitamin B-12 concentrations. In a controlled feeding trial, pregnant women in late pregnancy had lower serum vitamin B-12 concentrations than did nonpregnant women after a 12-wk intervention despite equivalent vitamin B-12 intakes (~8.6 μg/d) (15). The authors of the latter study also suggested that vitamin B-12 intakes ~3 times the current RDA for pregnancy (2.6 μg/d) may be required to mitigate the reduction in serum vitamin B-12 concentrations and to exert beneficial effects during pregnancy (15).

Several common SNPs in genes involved in vitamin B-12 absorption, metabolism, and transport have been shown to be associated with serum vitamin B-12 and plasma tHcy concentrations in nonpregnant women in the recent CHMS (16) and plasma MMA concentrations (17) and adverse pregnancy and birth outcomes (18, 21, 22, 32, 33). However, the associations of these SNPs in the fetal genome with cord blood concentrations of vitamin B-12 and its biomarkers are unknown. Among the 28 SNPs examined, we observed nominal associations between the MUT rs4267943 and TCN1 rs34324219 SNPs and cord
plasma MMA and serum vitamin B-12 concentrations, respectively. 

MUT encodes the mitochondrial enzyme, methylmalonyl-CoA mutase, which catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA. TCNJ encodes the vitamin B-12–binding protein haptocorrin. After controlling for the false-discovery rate, however, none of the SNPs were significantly associated with cord blood concentrations of serum vitamin B-12 or plasma tHcy and MMA, nor did any SNP interact with maternal blood concentration at delivery to influence cord blood concentration. These results suggest that maternal vitamin B-12 metabolism and status have a greater influence on cord blood concentrations of serum vitamin B-12 and its biomarkers than fetal genetic variants of vitamin B-12 metabolism.

The PREFORM study is the largest North American observational study assessing the factors (maternal vitamin B-12 intake and 28 fetal SNPs) that affect vitamin B-12 status in a diverse group of pregnant women and their newborns. There are a few limitations of our study. First, maternal DNA was not available for genotyping. Maternal genotype data would have strengthened our understanding of factors that influence maternal and cord blood concentrations of vitamin B-12 and its biomarkers. Second, the relatively small sample size and low frequency of several SNPs may have reduced the ability to detect an association between fetal genotype and cord blood concentrations. Third, we did not measure holoTC, the biologically active portion of vitamin B-12 in the circulation; the inclusion of holoTC might have provided a better estimate of vitamin B-12 status. Last, gestational age at delivery ranged from 28 to 42 wk. The inclusion of preterm deliveries (n = 16, <5%) may have slightly affected the mean concentration of the analytes reported at delivery.

The prevalence of serum vitamin B-12 concentrations in the range considered deficient and marginal was high in a cohort of pregnant Canadian women with dietary vitamin B-12 intakes above the current RDA for pregnancy and with widespread vitamin B-12 supplement use. Given the growing interest among women to adhere to vegetarian diets that may be lower in vitamin B-12 and the importance of vitamin B-12 in pregnancy, the functional ramifications of these observations need to be elucidated.

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HB, Y-IK, and DLO designed the study; CEV, SPM, LP, and AL conducted the research; AYL and HB contributed to the participant recruitment; TSH and K-JS contributed to the sample analysis; CEV analyzed data and drafted the manuscript; RC contributed to the data analysis; and YI, Y-IK, and DLO contributed to critically revising the manuscript for important intellectual content. All authors read and approved the final manuscript and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

12. Bor MV, Lydking-Olsen E, Moller J, Nexo E. A daily intake of approximately 6 μg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. Am J Clin Nutr 2006;83:52–8.


