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Maternal vitamin B_{12} status in early pregnancy and its association with birth outcomes in Canadian mother–newborn Dyads

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Abstract

Vitamin B_{12} (B_{12}) is a co-enzyme essential for fetal growth and development. Lower maternal B_{12} status has been associated with preterm birth (<37 gestational weeks) and low birth weight (<2500 g), which are linked to morbidity and mortality across the lifespan. In Canada, 17–25 % of women in early pregnancy had a serum total B_{12} concentration <148 pmol/l and maternal total B_{12} concentration decreased throughout pregnancy. This study aimed to determine the association between maternal B_{12} status and birth outcomes in Canadian mother–newborn dyads. A secondary analysis of 709 mother–newborn dyads in British Columbia (BC), Canada, was conducted. Bio-banked first- (*n* 656) and second-trimester (*n* 709) maternal serum samples of apparently healthy South Asian (50 %) and European (50 %) women from the BC Prenatal Genetic Screening Program were quantified for B_{12} biomarkers (total B_{12}, holotranscobalamin (holoTC), methylmalonic acid (MMA) and total homocysteine (tHcy)). Obstetric history and birth outcome data were obtained from the BC Perinatal Data Registry. All associations were determined using multiple linear regression. Maternal serum total B_{12}, holoTC, MMA and tHcy had a mean weekly decrease of 3·64 pmol/l, 1·04 pmol/l, 1·44 nmol/l and 0·104 µmol/l, respectively ($P < 0{\cdot}001$). Despite a total B_{12} concentration <148 pmol/l among 20–25 % of the women, maternal B_{12} biomarker concentrations were not associated with birth weight *z*-score, head circumference *z*-score and gestational age at birth ($P > 0{\cdot}05$). Additional research in women at high risk of adverse birth outcomes and the association between maternal B_{12} status and functional, for example, cognitive, outcomes is needed.

Key words: **Early pregnancy: Maternal vitamin B_{12}: Birth weight: Head circumference: Gestational age**

Vitamin B_{12} (B_{12}) is an essential nutrient for DNA synthesis, methylation reactions and neural myelination making it critical for fetal and neonatal growth and development[1]. Lower maternal B_{12} status has been associated with adverse birth outcomes including small for gestational age[2,3], low birth weight[4] and preterm birth[4]. These adverse birth outcomes have been associated with perinatal morbidity and mortality[5,6], as well as an increased risk of chronic disease and mortality as an adult[7,8]. Furthermore, a pooled analysis of case studies showed that infants born to B_{12}-deficient mothers had impaired neurodevelopment and motor deficits, such as developmental delay and hypotonia[9]. Studies suggest a possible association between lower maternal B_{12} status and birth head circumference[10,11], which has been suggested as an indicator of fetal brain growth[12].

Due to increased B_{12} requirements to support the growth and development of the fetus, pregnant women are at greater risk of B_{12} inadequacy. As well, establishing sufficient B_{12} stores in the fetus is dependent on maternal B_{12} status[13]. Because pregnancy-specific reference values for optimal B_{12} concentration have not been established, reference values for non-pregnant individuals are often used. However, the use of these reference values in pregnant women may not be appropriate, due to physiological changes during pregnancy, such as hemodilution, that affect the biochemical indicators of B_{12} status[14]. In Canada, approximately 17–25 % of pregnant women had a total B_{12} concentration <148 pmol/l (i.e. the cut-off for B_{12} deficiency in non-pregnant adults) in early pregnancy[15-17]. While maternal B_{12} status has been found to be associated with neural tube defects

Abbreviations: B12, Vitamin B12; BC, British Columbia; BCPDR, British Columbia Perinatal Data Registry; holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

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in Canadian pregnant women[18], there is limited research on the association between maternal B_{12} status and other birth outcomes, such as newborn anthropometrics and gestational age at birth, in Canada. Thus, the objectives of this study were to describe the change in maternal B_{12} biomarker concentrations during early pregnancy and to determine the association of first- and second-trimester maternal B_{12} status with birth outcomes (birth weight *z*-score, head circumference *z*-score and gestational age at birth) in a cohort of Canadian mother–newborn dyads.

Methods

Study design

This study is a secondary analysis of data collected in a retrospective cohort study investigating the B_{12} status of pregnant women who underwent prenatal genetic screening between November 2014 and May 2016 as part of the British Columbia (BC) Prenatal Genetic Screening Program[19]. Additional maternal and neonatal health data were obtained from the BC Perinatal Data Registry (BCPDR)[20]. The sample consists of a cohort of healthy pregnant women aged 19–44 years who are of South Asian (*n* 357) or European (*n* 352) ethnicity (self-reported) and residing in BC, as well as their newborns (*n* 709) born in the study catchment area of BC. The sample size (Fig. 1) was determined based on the primary objective of the original study, which was to compare the first- and second-trimester total B_{12} concentrations between pregnant women of South Asian and European descent[17]. A waiver of individual consent for the secondary use of deidentified clinical samples from the BC Prenatal Genetic Screening Program was approved by the University of British Columbia Children's and Women's Research Ethics Board (institutional approval no.: H15-00820).

Exclusion criteria

Women with multiple gestation, who were smoking during the pregnancy, HIV positive, diagnosed with diabetes mellitus Type I or II, who conceived through *in vitro* fertilisation or taking intravenous or oral steroid medication during pregnancy were excluded. Women who were identified to be at increased risk of trisomy 21 (Down Syndrome), 18, 13 or open neural tube defects according to the BC Prenatal Genetic Screening Program (Perinatal Services BC) were also excluded from the study. In addition, women with impaired renal function were excluded, as renal impairment has been associated with elevated methylmalonic acid (MMA) and total homocysteine (tHcy) concentrations. MMA and 2-methylcitric acid concentrations were used to identify renal insufficiency. Specifically, women were excluded if 2-methylcitric acid concentration exceeded MMA concentration[21] and MMA concentration was >210 nmol/l.

Maternal biomarker data

The biochemical analyses of the maternal B_{12} biomarkers were previously described[17]. In brief, data on maternal B_{12} biomarkers were obtained from the analysis of non-fasting maternal serum samples from the BC Prenatal Genetic Screening Program. Non-fasting samples were used, since B_{12} biomarkers are not impacted by fed or fasting state[22,23]. Maternal blood samples were collected at 8–14 gestational weeks (first trimester) and 15–21 gestational weeks (second trimester). Maternal serum total B_{12} and holotranscobalamin (holoTC) concentrations, which are the direct indicators of B_{12} status, were quantified using fully automated immunoassays (Access 2 Immunoassay System by Beckman Coulter and Architect Immunoassay Analyzer by Abbott Technologies, respectively). Total B_{12} concentrations > 1107 pmol/l (first trimester: *n* 1; second trimester: *n* 3) and holoTC concentrations > 128 pmol/l (first trimester: *n* 125/636, 20 %; second trimester: *n* 83/615, 13 %) were not quantified as they were outside the assay range. Maternal serum MMA and tHcy, which are the functional indicators of B_{12} status, were quantified by stable isotope dilution-LC-tandem MS (LC-MS/MS). In addition, information on maternal age, self-reported ethnicity and weeks of gestation at blood collection, obtained during the prenatal genetic screening visit for blood collection, were abstracted from medical charts.

British Columbia Perinatal Data Registry health data collection

Data on obstetric history, current pregnancy and birth outcomes were retrieved from the BCPDR. The BCPDR contains data abstracted from obstetrical and neonatal medical records on nearly 100 % of births in the province of BC from over sixty acute care facilities, as well as births occurring at home attended by BC registered midwives, including women who had pregnancies ending in a live or still birth of at least 20 gestational weeks or 500 g birth weight[24]. Gestational age at birth in completed weeks was calculated using a hierarchical algorithm based on information on last menstrual period, first ultrasound in early pregnancy, newborn clinical examination and maternal chart (online Supplementary Table S1).

Sociodemographic data collection

The forward sortation area indicating the first three characters of each mother's resident postal code was retrieved from the BCPDR and linked to aggregated socio-economic data at the forward sortation area level from the 2016 Canadian census using the Canadian Census Analyzer (University of Toronto, 2018). Forward sortation area-level (or neighbourhood-level) indicators of socio-economic status obtained from the census include median family income (including lone-parent families and couples with children), which was adjusted for the average family size in each forward sortation area (i.e. divided by the square root of average family size). The adjusted median income for all forward sortation areas in BC was categorised into quintiles. The respective quintiles were then assigned to each of the women. In addition, the proportion of individuals aged 25–64 years who did not have a high school diploma or equivalency certificate in each mother's resident forward sortation area was used to calculate forward sortation area-level education. The models were adjusted for both neighbourhood income and education, as previous research suggested that these measures capture different facets of socio-economic status[25].

Standardisation of birth weight and head circumference

The newborn anthropometric outcomes, birth weight and head circumference, were standardised for gestational age at birth and

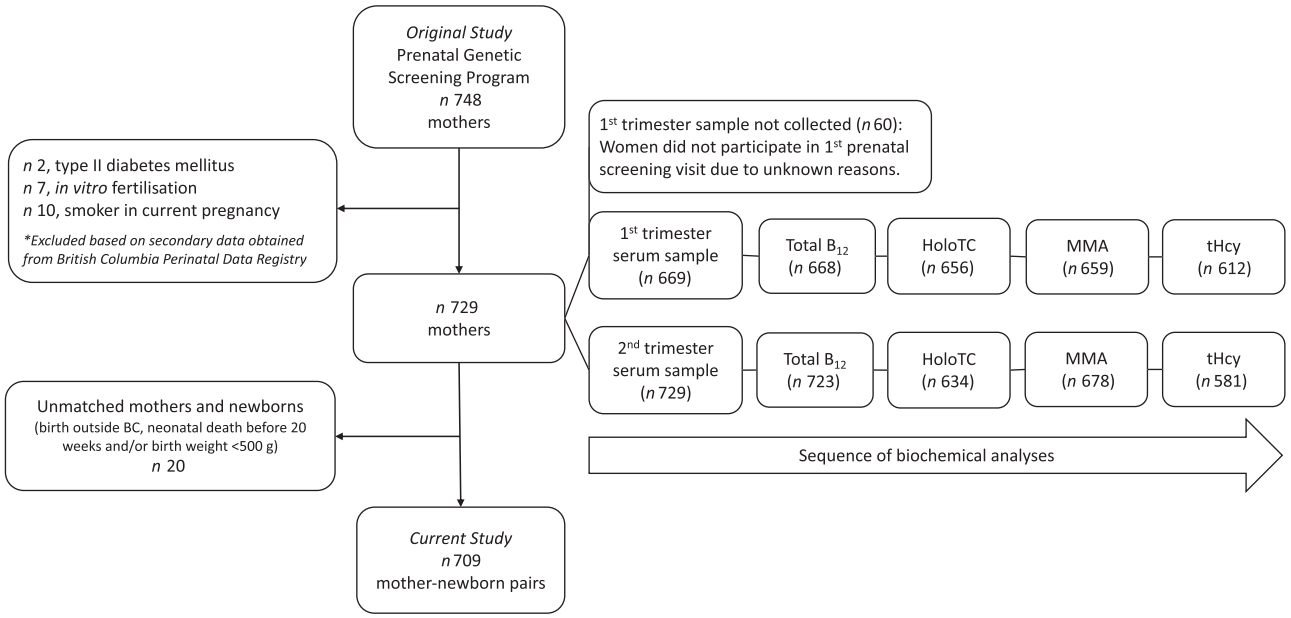

Fig. 1 Flow diagram for the derivation of mother–newborn pairs from the original study sample. Sequence of biochemical analyses refers to the prioritisation of maternal biomarker analyses, which was dependent on serum volume availability and successful biomarker quantitation. B_{12}, vitamin B_{12}; holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

sex using newborn anthropometric charts. The models were not adjusted for gestational age at birth, as gestational age at birth may be a possible mediating variable in the association between maternal B_{12} status and newborn anthropometrics and adjusting for gestational age at birth may lead to bias. Furthermore, the use of anthropometric charts accounts for the non-linear association between birth weight and gestational age at birth, which are closely related, and standardised measurements are more appropriate for outcomes associated with an ongoing risk throughout pregnancy[26], such as lower birth weight and birth head circumference.

Neonatal birth weight and head circumference were standardised for gestational age at birth and sex based on the Kramer[27] and Barbier[28] neonatal charts, respectively. Fetal charts, such as the WHO Fetal Growth Charts[29], which are considered a more accurate representation of optimal growth for preterm newborns, were not used as they were a poor fit for the sample of newborns in this study. Given the low proportion (<10 %) of preterm births in this cohort, the Kramer and Barbier neonatal charts were used to standardise birth weight and head circumference, as they were based on Canadian populations. Although the Kramer and Barbier charts were not derived using the same neonatal data, the birth weights of newborns in the Barbier study were shown to be comparable to that of the Kramer study[28]. Since neonatal anthropometric charts were used in lieu of fetal anthropometric charts, sensitivity analyses excluding preterm births were conducted.

Data analysis

Descriptive statistics. The characteristics of the study sample were summarised using descriptive statistics (absolute value, percentage, median and interquartile range). Normality of individual numerical variables was assessed visually using histograms, as well as quantile–quantile plots.

Change in maternal vitamin B_{12} biomarker concentrations. The association between gestational weeks at maternal serum sample collection and maternal serum total B_{12}, MMA and tHcy concentrations was determined using linear mixed effects modelling; the association between gestational weeks at maternal sample collection and maternal holoTC was estimated using multilevel Tobit regression because 20 % and 13 % of first- and second-trimester holoTC concentrations, respectively, were higher than the upper limit of the assay range, that is, >128 pmol/l. Sample ID was included as a random effect to account for multiple measures within individuals.

Association between maternal vitamin B_{12} status and birth outcomes. Univariable linear regression, as well as multivariable linear regression, models adjusted for confounding variables were used to determine the association between maternal B_{12} status, as indicated by first- and second-trimester serum total B_{12}, holoTC, MMA and tHcy and birth outcomes (birth weight z-score, head circumference z-score and gestational age at birth). Given a sample size of about 600, we had 95 % power to detect $R^2 = 0{\cdot}04$ with $\alpha = 0{\cdot}05$ and ten independent predictors in the regression models for the association between maternal B_{12} biomarkers and birth outcomes. All multivariable models were adjusted for maternal ethnicity, maternal age, parity, pre-pregnancy BMI, gestational weight gain, gestational diabetes mellitus, hypertensive disorder of pregnancy and neighbourhood-level income and education. The models with gestational age at birth as the outcome were additionally adjusted for newborn sex. These confounding variables were determined *a priori* based on a literature review to identify factors associated with maternal B_{12} status and birth outcomes, as well as using directed acyclic graphs. The maternal B_{12} biomarkers and trimesters were analysed separately. Total B_{12} concentrations > 1107 pmol/l were excluded from analyses, as only a small proportion of total

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B_{12} samples were above the assay range. HoloTC concentrations > 128 pmol/l were included in the regression analyses, since a large proportion of the holoTC samples exceeded the assay range. Discontinuous regression, in which a dummy variable (holoTC value > 128 pmol/l = '1'; non-censored holoTC value = '0') was added to the regression model, was applied for all models that included holoTC as an independent variable. A sensitivity analysis for all regression models with holoTC was conducted comparing the discontinuous regression models to models that assigned a concentration of 129 pmol/l for holoTC concentrations >128 pmol/l. All linear regression assumptions were tested for each model. Linearity was visually assessed using augmented component-plus-residual plots, and homoscedasticity was visually assessed using residual plots. Non-linear associations were explored by log-transforming the B_{12} biomarker and with polynomial regression (i.e. addition of quadratic term). Regression models with restricted cubic splines were used to further explore if there were any non-linear associations between each maternal B_{12} biomarker and birth outcome. The restricted cubic spline models included five knots, which according to Harrell[30] is reasonable with larger sample sizes (e.g. $n \geq 100$). The locations of the knots were determined using equally spaced quantiles suggested by Harrell[30] (i.e. 5th, 27·5th, 50th, 72·5th and 95th percentiles). Non-linearity in each model was tested using Wald's statistics (null hypothesis: beta coefficient for 2nd, 3rd and 4th splines equals zero; *P*-value <0·05 considered significant), as well as visually, using graphs of the adjusted predictions and marginal effects. Additional sensitivity analyses excluding influential outliers, which were identified visually using augmented component-plus-residual plots, were conducted.

A significance level (α) of 0·05 for two-tailed tests was used. All analyses were conducted in Stata 15 (StataCorp. 2017. Stata Statistical Software: Release 15. StataCorp LLC).

Handling missing data. Several methods for handling missing data were used for all analyses that included pre-pregnancy BMI and gestational weight gain due to a high proportion of missing data (21 % and 39 %, respectively); this includes the missing indicator method, complete case analysis and multiple imputation[31]. The missing indicator method was used as the primary analysis. In this method, pre-pregnancy BMI and gestational weight gain were converted to categorical variables using WHO[32] and Institute of Medicine[33] guidelines, respectively, including a category for missing data. Complete case analysis, which involves the exclusion of individuals with any missing data, and multiple imputation by chained equations were also conducted as a sensitivity analysis. A description of the methods for the multiple imputation model is included in the Supplementary Material.

Results

Maternal and newborn characteristics

The study included 709 mother–newborn dyads. One mother was excluded due to suspected renal insufficiency. The maternal characteristics of the entire sample are summarised in Table 1. Approximately 20 % (*n* 134/656) and 51 % (*n* 332/656) of women in the first trimester and 25 % (*n* 180/706) and 58 % (*n* 408/706) of women in the second trimester had a total B_{12} concentration <148 pmol/l (cut-off for B_{12} deficiency in non-pregnant adults) and <221 pmol/l (cut-off for low B_{12} in non-pregnant adults), respectively. Newborn characteristics are summarised in Table 2. Nine women had a stillbirth, defined as fetal death after at least 20 weeks of pregnancy or fetal weight of at least 500 grams.

Gestational weeks and Biomarker concentrations

Each of the maternal B_{12} biomarkers, total B_{12}, holoTC, MMA and tHcy, was inversely associated with gestational weeks. The mean (95 % CI) decrease in maternal serum total B_{12} and holoTC was −3·64 (95 % CI −4·64, −2·65) pmol/l (*n* 693; $P < 0{\cdot}001$) and −1·04 (95 % CI −1·42, −0·660) pmol/l (*n* 648; $P < 0{\cdot}001$) with each gestational week, respectively. For the functional biomarkers, MMA and tHcy, the mean (95 % CI) decrease with each gestational week was −1·44 (95 % CI −2·30, −0·587) nmol/l (*n* 689; $P = 0{\cdot}001$) and −0·104 (95 % CI −0·121, −0·086) μmol/l (*n* 674; $P < 0{\cdot}001$), respectively.

Maternal vitamin B_{12} status and birth outcomes

None of the maternal B_{12} biomarkers in either trimester was linearly associated with birth weight *z*-score, head circumference *z*-score and gestational age at birth (β-coefficient $P > 0{\cdot}05$; Tables 3–5). Each model met linear regression assumptions. Non-linear associations were not observed using restricted cubic spline models. For the sensitivity analysis, excluding preterm births did not significantly affect the β-coefficient estimates in each of the models (data not shown). Furthermore, the estimates did not differ among the methods used for handling missing data, including the missing indicator method (results presented in Tables 3–5), complete case analysis (data not shown) and multiple imputation (online Supplementary Tables S3–S5). The results remained not significant following the removal of influential outliers.

Discussion

Previous studies investigating the association between maternal B_{12} status, primarily as total B_{12} and tHcy concentrations, and birth outcomes have shown inconsistent findings. Despite a small but statistically significant decrease in maternal B_{12} status with each gestational week, the current study did not observe an association between each maternal B_{12} biomarker concentration (total B_{12}, holoTC, MMA and tHcy) in the first and second trimesters and the birth outcomes, birth weight *z*-score, head circumference *z*-score and gestational age at birth.

Maternal vitamin B_{12} status and birth outcomes

The lack of linear association between maternal total B_{12} concentration and birth weight *z*-score in the current study is consistent with a recent meta-analysis of eighteen studies by Rogne *et al.*[4]. However, a subgroup analysis of low- and middle-income countries consisting predominantly of studies in India found a mean (95 % CI) increase in birth weight standard

Table 1. Maternal characteristics*
(Median and interquartile ranges (IQR); absolute numbers and percentages, *n* 709)

Characteristics	*n*	%	Median	IQR	Minimum-Maximum
Maternal age (years)	709		31	28, 34	
Gestational weeks at sample collection					
First trimester	693		11·4		8·3–13·9*
Second trimester	693		16·1		14·9–20·9*
Serum total B_{12} (pmol/l)					
First trimester	655		219	158, 297	
Second trimester	703		200	147, 272	
Serum holoTC (pmol/l)					
First trimester	645		84	60, 116	
Second trimester	624		78	55, 108	
Serum MMA (nmol/l)					
First trimester	646		120	100, 159	
Second trimester	661		113	92, 150	
Serum tHcy (μmol/l)					
First trimester	599		5·0	4·5, 5·7	
Second trimester	569		4·4	3·8, 5·1	
Ethnicity					
European	352	50			
South Asian	357	50			
Parity					
Nulliparous	358	50			
Multiparous	343	48			
Pre-pregnancy BMI					
Underweight (<18·5 kg/m^2)	22	3			
Normal weight (18·5–24·9 kg/m^2)	334	47			
Overweight (25–29·9 kg/m^2)	132	19			
Obese (≥30 kg/m^2)	70	10			
Data not available	143	20			
Gestational weight gain†					
Inadequate	93	13			
Adequate	148	21			
Excessive	179	25			
Data not available	281	40			
Health conditions					
Gestational diabetes	107	15			
Hypertensive disorder of pregnancy	25	4			
Both	<5	<0·7			
No risk factors‡	383	54			
Neighbourhood income quintile					
First	210	30			
Second	130	18			
Third	176	25			
Fourth	114	16			
Fifth	70	10			
Neighbourhood education (%)					
Less than secondary	700		10	7, 14	
Secondary	700		28	24, 31	
Post-secondary	700		61	56, 68	

B_{12}, vitamin B_{12}; holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.
* Percentages may not add up to 100% due to missing data.
† Gestational weight gain based on Institute of Medicine guidelines. Adequate weight gain defined according to maternal pre-pregnancy BMI: BMI < 18·5 kg/m^2, 12·5–18 kg; BMI 18·5–24·9 kg/m^2, 11·5–16 kg; BMI 25–29·9 kg/m^2, 7–11·5 kg; BMI ≥ 30 kg/m^2, 5–9 kg.
‡ No specific risk factors in the current or past pregnancy or mother's medical history.

deviation score of 0·08 (95 % CI 0·03, 0·14) with each 1 SD increase in maternal total B_{12} concentration[4]. Similarly, a meta-analysis of nineteen studies by Hogeveen, Blom and Heijer[34] also observed a 0·062 (95 % CI 0·025, 0·10) decrease in birth weight standard deviation score for each 1 SD increase in maternal tHcy concentration. These findings, although statistically significant, may not be clinically significant due to the small effect sizes, which were similarly observed in the current study. The lack of association between first- and second-trimester maternal tHcy and birth weight *z*-score in the current study may be due to the low concentrations of serum tHcy (i.e. median of 5·0 and 4·4 μmol/l in first and second trimester, respectively) compared with those described in the meta-analysis by Hogeveen, Blom and Heijer[34]. The low tHcy concentrations in these pregnant women are likely the result of their high folate concentrations[17], with folate being the main determinant of circulating tHcy[35]. The high folate concentrations may be attributed to the high prevalence of folic acid containing prenatal supplement use in Canada[36] – with most supplements providing 1 mg/d of folic acid – and to a lesser extent, mandatory folic

Table 2. Newborn characteristics*
(Medians and interquartile ranges (IQR); absolute numbers and percentages, *n* 709)

Characteristics	*n*	%	Median	IQR
Discharged to				
Death/stillbirth	9	1		
Home	683	96		
Other hospital	9	1		
Sex†				
Female	349	50		
Male	351	50		
Apgar score at 5 minutes†				
<7	17	2		
≥7	675	96		
Gestational age at birth (gestational weeks)†	699		39	38, 40
Preterm, i.e., <37 gestational weeks†	46	7		
Birth weight (grams)†	700		3380	3086, 3691
Low birth weight, i.e., <2500 g†	20	3		
Standardised birth weight†,‡				
Small for gestational age	34	5		
Appropriate for gestational age	584	83		
Large for gestational age	81	12		
Head circumference (cm)†	689		35	34, 36

* Percentages may not add up to 100 % due to missing data.
† Excluding stillbirths (*n* 9).
‡ Based on Kramer birth weight charts compared with newborns of the same sex and gestational age at birth. Small for gestational age: <10th percentile; appropriate for gestational age: 10th–90th percentile; large for gestational age: >90th percentile.

Table 3. Association between maternal vitamin B_{12} (B_{12}) status and birth weight *z*-score in Canadian mother–newborn dyads*
(β-coefficients and 95 % confidence intervals)

	Unadjusted model					Adjusted model†				
Maternal B_{12} biomarker	*n*	β	95 % CI	*P*	R^2	*n*	β	95 % CI	*P*	R^2
First trimester										
HoloTC‡	636	0·00956	–0·0217, 0·0408	0·55	0·0006	628	–0·00617	–0·0364, 0·0241	0·69	0·104
Total B_{12}	647	0·00129	–0·00528, 0·00786	0·70	0·0002	639	–0·00402	–0·0107, 0·00266	0·24	0·101
MMA	637	–0·00331	–0·0120, 0·00542	0·46	0·0009	630	0·00233	–0·00624, 0·0109	0·59	0·099
tHcy	592	0·0106	–0·0600, 0·0812	0·77	0·0001	585	0·0387	–0·0311, 0·108	0·28	0·096
Second trimester										
HoloTC‡	615	–0·00382	–0·0336, 0·0260	0·80	0·0015	607	–0·00753	–0·0367, 0·0217	0·61	0·108
Total B_{12}	693	0·00164	–0·00529, 0·00857	0·64	0·0003	685	–0·00277	–0·00969, 0·00415	0·43	0·109
MMA	652	–0·00457	–0·0131, 0·00397	0·29	0·0017	644	0·00122	–0·00710, 0·00953	0·77	0·107
tHcy	562	–0·0103	–0·0766, 0·0561	0·76	0·0002	555	0·0193	–0·0457, 0·0844	0·56	0·106

holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.
* Unadjusted R^2 reported for univariable model and adjusted R^2 reported for multivariable models. β-coefficients represent change in birth weight *z*-score with each 10-unit increase in maternal serum holoTC, total B_{12} and MMA concentration and 1-unit increase in tHcy.
† All multivariable models were adjusted for maternal age, maternal ethnicity, parity, GWG, pre-pregnancy BMI, hypertensive disorder of pregnancy, gestational diabetes, neighbourhood income and neighbourhood education.
‡ Discontinuous regression.

acid fortification of flour products. The comparable tHcy concentration of the pregnant women in this study to that of other studies of healthy pregnant women in Canada[15,16] supports this hypothesis.

In contrast to the current study, the Generation R Study, a prospective cohort study of 5890 pregnant women in the Netherlands, observed a 1·6 cm (95 % CI 0·1, 3·1) lower birth head circumference in the newborns of mothers with an early pregnancy tHcy concentration in the highest quintile of tHcy (≥8·31 μmol/l) compared with the lowest quintile (≤5·80 μmol/l)[37]. The inverse association between maternal tHcy concentration and birth head circumference may be due to the higher concentration and greater variability in maternal tHcy concentration (median (90 % range): 6·9 (4·9, 10·5) μmol/l)[37], which may be attributed to the 19 % of women who reported no folic acid supplement use and the absence of food fortification with folic acid in the Netherlands. The tHcy concentrations were lower in this Canadian cohort compared with the Generation R Study, which possibly contributed to the null findings between maternal tHcy and birth head circumference.

The meta-analysis by Rogne *et al.*[4] did not observe a linear association between maternal total B_{12} concentration and gestational age at birth; however, they found an 11 % (95 % CI 3, 18) decreased risk of preterm birth with each standard deviation increase in maternal total B_{12} concentration after adjusting for maternal age, parity and BMI or weight. The inverse association between maternal B_{12} status and preterm birth in the meta-analysis by Rogne *et al.*[4] suggests that lower maternal B_{12} status is a

Table 4. Association between maternal vitamin B_{12} (B_{12}) status and head circumference *z*-score in Canadian mother–newborn dyads* (β-coefficients and 95 % confidence intervals)

	Unadjusted model					Adjusted model†				
Maternal B_{12} biomarker	*n*	β	95 % CI	*P*	R^2	*n*	β	95 % CI	*P*	R^2
First trimester										
HoloTC‡	626	−0·00674	−0·0445, 0·0311	0·73	0·0002	625	−0·00554	−0·0433, 0·0322	0·77	0·044
Total B_{12}	637	−0·00353	−0·0115, 0·00446	0·39	0·0012	636	−0·00278	−0·0112, 0·00562	0·52	0·042
MMA	628	−0·00086	−0·0114, 0·00972	0·87	< 0·0001	627	−0·000265	−0·0110, 0·0105	0·96	0·042
tHcy	584	0·00329	−0·0827, 0·0893	0·94	< 0·0001	583	0·0202	−0·0672, 0·107	0·65	0·043
Second trimester										
HoloTC‡	605	−0·0282	−0·0641, 0·00771	0·12	0·0044	604	−0·0243	−0·0604, 0·0118	0·19	0·050
Total B_{12}	683	−0·00766	−0·0160, 0·000646	0·07	0·0048	682	−0·00715	−0·0157, 0·00143	0·10	0·049
MMA	642	0·00292	−0·00744, 0·0133	0·58	0·0005	641	0·00450	−0·00589, 0·0149	0·40	0·051
tHcy	553	0·0710	−0·00741, 0·149	0·08	0·0057	552	0·0762	−0·00237, 0·155	0·06	0·064

holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.
* Unadjusted R^2 reported for univariable model and adjusted R^2 reported for multivariable models. β-coefficients represent change in head circumference *z*-score with each 10-unit increase in maternal serum holoTC, total B_{12} and MMA concentration and 1-unit increase in tHcy.
† All multivariable models were adjusted for maternal age, maternal ethnicity, parity, GWG, pre-pregnancy BMI, hypertensive disorder of pregnancy, gestational diabetes, neighbourhood income and neighbourhood education.
‡ Discontinuous regression.

Table 5. Association between first trimester maternal vitamin B_{12} (B_{12}) status and gestational age at birth in weeks in Canadian mother–newborn dyads* (β-coefficients and 95 % confidence intervals)

	Unadjusted model					Adjusted model†				
Maternal B_{12} biomarker	*n*	β	95 % CI	*P*	R^2	*n*	β	95 % CI	*P*	R^2
First trimester										
HoloTC‡	636	0·0146	−0·0414, 0·0706	0·61	0·0052	628	−0·00913	−0·0651, 0·0469	0·75	0·0578
Total B_{12}	647	0·00400	−0·00790, 0·0159	0·51	0·0007	639	−0·00653	−0·0190, 0·00594	0·30	0·0575
MMA	637	−0·0114	−0·0272, 0·00435	0·16	0·0032	630	−0·00584	−0·0218, 0·0101	0·47	0·0547
tHcy	592	0·0634	−0·0643, 0·191	0·33	0·0016	585	0·0335	−0·0961, 0·163	0·61	0·0549
Second trimester										
HoloTC‡	615	0·0486	−0·00474, 0·102	0·07	0·0054	607	0·0148	−0·0391, 0·0687	0·59	0·0636
Total B_{12}	693	0·00495	−0·00731, 0·0172	0·43	0·0009	685	−0·00699	−0·0196, 0·00563	0·28	0·0646
MMA	652	0·00166	−0·0137, 0·0170	0·83	0·0001	644	0·00841	−0·00703, 0·0239	0·29	0·0560
tHcy	562	0·00226	−0·115, 0·120	0·97	< 0·0001	555	−0·0202	−0·139, 0·0991	0·74	0·0497

holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.
* Unadjusted R^2 reported for univariable model and adjusted R^2 reported for multivariable models. β-coefficients represent change in gestational age at birth (weeks) with each 10-unit increase in maternal serum holoTC, total B_{12} and MMA concentration and 1-unit increase in tHcy.
† All multivariable models were adjusted for maternal age, maternal ethnicity, parity, GWG, pre-pregnancy BMI, hypertensive disorder of pregnancy, gestational diabetes, neighbourhood income, neighbourhood education and newborn sex.
‡ Discontinuous regression.

risk factor for preterm birth. Preterm birth and other adverse birth outcomes were not explored as outcomes in the current cohort due to their low prevalence.

Maternal biomarker concentrations

The small decrease in maternal total B_{12}, holoTC and tHcy concentrations between the first and second trimester is consistent with previous longitudinal studies of maternal B_{12} status in early pregnancy[38,39]. Circulating maternal MMA concentration in early pregnancy has been described to remain largely unchanged[14,40]. In contrast, the current study observed a decrease in maternal MMA concentration with each gestational week, albeit this change was small. The decrease in maternal B_{12} status may be attributed to normal physiological changes that occur during pregnancy, such as hemodilution, hormonal changes and active maternal–fetal transport of B_{12}[14,41]. Mørkbak *et al.*[42] found that the 50 % decrease in maternal total B_{12} concentration from mid to late pregnancy was primarily due to a decrease in haptocorrin, and not holoTC, concentration. In comparison with the Mørkbak *et al.*[42] study, the serum samples in the current study were collected earlier in pregnancy between 8 and 21 gestational weeks, which may explain the less marked decrease in total B_{12} concentration.

The proportion of women with a total B_{12} concentration < 148 pmol/l (20 and 25 % in the first and second trimester, respectively) was similar to the prevalence of B_{12} insufficiency reported in a systematic review and meta-analysis by Sukumar *et al.*[43] (first trimester 21 %; second trimester 19 %). This suggests that maternal total B_{12} concentration in this cohort is comparable to those of other studies. Although 20–25 % of the pregnant women had a total B_{12} concentration < 148 pmol/l, the lack of association between maternal B_{12} status and birth outcomes suggests the women were likely B_{12} replete. Studies from India[2,3] and the meta-analysis by Rogne *et al.*[4] observed an association between maternal total B_{12} concentration, categorised using tertiles and cut-offs and birth weight. Due to the lack of pregnancy-specific cut-offs for all B_{12} biomarkers, maternal

B_{12} biomarker concentrations were only assessed as continuous variables in the current study.

Maternal nutrient status

The potential influence of other micronutrients, such as Fe, cannot be fully ruled out due to the lack of data on diet and supplement use, as well as nutrient status indicators. Considering the co-enzymatic role of B_{12}, it may not be as influential as other nutrients, such as folate, which serves as a methyl donor in one-carbon metabolism. This is supported by the small effect sizes observed for the association between maternal B_{12} biomarkers and each of the birth outcomes. Other pregnancy cohort studies have reported a high proportion (93–97 %) of prenatal multivitamin supplement use in early pregnancy[44,45]. If this is the case in the current study, the women are less likely to be deficient with respect to their overall micronutrient status.

Study strengths and limitations

Strengths of this study include the large sample size and the assessment of both direct and functional B_{12} biomarkers in early pregnancy. The longitudinal nature of the study and the narrow range in gestational weeks at sample collection also allowed for the analysis of first and second-trimester-specific maternal B_{12} status. As well, additional data were obtained from the BCPDR, which enabled adjustment for confounding in the regression models. Last, there was no consent bias, since consent for access to the bio-banked serum samples was waived. Due to the selection for healthy women, the findings may not be generalisable to the general Canadian population. Furthermore, we were unable to perform analyses for adverse birth outcomes (i.e. low birth weight, small for gestational age and preterm birth) as a result of the limited number of cases in the current study.

Conclusion

In apparently healthy pregnant women, maternal B_{12} biomarker concentrations in early pregnancy were not associated with birth weight *z*-score, head circumference *z*-score and gestational age at birth. The findings of this study contribute to the body of research on the role of maternal B_{12} status in fetal growth and development, specifically in Canadian pregnant women. Studies of the association between maternal B_{12} biomarkers and birth outcomes are needed in women at higher risk of adverse birth outcomes in Canada. Furthermore, research is needed in infant outcomes after birth that have been associated with maternal B_{12} status, such as cognitive function.

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Y. L. and A. T. formulated the research question; Y. L. and A. T. designed and conducted the study; A. T. analysed the data; G. S., A. M. and H. D. V. provided input on study design, execution and data interpretation; A. T. and Y. L. interpreted the findings and wrote the article. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

All inferences, opinions and conclusions drawn in this publication are those of the authors and do not reflect the opinions or policies of Perinatal Services BC.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114521000581

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