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Pregnant women of South Asian ethnicity in Canada have substantially lower vitamin B_{12} status compared with pregnant women of European ethnicity

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Abstract

Maternal vitamin B_{12} (B_{12}) status has been inversely associated with adverse pregnancy outcomes and positively with fetal growth and infant development. South Asians, Canada's largest ethnic minority, are prone to B_{12} deficiency. Yet, data are lacking on B_{12} status in South Asian pregnant women in North America. We sought to determine B_{12} status, using multiple biomarkers, in 1st and 2nd trimester pregnant women of South Asian and, for comparison, European ethnicity living in Vancouver, Canada. In this retrospective cohort study, total B_{12}, holotranscobalamin (holoTC), methylmalonic acid (MMA), and total homocysteine concentrations were quantified in two routinely collected (mean gestational week: 11·5 (range 8·3–13·9) and 16·5 (range 14·9–20·9)), banked serum samples of 748 healthy pregnant South Asian (*n* 371) and European (*n* 377) women. South Asian pregnant women had significantly lower B_{12} status than European pregnant women at both time points, as indicated by lower serum total B_{12} and holoTC concentrations, and higher MMA concentrations (all $P \leq 0{\cdot}001$). The largest difference, which was substantial (Cohen's $d \geq 0{\cdot}5$), was observed in mean serum total B_{12} concentrations (1st trimester: 189 (95 % CI 180, 199) *v.* 246 (95 % CI 236, 257) pmol/l; 2nd trimester: 176 (95 % CI 168, 185) *v.* 226 (95 % CI 216, 236) pmol/l). Further, South Asian ethnicity was a significant negative predictor of B_{12} status during pregnancy. South Asian women living in Vancouver have substantially lower B_{12} status during early pregnancy. Future research identifying predictors and health consequences of this observed difference is needed to allow for targeted interventions.

Key words: Maternal vitamin B_{12} status: Pregnancy: South Asian ethnicity: Holotranscobalamin: Methylmalonic acid

Vitamin B_{12} (B_{12}) is involved in DNA synthesis, methylation of biomolecules, and neuron myelination, which are crucial processes for healthy fetal development and growth[1]. Maternal B_{12} status during pregnancy has been inversely associated with a wide array of adverse health outcomes for mother and offspring, including neural tube defects[2–4], restricted fetal growth[5–7], and offspring predisposition for non-communicable diseases[8–10]. Notably, South Asians have been described to have an increased risk for such adverse pregnancy outcomes, including neural tube defects[4] as well as a fetal anthropometry associated with cardiometabolic diseases[11–13]. Studies from the Indian subcontinent showed low maternal B_{12} status to be related to an increased risk of intra-uterine growth retardation[5,6], low birth weight[7] and offspring insulin resistance[8,9]. South Asians are the largest ethnic minority in Canada[14] and the UK[15], and one of the largest growing ethnic minorities in the USA[16]. As such, it is imperative to investigate B_{12} status in South Asian pregnant women in these countries.

Some populations and ethnic groups, including South Asians, with low intakes of animal-source foods are at increased risk for inadequate B_{12} status[17–19]. Prevalence of B_{12} deficiency in non-pregnant South Asian populations in Canada, the USA and the UK was reported to be higher compared with that in population groups of European or other ethnicities in a limited number of smaller studies[20–23]. Logistic challenges and cultural barriers have been identified limiting health research in South Asians[24]. The prevalence of B_{12} deficiency, defined as serum total B_{12} concentration <148 pmol/l, among reproductive-aged and pregnant women in Canada[25–30] and the UK[31] was reported to range between 5 and 40 %. A recent cross-sectional study in pregnant women (20–35 weeks of gestation) residing in Vancouver, Canada, in which 18 % of the total sample had a total B_{12} concentration <148 pmol/l, revealed a markedly higher prevalence among women whose self-identified ethnicity was South Asian (61·5 % (*n* 16/26)) compared with European (16 % (*n* 24/150))[30]. Taken together, B_{12} status

Abbreviations: B_{12}, vitamin B_{12}; holoTC, holotranscobalamin; MCA, 2-methylcitric acid; MMA, methylmalonic acid; tHcy, homocysteine.

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appears to be low in Canadian pregnant women and South Asians are vulnerable to B_{12} deficiency; yet, larger studies addressing ethnic differences are needed to confirm our preliminary findings on B_{12} status in South Asian pregnant women living in Canada.

Multiple biomarkers are available to assess B_{12} status and no consensus has yet been reached on a valid assessment of B_{12} deficiency in pregnant women. Total B_{12} is the most commonly used direct B_{12} biomarker; however, it has been criticised for its limited sensitivity[32]. Further, total B_{12} concentration decreases throughout gestation[33] leading to a potential misclassification of B_{12} status using non-pregnant cutoffs. Holotranscobalamin (holoTC) is the fraction of circulating B_{12} that is bioavailable to tissues including the placenta[34]. Circulating holoTC concentrations have been suggested to remain largely unchanged during pregnancy[35,36]. As such, holoTC may provide a more meaningful and accurate direct measure of B_{12} status in pregnant women. Current recommendations advise the use of one direct and one functional biomarker for B_{12} status assessment[37]. The functional B_{12} biomarkers are methylmalonic acid (MMA) and total homocysteine (tHcy). MMA is considered the more specific functional biomarker, because tHcy is determined by other B-vitamins in addition to B_{12}. Yet, elevated tHcy concentration has been associated with adverse pregnancy outcomes, independent of B-vitamin status[38]. MMA is confounded by renal insufficiency[39] as is tHcy[40]. The ratio of 2-methylcitric acid (MCA):MMA may be used to assess renal function when creatinine concentrations are not available, because MCA concentrations were shown to be substantially higher than MMA concentrations, that is, MCA > MMA, in patients with renal failure compared with those with normal renal function[41]. Given the limitations of each biomarker, quantification of all four biomarkers allows for the most comprehensive assessment of B_{12} status.

Maternal B_{12} adequacy is critical for maternal health and optimal fetal growth and development. In light of South Asians being the largest ethnic minority in Canada and the higher risk of B_{12} deficiency for this ethnic minority, we aimed to assess B_{12} status, using multiple biomarkers, in pregnant women of South Asian and, for comparison purposes, European ethnicity, residing in Vancouver, Canada.

Methods

Study design and setting

This retrospective cohort study utilised residual serum samples, which had been routinely collected during prenatal genetic screening in British Columbia (BC), Canada (BC Prenatal Genetic Screening Program[42]). The programme is offered free of charge and to all pregnant women residing in BC (Canada)[43]. Participation is entirely voluntary and ranges from 38·5 % among pregnant women aged 20–24 years to 68·8 % among those aged 35–39 years[44]. The screening involves non-fasting blood collections at two time points: approximately 11 (range 9–13) gestational weeks (1st trimester) and 17 (range 15–19) gestational weeks (2nd trimester). Demographic and other health-related information are recorded during the blood collection.

The University of British Columbia Children's and Women's Research Ethics board (Vancouver, Canada) reviewed this study and approved a waiver of individual consent for the secondary use of deidentified clinical samples from the BC Prenatal Genetic Screening Program (institutional approval no.: H15-00820).

Study sample and data collection

Serum samples were included in the study if the women had identified themselves during the registration process for the BC Prenatal Genetic Screening Program as being either of 'South Asian' or 'Caucasian' (i.e. European origin and, for clarity, from here forth referred to as European) ethnicity and were aged 19–45 years old. In addition, only samples collected within the Lower Mainland (Metro Vancouver, BC, Canada) were retrieved for the study. Samples of women who had a multiple gestation, a pregnancy after *in vitro* fertilisation or steroid use, diabetes mellitus (I/II), history of smoking, or a positive screen for a chromosomal abnormality or an open neural tube defect in this or a past pregnancy were excluded from the study. Information on maternal age, self-identified ethnicity and gestational week at blood collection (estimated by fetal ultrasound crown-rump-length) was obtained from medical charts completed during the prenatal screening visit. The serum samples retrieved for this study were collected between November 2014 and May 2016.

Maternal non-fasting blood samples were collected into serum separator tubes and left at room temperature for serum to separate, following standard protocol. Samples were subsequently stored at 4°C, centrifuged within 24 h of sample collection at 1890 ***g*** for 5 min at 4°C, and transferred to −80°C within 4 d. Samples were thawed once allowing for deidentification. Specifically, samples were thawed on ice, separated into aliquots (one aliquot per biomarker assay) labelled with a unique study identification number (ID), and subsequently stored at −80°C until samples were thawed for specific biomarker analyses. The two freeze-thaw cycles in total for this study will not have affected the B_{12} biomarker concentrations: serum MMA concentrations are unaffected by up to five freeze-thaw cycles[45], tHcy is not affected by repeated freezing and thawing[46], total B_{12} is stable for ten repeated freeze-thaw cycles[47], and up to three freeze-thaw cycles are acceptable for holoTC measurements according to the manual of the assay manufacturer (Abbott Laboratories). Further, the use of non-fasting samples is acceptable for B_{12} status assessment as B_{12} biomarker concentrations remain unchanged during the postprandial stage[48,49].

Laboratory analyses

Serum samples were analysed, sequentially, for the B_{12} biomarkers total B_{12}, holoTC, MMA with MCA, and tHcy as well as folate between January 2016 and September 2016. If the sample volume was insufficient for an aliquot, the analyte was omitted in the respective sample. Samples were analysed at random to avoid analytical bias between ethnicities.

Serum total B_{12} and holoTC were quantified by fully automated immunoassays (Access 2 by Beckman Coulter and Architect by Abbott Laboratories, respectively) according to manufacturers' protocols at the pathology laboratories at BC Children's Hospital and St. Paul's Hospital, respectively.

The inter-assay CV for four serum total B_{12} control samples (Bio-Rad, mean concentration of 93·6, 245, 335, 407 pmol/l) ranged from 2·4 to 7·1 % (analysed weekly over 4 months; *n* 18). Manufacturer (Abbott Laboratories) control samples for holoTC were run once per batch for the three batches of study samples, yielding mean concentrations of 46 (SD 1·5) pmol/l (CV: 3·3 %) and 16 (SD 2·0) pmol/l (CV: 12 %) for the high and low control sample, respectively. The upper limits of the analytical measurement range for the total B_{12} and holoTC assays were 1107 and 128 pmol/l, respectively. Due to the limited volume of residual serum samples, repeated analyses of samples with total B_{12} and holoTC concentrations above the upper limit of the measurement range were not possible.

MMA in serum was determined by stable isotope dilution-liquid chromatography-tandem MS (LC-MS/MS) as previously described[50]. Simultaneously with MMA, MCA was quantified using MCA (Sigma-Aldrich) as standard and d_3-MMA (Cambridge Isotopes Ltd) as internal standard. Results are reported as sum of all stereoisomers. MCA > MMA was used as an indicator of renal insufficiency[41]. Although MCA > MMA has not yet been confirmed as an indicator of renal insufficiency in pregnancy, one woman was excluded from the analyses on this basis. The inter-assay CV for an in-house control sample was 7 % for MMA and 14 % for MCA (analysed over 19 d); the intra-assay CV was <5 % for all analyses.

Serum tHcy was quantified using stable isotope dilution LC-MS/MS. In brief, after reduction with dithiothreitol and protein precipitation, samples were injected into an LC system (Agilent 1260; Agilent Technologies). Compounds were separated by a normal phase column (Fortis H_2O, 2·1 × 150 mm, 5 µm; Fortis Technologies). The mobile phase consisted of (A) 0·2 % heptafluorobutyric acid in water and (B) 0·2 % heptafluorobutyric acid in acetonitrile using a gradient run (A:B 95:5 (v/v) to 20:80 (v/v)). The affluent was directed into an MS/MS system (API4000; SCIEX Pte). Quantification was performed with seven-point calibration curves (1·14–114 µmol/l) made using L-homocysteine (Sigma-Aldrich) as calibrator and d_4-homocysteine (Cambridge Isotopes Ltd) as internal standard. The inter-assay CV for an in-house control sample was 9·4 % (analysed over 17 d). Two external quality control samples (ClinChek 23082 and IRIS Technologies International) were quantified with every analysis and were within their acceptable range of 9·0 (SD 1·8) and 25·9 (SD 5·1) µmol/l, respectively.

Serum folate was analysed using the microbiological assay according to the method developed by O'Broin & Kelleher[51] and Molloy & Scott[52]. The assay was performed using the chloramphenicol-resistant *Lactobacillus rhamnosus* (ATCC 27773) and 5-methyltetrahydrofolate ((6S)-5-methyl-5,6,7,8-tetrahydropteroyl-L-glutamic acid, sodium salt; Merck Eprova) as calibrator. Control samples were included in each of the twenty-two batch runs. The analyses yielded folate contents of 28·3 nmol/l (13 ng/ml) (inter-assay CV: 8·8 %) for the NIBSC 95/528 control sample (13 ng/ml) and of 42·8 nmol/l (inter-assay CV 10·1 %) for an in-house serum control sample.

Biomarker cutoffs

To date, there are no established cutoffs to define B_{12} status in pregnancy for any of the four available B_{12} biomarkers. Pregnant women were, thus, classified using non-pregnant adult cutoffs to allow for comparison with previous research. It is commonly accepted that serum total B_{12} concentrations <148 pmol/l[53] or serum holoTC concentrations <35 pmol/l[54–56] indicate B_{12} deficiency in non-pregnant adults. Serum total B_{12} concentrations <221 pmol/l[2] and serum holoTC concentrations <55 pmol/l[3] have been associated with an increased risk for neural tube defect-affected pregnancies and will be referred to as inadequate B_{12} status. Elevated and mildly elevated MMA concentrations were defined as serum MMA concentrations >370[57] and >210 nmol/l[58], respectively. Overt B_{12} deficiency was defined as combined serum total B_{12} concentrations <148 pmol/l and serum MMA concentrations >370 nmol/l.

Serum tHcy concentrations >13 µmol/l have been used to define elevated tHcy concentrations in pregnant women and populations with folic acid fortification[26,58–60]. In Canada, mandatory fortification of flour with folic acid has been in place since 1998[61]. In addition, tHcy concentrations <9 µmol/l have been described as 'desired'[58]. Folate deficiency and inadequate folate status were defined as having serum folate concentration <6·8 and <13·4 nmol/l, respectively, as proposed by the World Health Organization[62].

Statistical analyses

The study aimed to retrieve sets of two serum samples (1st trimester and 2nd trimester) collected from 600 women of South Asian (*n* 300) and European (*n* 300) ethnicity during pregnancy. This sample size allowed for the detection of a difference in serum total B_{12} concentrations between pregnant women of South Asian and European ethnicity of 40 pmol/l with a power of 0·80 at a confidence level (α) of 0·05. Sampling was continued until sufficient complete sets (1st and 2nd trimester) had been obtained. Normality of data was tested visually (Kernel's density distribution) and by Shapiro–Wilk test. Non-normally distributed data are presented as geometric means and 95 % CI. Given the censored data (at 128 pmol/l), mean (95 % CI) holoTC concentrations were estimated by Tobit regression after ln-transformation. Differences in biomarker concentrations, maternal age, and gestational week at sample collection between women of South Asian and European ethnicity were determined by Wilcoxon's rank-sum test; differences in prevalence of B_{12} deficiency and inadequate B_{12} status were determined by Pearson's χ^2 test or likelihood-ratio χ^2 test, when cell size <5 (i.e. for tHcy and folate). Cohen's *d* was used to estimate the effect size of the difference in biomarker concentrations between women of South Asian and European ethnicity. The association between maternal B_{12} status and ethnicity was tested by three mixed-effects generalised linear models. In these models, either maternal serum total B_{12} (ln-transformed), serum holoTC (ln-transformed and 133 (20 %) and 90 (14 %) participants with serum holoTC ≥128 pmol/l during 1st and 2nd trimester, respectively, excluded), or serum MMA (ln-transformed) concentration served as the dependent variable and maternal ethnicity (South Asian), maternal age (years), and gestational week at sample collection (weeks) as independent variables. The models accounted for repeated

measures by including the study ID as a random effect to account for autocorrelation among samples from the same person. All statistical analyses were performed in Stata 14.2 (StataCorp LP) for Windows 10 (Microsoft Corp.), with the level of significance set at $P < 0{\cdot}05$.

Results

To meet the sampling goal of the study, samples and data of 748 women, aged 19–44 years, were retrieved for this study. The mean gestational week at the two study time points was 11·5 (range 8·3–13·9) weeks for the 1st trimester and 16·5 (range 14·9–20·9) weeks for the 2nd trimester (Table 1). There were no significant differences in gestational weeks at sample collection between pregnant women of South Asian and European ethnicity. Further, there were no significant differences in maternal characteristics between pregnant women of South Asian and European ethnicity after exclusion of women with missing data for total B_{12}, holoTC, MMA, tHcy or folate (data not shown).

Vitamin B_{12} biomarker concentrations were quantified in at least 300 samples from women of each South Asian and European ethnicity (in total *n* 600) per time point, that is, the calculated target sample size, except for tHcy concentrations (Fig. 1). Missing data, for example tHcy concentrations, resulted from insufficient sample volume to quantify the respective analyte. The results of B_{12} biomarker concentrations are summarised in Table 2. Pregnant women of South Asian compared with European ethnicity had significantly lower serum total B_{12} and serum holoTC concentrations and significantly higher serum MMA concentrations at both time points. The effect size of the difference was largest for 1st and 2nd trimester serum total B_{12} (Cohen's *d*: 0·52 and 0·45, respectively) and smallest for holoTC concentrations (Cohen's *d*: 0·28 and 0·20, respectively). There was no difference in serum tHcy and folate concentrations between pregnant women of South Asian and European ethnicity.

A significantly higher prevalence of women of South Asian compared with European ethnicity were classified as B_{12} deficient and having inadequate B_{12} status, respectively, as indicated by serum total B_{12} (<148 and <221 pmol/l) and serum holoTC (<35 and <55 pmol/l) at either time point in the study (Table 3). The prevalence of women classified as B_{12} deficient in this study differed substantially depending on biomarker and time point of sample collection. It ranged from 5·3 % (serum holoTC <35 pmol/l, both time points) to 25·4 % (serum total B_{12} < 148 pmol/l, 2nd trimester) (Table 3). More than half of all women were classified as having inadequate B_{12} status, indicated by serum total B_{12} < 221 pmol/l at both visits. However, only ≤25 % were classified as having inadequate B_{12} status at both visits when using serum holoTC <55 pmol/l as indicator. The prevalence of women with elevated serum MMA concentrations (>370 nmol/l) and mildly elevated serum MMA concentrations (>210 nmol/l) was approximately 6·5 and 12 %, respectively, at both time points. This indication of functional B_{12} deficiency was not reflected in elevated serum tHcy concentrations (>13 or 9 μmol/l, approximately 0 %). During the 1st and 2nd trimester, 4·8 % (32/669) and 4·9 % (34/697) of all women, respectively, were classified as having overt B_{12} deficiency (total B_{12} < 148 pmol/l and MMA >370 pmol/l) with a significantly higher prevalence in women of South Asian ethnicity (8·9 and 8·3 %, respectively) compared with those of

Table 1. Maternal characteristics*
(Mean values and ranges)

	All		European		South Asian		
Characteristics	Mean	Range	Mean	Range	Mean	Range	*P*
n	748		377		371		
Age (years)	31·0	19–44	30·9	19–40	31·1	20–44	0·8
Gestational week at 1st trimester visit (weeks)	11·5	8·3–13·9	11·5	8·3–13·9	11·4	8·3–13·9	0·3
Gestational week at 2nd trimester visit (weeks)	16·5	14·9–20·9	16·5	14·9–20·9	16·6	15·0–20·9	0·3

* Maternal ethnicity, that is, European or South Asian, was self-reported. *P* value is reported for the difference between pregnant women of European and South Asian ethnicity as determined by Wilcoxon's rank-sum test.

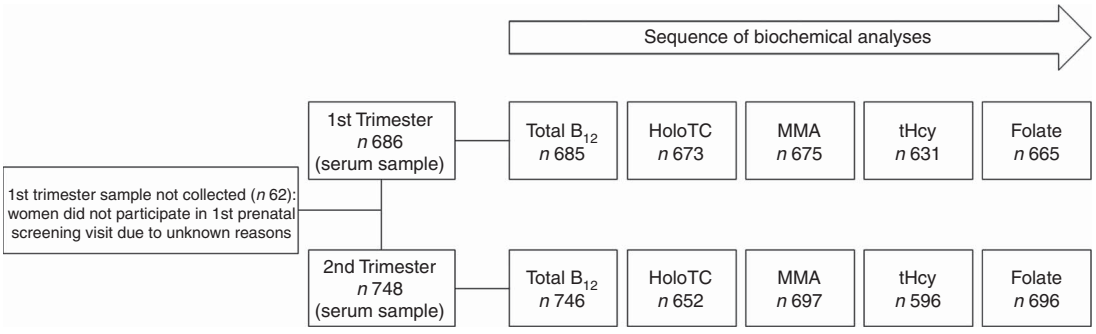

Fig. 1. Flow diagram of serum sample collection and biomarker analyses. The sequence of biochemical analyses reflects the prioritisation of biomarker analyses in dependence of available serum volume and successful biomarker quantitation. B_{12}, vitamin B_{12}; HoloTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

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Table 2. Serum total vitamin B_{12} (B_{12}), holotranscobalamin (holoTC), methylmalonic acid (MMA), total homocysteine (tHcy) and folate concentrations of women of European and South Asian ethnicity during 1st and 2nd trimester of pregnancy
(Geometric means and 95 % confidence intervals)

	1st Trimester						2nd Trimester					
	All		European		South Asian		All		European		South Asian	
Biomarkers	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI
Total B_{12} (pmol/l)	216	209, 224	247***	237, 258	188***	179, 197	200	193, 206	226***	216, 236	176***	168, 185
n	685		351		334		746		377		369	
HoloTC (pmol/l)†	83·5	80·2, 86·9	89·9**	85·7, 94·2	77·0**	72·4, 81·9	76·8	73·8, 79·8	81·2*	77·4, 85·1	72·5*	68·2, 77·0
n	673		340		333		652		325		327	
MMA (nmol/l)	131	127, 135	120***	115, 124	143***	137, 151	125	121, 130	114***	109, 119	138***	130, 146
n	675		343		332		697		348		349	
tHcy (μmol/l)	4·98	4·90, 5·07	4·99	4·88, 5·10	4·97	4·85, 5·10	4·42	4·33, 4·50	4·42	4·30, 4·54	4·41	4·29, 4·53
n	636		340		296		596		298		298	
Folate (nmol/l)	65·6	63·7, 67·6	66·1	63·5, 68·8	65·1	62·4, 68·0	67·7	65·7, 69·6	67·3	64·6, 70·1	68·4	65·7, 71·2
n	665		334		331		696		348		348	

Significant differences within 1st or 2nd trimester between pregnant women of European and South Asian ethnicity: * $P \leq 0{\cdot}01$, ** $P \leq 0{\cdot}001$, *** $P \leq 0{\cdot}0001$, as tested by Wilcoxon's rank-sum test; concentrations determined in non-fasting serum samples.
† Mean (95 % CI) holoTC concentrations were estimated by Tobit regression.

Table 3. Prevalence of pregnant women of European and South Asian ethnicity classified as vitamin B_{12} (B_{12}) inadequate or B_{12} deficient according to cutoffs indicated in the first column
(Prevalences (%) and absolute numbers (*n*))

	1st Trimester						2nd Trimester					
	All		European		South Asian		All		European		South Asian	
Biomarkers	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
Total B_{12}												
<221 pmol/l†	50·7	347	38·7***	136	63·2***	211	58·0	433	48·3***	182	68·0***	251
<148 pmol/l‡	20·1	138	10·6***	37	30·2***	101	25·4	189	15·1***	57	35·8***	132
HoloTC												
<55 pmol/l†	19·5	131	12·0***	41	27·1***	90	25·0	163	19·6**	64	30·3**	99
<35 pmol/l‡	5·3	36	1·5***	5	9·3***	31	5·3	35	2·5**	8	8·2**	27
MMA												
>210 nmol/l†	12·6	85	5·3***	18	20·3***	67	11·9	83	5·5***	19	18·3***	64
>370 nmol/l‡	6·1	41	2·1***	7	10·2***	34	7·0	49	3·2***	11	10·9***	38
tHcy												
>9 μmol/l†	0·32	2	0·30	1	0·34	1	0·19	1	0		0·37	1
>13 μmol/l‡	0		0		0		0·19	1	0		0·37	1

holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.
Significant differences within the 1st or 2nd trimester between pregnant women of European and South Asian ethnicity: ** $P \leq 0{\cdot}001$, *** $P \leq 0{\cdot}0001$ as tested by Pearson's χ^2 test (total B_{12}, holoTC, MMA) or likelihood-ratio χ^2 test (tHcy); concentrations determined in non-fasting serum samples.
† B_{12} inadequate.
‡ B_{12} deficient.

European ethnicity (0·9 and 1·4 %, respectively) at both time points ($P < 0{\cdot}001$). Folate status was adequate in all but one woman of South Asian ethnicity who had inadequate folate status (<13·4 nmol/l) in the 1st trimester.

To examine the association between maternal ethnicity and B_{12} status, we determined the predictors of maternal serum total B_{12}, holoTC, and MMA concentration (ln-transformed). Being of South Asian ethnicity predicted a 22 % ($P < 0{\cdot}0001$) and 12 % ($P < 0{\cdot}0001$) lower mean serum total B_{12} and holoTC concentration, respectively, and a 21 % ($P < 0{\cdot}0001$) higher mean serum MMA concentration (Table 4). Gestational week at sample collection was a significant negative predictor of maternal B_{12} biomarker concentrations ($P < 0{\cdot}005$), whereas maternal age was not associated with maternal B_{12} status.

Discussion

In this retrospective cohort study of 748 pregnant women residing in Canada, South Asian compared with European women had significantly lower B_{12} status, as indicated by lower serum total B_{12} and holoTC concentrations, and higher serum MMA concentrations during early pregnancy. For example, the difference in mean serum total B_{12} concentration between South Asian and European women in the 1st and 2nd trimester was approximately 23 % (57 and 50 pmol/l, respectively). In addition, South Asian ethnicity was a significant negative predictor of B_{12} status, as assessed by total B_{12}, holoTC, and MMA concentrations. During the 1st and 2nd trimesters, the prevalence of pregnant women who were classified as overtly B_{12}

Table 4. Estimates for South Asian ethnicity to predict maternal serum total vitamin B_{12} (B_{12}), holotranscobalamin (holoTC), and methylmalonic acid (MMA) concentrations during 1st and 2nd trimester, determined using mixed effects models adjusted for maternal age and gestational week at sample collection and performed on ln-transformed data while accounting for repeated measures*
(Estimates (*B*) and 95 % confidence intervals)

Characteristics	*n*	*B*	95 % CI	*P*
Total B_{12} (pmol/l)	668	−0·24	−0·30, −0·18	<0·0001
HoloTC (pmol/l)	560	−0·13	−0·20, −0·07	<0·0001
MMA (nmol/l)	664	0·19	0·13, 0·25	<0·0001

* Exponentiated *B* describe the factor change of biomarker concentrations when being of South Asian compared with European ethnicity, for example, serum total B_{12} concentration is multiplied by a mean of $e^{-0.24} = 0.78$ when a pregnant woman is of South Asian compared with European ethnicity and as such being of South Asian ethnicity compared with European ethnicity predicts a 22 % lower mean serum total B_{12} concentration; *P* values were determined by likelihood ratio test after adding ethnicity into the models.

deficient (total B_{12} < 148 pmol/l and MMA >370 pmol/l) was ten and six times higher, respectively, among South Asian women (who had prevalences of approximately 9 and 8 %, respectively) compared with that of European women (approximately 1 % at both time points). Thus, findings from this study in Vancouver suggest that women of South Asian compared with those of European ethnicity have markedly lower B_{12} status during the 1st and 2nd trimesters of pregnancy.

The 30 and 36 % prevalence of South Asian pregnant women with total B_{12} concentration <148 pmol/l in 1st and 2nd trimesters, respectively, in the present study is lower than findings in pregnant women living on the Indian subcontinent. Studies from rural and urban India reported a substantially higher prevalence of approximately 50–70 % of pregnant women having total B_{12} concentrations <150 pmol/l during early pregnancy[6,9,18]; one study from Nepal found a 28 % prevalence during the 1st trimester[8,19]. In addition, studies from India reported 70–95 % of pregnant women to have MMA concentrations >260 nmol/l[6,9], which is also substantially higher than the approximately 12 % prevalence of MMA concentrations >260 nmol/l among South Asian pregnant women during either trimester observed in the present study (data not shown). The prevalence of South Asian women with mildly elevated MMA concentrations (>210 nmol/l) in the present study was approximately 20 %. On the other hand, the 10 and 15 % prevalence of European women with total B_{12} concentrations <148 pmol/l during 1st and 2nd trimester, respectively, was comparable with previous reports of B_{12} status during early pregnancy in Canada with 5–17 % of predominantly European pregnant women being classified as B_{12} deficient (total B_{12} < 148 pmol/l)[26,63]. A recent cross-sectional study in Vancouver found 16 % of European pregnant women (*n* 150) to have serum total B_{12} concentration <148 pmol/l at 20–35 weeks of gestation[30]. In the present study, 1·5 and 2·5 % of European women had holoTC concentrations <35 pmol/l during 1st and 2nd trimester, respectively. Similarly, the prevalence of holoTC concentrations <35 pmol/l in the Alberta Pregnancy Outcomes and Nutrition study was negligible[55]. Other studies in Canadian pregnant women did not use holoTC as an indicator of B_{12} status[26,30]. As such, whereas B_{12} biomarker concentrations did not reflect as low a B_{12} status in South Asian pregnant women in the present study as was reported for women residing on the Indian subcontinent, the prevalence of South Asians classified as B_{12} deficient was substantial and at least twice as high (depending on biomarker and cutoff) compared with pregnant women of European ethnicity in the present and other Canadian cohorts.

Overall, the prevalence of pregnant women classified as B_{12} deficient depends on the biomarker and cutoffs used; in the present study, using total B_{12} < 148 pmol/l resulted in a prevalence of deficiency at least three-times higher than any other indicator. This tendency was also reflected in the prevalence of pregnant women classified as B_{12} inadequate. We acknowledge that physiological changes during pregnancy may impact biomarker concentrations independent of B_{12} status and pregnancy-specific cutoffs are currently lacking. Yet, infants of mothers, living in rural Nepal who had serum total B_{12} concentrations <148 pmol/l at approximately 11 weeks of gestation (*n* 147/524), have previously been reported to have a significantly higher (approximately 27 %) estimated insulin resistance using the homeostasis model assessment (HOMA-IR) at age 6–8 years[8]. Further, Indian mothers with total B_{12} concentration < 148 pmol/l during 1st and 2nd trimester (median 115 (interquartile range (IQR) 104, 125) and 112 (IQR 99, 122) pmol/l, respectively) had higher odds (OR 5·98; 95 % CI 1·72, 20·74 and 9·28; 95 % CI 2·90, 29·68, respectively) of intrauterine growth retardation than mothers with higher total B_{12} concentrations (median 224 (IQR 206, 268) and 210 (IQR 177, 217) pmol/l, respectively)[5]. In a recent meta-analysis, maternal total B_{12} concentration <148 pmol/l was related to an increased risk of low birth weight (risk ratio 1·15; 95 % CI 1·01, 1·31) and preterm birth (risk ratio 1·21; 95 % CI 0·99, 1·49)[7]. Inadequate maternal B_{12} status (total B_{12} < 221 pmol/l or holoTC <55 pmol/l) at <28 d of gestation has been associated with an increased risk for neural tube defects[2,3]. Thus, although further research is needed to develop and evaluate pregnancy-specific cutoffs for B_{12} deficiency, low B_{12} status and especially total B_{12} concentrations <148 pmol/l have been associated with adverse pregnancy outcomes in South Asians and other ethnicities.

One of the pregnancy-related changes highlighting the need for pregnancy-specific cutoffs is the decrease in circulating total B_{12} concentration during healthy pregnancy, which has previously been described[33,59,64,65]. It may be due to hemodilution or other physiological changes. In the present study, gestational week at sample collection was a negative predictor of all B_{12} biomarker concentrations, that is total B_{12}, holoTC, MMA and tHcy. As such, these findings support the hypothesis that circulating B_{12} biomarker concentrations, especially total B_{12} concentration, decrease throughout pregnancy, emphasising the need for pregnancy-specific cutoffs for B_{12} biomarkers.

Low intakes of animal-source foods, low socioeconomic status, and a lower prevalence of supplement use have been suggested as predictors of B_{12} deficiency in South Asians[66–68]. No dietary or demographic data were available in the present retrospective study to determine predictors of the reported differences in B_{12} status. In addition, some genetic factors may

influence B_{12} biomarker concentrations, independent of B_{12} intake[69]. Variants in the *FUT2* gene have been identified as predictors of low serum total B_{12} concentrations in South Asians[70] and have been suggested to explain part of the association between low serum total B_{12} concentration and obesity[71]. An increased prevalence of obesity has been described in South Asian populations, including South Asian pregnant women in the UK[68,72]. Future research is warranted to identify dietary, socioeconomic and genetic predictors of low B_{12} status in pregnant women, especially in those of South Asian ethnicity, living in Canada to allow for targeted interventions.

None of the women in the present study had folate deficiency and <1 % had tHcy concentration >9 μmol/l at both time points. Folate status in Canadian pregnant women[55,73] and reproductive-aged women[61] was reported to be substantially elevated. The high folate status in Canadian pregnant women is explained by the prevalent use of prenatal supplements (>90 %[55,73]) and the high dosage of folic acid (most commonly 1 mg/d) in prenatal supplements available on the Canadian market. Elevated tHcy was not found in Canadian pregnant women[26] and was observed in 5 % of the general Canadian population[25]. The high folate status in Canadian pregnant women likely explains the low tHcy concentration as described previously[26] and found in the present study. Further, pregnant women have lower tHcy concentration compared with non-pregnant women[74], likely due to an increase in remethylation[75,76], emphasising the need for pregnancy-specific cutoffs of all B_{12} biomarkers. This study contributes to the body of evidence that folate status in pregnant women in Canada is high, whereas B_{12} status may be low[26,61,77,78].

Our study has several strengths, including the longitudinal approach, the lack of consent bias, and the large sample size, which allowed for sufficient power to detect a meaningful difference in B_{12} status between South Asian and European pregnant women. In addition, we performed a comprehensive assessment of B_{12} status using multiple direct and functional biomarkers over two time points during pregnancy. Yet, we acknowledge some limitations. As this study involved retrospective access to bio-banked serum samples, the sampled population may be biased. Records estimate that, depending on the age group, between 35·8 and 68·8 % of pregnant women living in BC, Canada, chose to participate in the genetic screening programme in 2015[44]. Yet, the women participating in the programme might be older, have experienced previous pregnancy complications, or may be more anxious, educated or health conscious than the general population of pregnant women[43,79]. We conclude that although women were not actively recruited, the study may have a sampling bias and thus, may not be representative of the general population.

In summary, women of South Asian compared with European ethnicity living in Canada have a substantially lower B_{12} status in early pregnancy. Given that B_{12} is important for a healthy pregnancy and that South Asians are Canada's largest ethnic minority, these results warrant future research on identifying predictors and potential health consequences of perinatal B_{12} inadequacy, especially in women of South Asian ethnicity.

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T. H. S. and Y. L. designed the study and led the sample and data analysis; G. S. and H. D. V. provided input on study execution; G. S., A. M., S. I. B. and H. D. V. contributed to data interpretation. B. J. led the serum total B_{12} analyses. T. H. S. wrote the initial draft of the manuscript; and all authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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