Vitamin B₁₂ and Placental Expression of Transcobalamin in Pregnant Adolescents

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ABSTRACT

Background: Transcobalamin is a key placental protein involved in transport of vitamin B₁₂ to the fetus. However, few data currently exist on the ability of the placenta to modify vitamin B₁₂ transporter expression, particularly in high-risk populations such as pregnant adolescents.

Objective: This study was conducted to determine the impact of maternal and neonatal serum vitamin B₁₂ concentrations on placental transcobalamin (TC) expression in a cohort of healthy pregnant adolescents in the United States.

Design: Serum vitamin B₁₂ concentrations were measured in maternal blood samples at mid-gestation (26.4 ± 2.8 weeks) and delivery (39.8 ± 1.4 weeks) and infant cord blood samples at birth. Placentas were collected at delivery and TC mRNA expression ($\Delta\Delta Ct$) and TC protein abundance (TC: α -actin) were evaluated. Linear and binomial regression models were used to examine the associations of maternal (mid-gestation, delivery) and neonatal serum vitamin B₁₂ concentrations with placental TC mRNA expression and protein abundance (n=63).

Results: Maternal serum vitamin B₁₂ concentrations at mid-gestation or delivery were not significantly associated with placental TC mRNA expression or TC protein abundance ($p > 0.05$). Higher placental TC protein abundance was associated with higher neonatal serum vitamin B₁₂ concentrations ($p = 0.003$).

Conclusions: Higher placental TC protein abundance was associated with higher neonatal serum vitamin B₁₂ concentrations.

1 INTRODUCTION¹

2
3 Vitamin B₁₂ deficiency (<148 pmol/L) is a major public health problem worldwide [1, 2].
4 Maternal vitamin B₁₂ deficiency has been associated with increased risk of common pregnancy
5 complications [3] and impaired growth and brain development in offspring [4, 5].

6 Maternal and fetal vitamin B₁₂ concentrations are thought to be closely associated
7 throughout pregnancy [6, 7]. Previous cross-sectional studies in Norway, Turkey, and Brazil
8 have noted correlations between maternal and infant vitamin B₁₂ status at delivery [8-10]. In a
9 study in Germany, maternal serum holotranscobalamin (holoTC) and total vitamin B₁₂
10 concentrations at delivery were significantly correlated with cord blood holoTC concentrations
11 (p<0.05) [6]. However, there is a lack of prospective studies investigating the determinants of
12 vitamin B₁₂ status early in life, and the mechanisms involved are unknown.

13 Placental transporter proteins modulate nutrient transfer to the fetus during gestation [11].
14 Transcobalamin (TC) and haptocorrin are the primary transporters of vitamin B₁₂;
15 transcobalamin binds to over 70% of vitamin B₁₂ transported across the placenta, compared to 10
16 to 30% in maternal circulation [12-14]. Although it is known that the placenta produces TC,
17 which may be released into the maternal and fetal circuit, the mechanisms and pathways of
18 vitamin B₁₂ transport from the maternal to the fetal circuit are largely unknown [15, 16].

19 Few data exist on the ability of the placenta to modify vitamin B₁₂ transport in response
20 to maternal vitamin B₁₂ status. Pregnant adolescents, which represent over 5% of the U.S.
21 obstetric population [17], are at an even greater risk for micronutrient deficiencies and pregnancy

¹**Abbreviations Used:** 95% CI, 95% confidence interval; BMI, body mass index; HoloTC, holotranscobalamin; MMA, methylmalonic acid; NTD, neural tube closure defect; qPCR, real-time quantitative reverse transcriptase-polymerase chain reaction; RR, risk ratio; SAM, S-adenosyl methionine; SAH, S-adenosyl-L-homocysteine; SD, standard deviation; SE, standard error; TC, transcobalamin; tHcy, total homocysteine

22 complications [18, 19]. No data currently exist on the role of placental vitamin B₁₂ transporters
23 and vitamin B₁₂ status in adolescent populations. We conducted this study to examine the
24 associations of maternal and neonatal serum vitamin B₁₂ concentrations and placental
25 transcobalamin expression in a cohort of healthy pregnant adolescents. We hypothesized that 1)
26 lower maternal serum vitamin B₁₂ concentrations at mid-gestation and delivery would be
27 associated with higher placenta transcobalamin expression, to maximize vitamin B₁₂ transport to
28 the fetus; and 2) higher placental transcobalamin expression would be associated with higher
29 vitamin B₁₂ concentrations in cord blood.

30

SUBJECTS AND METHODS

Study Population. Participants in this study were enrolled in two USDA-funded cohort studies of maternal and fetal bone health and iron status in pregnant adolescents (13-18y). Pregnant adolescents were recruited from the Rochester Adolescent Maternity Program in Rochester, NY. Adolescents were eligible to participate if they were between 12 and 30 weeks of gestation at enrollment, healthy, carrying a single fetus; and excluded if they had any known medical complications. Research protocols and study procedures were approved by the Institutional Review Boards at Cornell University and the University of Rochester, and informed consent was obtained from all participants.

Follow-up Procedures. Structured interviews were conducted at baseline to collect information on demographic characteristics and obstetric history. Adolescents in the bone health study attended up to three visits across pregnancy, timed to coincide with early, mid-, and late gestation. Detailed clinical, dietary (24-hour recall), anthropometric, and biochemical data were collected at each visit and prenatal supplementation use was recorded. Maternal blood samples were collected at mid-gestation and delivery, and placental samples and cord blood were collected at delivery. Adolescents enrolled in the anemia study were assessed only at delivery, at which time maternal and cord blood samples were obtained. All adolescents were prescribed prenatal supplements containing 27 mg iron and 400 µg folic acid.

Laboratory Investigations. Maternal blood samples were collected at mid-gestation in the bone study and at delivery in all adolescents. Non-fasting venous maternal blood samples and cord blood samples were allowed to clot at room temperature, separated by centrifugation, processed, and stored $\leq -80^{\circ}\text{C}$ until analysis. Maternal serum and cord blood vitamin B₁₂

concentrations were evaluated by electrochemiluminescence using the Immulite 2000 immunoassay system (Seimens Medical Solutions Diagnostics, Los Angeles, CA). Three levels of controls were used, to ensure instrument precision (Bio-Rad).

Placentas were collected and processed using a standard protocol immediately following delivery. The placenta and umbilical cord were weighed and placental dimensions were recorded. The fetal membrane was removed and four to five samples from the middle section of different placental cotyledons were sectioned, combined, and distributed into aliquots to reduce interplacental variability. Samples were stored in RNALater for qPCR analysis (Ambion, Austin, TX) or flash frozen and stored $\leq -80^{\circ}\text{C}$ for Western blot analysis.

Transcobalamin mRNA expression in placental tissue was evaluated by qPCR. Total RNA was extracted from placental tissue using the RNEasy Microarray Tissue Mini Kit (QIAGEN, Valencia, CA) and stored $\leq -80^{\circ}\text{C}$ until RNA quantification; quality assessment was measured using the Experion Automated Electrophoresis System (Bio-Rad, Hercules, CA). cDNA was generated by reverse transcription using the Transcriptor First Stand cDNA Synthesis Kit (Roche Diagnostics, Indianapolis, IN). TC primers were designed from the Roche Universal Probe Library catalog (#04689151001; forward: 5'tgggaccaaggaagacctc 3'; reverse: 5'accgtcatcctcgctgaa 3'). β -actin was used as an endogenous control; primers were developed (NCBI:NM_001101.3) and purchased from Integrated DNA Technologies (Coralville, IA). qPCR reactions were prepared in triplicate with 10 μL of reaction mixture in each well (2 μL cDNA, 5 μL SYBR Green Master Mix, 0.35 μL of 20 $\mu\text{mol/L}$ forward primer, 0.35 $\mu\text{mol/L}$ reverse primer, 2.3 μL DI water, and reactions were analyzed using the LightCycler 480 Instrument (Roche Diagnostics); DI-water was used as a negative control. Transcobalamin mRNA expression was normalized to β -actin expression ($\Delta\text{Ct} = \# \text{ cycles for TC} - \# \text{ cycles for } \beta \text{-actin}$)

and compared to control placental samples using the $2^{-\Delta\Delta C_t}$ method ($2^{-\Delta\Delta C_t} = 2^{(\Delta C_t \text{ sample} - \Delta C_t \text{ control placenta})}$) [20, 21], accounting for inter-assay variation.

Western blot analysis was conducted to determine placental TC protein abundance. Placental tissue was thawed on ice and rinsed with 0.9% saline solution containing protease inhibitors (Sigma-Aldrich, St. Louis, MO) to remove surface hemoglobin. Placental tissue in hypertonic lysis buffer containing protease inhibitors was homogenized on ice using a polytron homogenizer. Placental homogenate was then centrifuged at 13,000g for 20 minutes at 4°C. Following centrifugation, the pellet was discarded and the supernatant was used for protein quantification or stored $\leq -80^\circ\text{C}$. Protein concentrations were quantified using the Bio-Rad assay (Bio-Rad, Hercules, CA, USA). Lysates were diluted in SDS sample buffer and stored $\leq -80^\circ\text{C}$ until analysis.

Placental samples were separated on a 10% SDS-PAGE gel and transferred to a polyvinylidene difluoride 156fluorescence membrane (Millipore, Billerica, MA). The membrane was blocked in Odyssey blocking buffer (Li-Cor, Lincoln, NB) for one hour at room temperature and probed overnight at 4°C with mouse-anti-TC antibody (R&D Systems, Minneapolis, MN) (1:1000 dilution) and rabbit anti- α -actin antibody (Abcam, Cambridge, MA) (1:10,000 dilution in a 1:1 dilution of Odyssey blocking buffer to 1xPBS containing 0.1% Tween). Fluorescent secondary antibodies were used to detect protein targets (Li-Cor, Lincoln, NE): IR800 conjugated goat anti-mouse IgG (1:5000 dilution) and an IR680-conjugated goat anti-rabbit IgG (1:5000 dilution). The TC and α -actin bands were quantified using the Odyssey infrared imaging system (Li-Cor) and the ratio of TC to α -actin protein abundance was calculated. Control placental tissue was run on each gel, and the ratio of TC to α -actin protein abundance was used to determine a signal adjustment factor between gels. Human transient overexpression lysate of

TC and an empty vector transfected control HEK293 cell lysate (OriGene, Rockville, MD) were used as positive and negative controls, respectively (**Figure 1**).

Statistical Analyses. Linear regression and Spearman correlations were used to examine the associations of maternal and neonatal serum vitamin B₁₂ concentrations with placental TC mRNA expression ($\Delta\Delta C_t$) and protein abundance (TC: α -actin). Linear regression was used to evaluate predictors of placental TC mRNA expression and protein abundance. We examined if associations between maternal and neonatal serum vitamin B₁₂ concentrations varied by TC mRNA expression or protein abundance.

Conventional cut-offs were used to categorize variables where available; otherwise, medians were used to classify variables. Vitamin B₁₂ deficiency and insufficiency were defined as vitamin B₁₂ <148 pmol/L and <221 pmol/L, respectively [22]. Anemia was race-adjusted and defined as hemoglobin <11 g/dL, during the first and third trimesters, <10.5 g/dL during the second trimester, and <11 g/dL at delivery [23]. Maternal body mass index was defined as the ratio of weight (kg) to height (m) squared (kg/m²). Infant ponderal index was calculated as the ratio of weight (g) to length (cm) cubed (g/cm³*100). Dietary vitamin B₁₂ and folate intake was a measurement of diet only; prenatal supplementation use was analyzed as number of supplements per week. Non-normally distributed variables were natural-logarithmically transformed to ensure normality before analysis; values in Tables 1 and 2 are presented as non-transformed values for interpretation purposes.

In predictor analyses, variables with univariate p-values <0.20 were included in multivariate models, eliminated using backward selection, and retained if p-values were <0.05. Observations with missing covariate data were retained in analyses using the missing indicator method [24]. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, US).

RESULTS

Participant Characteristics. Characteristics of participants included in this study are presented in **Table 1**. Participants in the TC sub-study with RNA available for analyses (n=63) were similar to the overall cohort (n=177), on sociodemographic, obstetric, dietary, and nutritional characteristics.

Vitamin B₁₂ Concentrations. Maternal **serum** vitamin B₁₂ concentrations at mid-gestation and delivery and neonatal **serum** vitamin B₁₂ concentrations are presented in **Table 2**. At mid-gestation, no women were vitamin B₁₂ deficient (<148 pmol/L) and 19.5% were vitamin B₁₂ insufficient (<221 pmol/L); at delivery, 18.2% were vitamin B₁₂ deficient and 54.6% were insufficient. The prevalence of maternal vitamin B₁₂ insufficiency was significantly higher at delivery than mid-gestation (**p<0.05**). Maternal vitamin B₁₂ concentrations at mid-gestation and delivery were significantly correlated (r=0.84, **p<0.0001**) and maternal vitamin B₁₂ levels were significantly lower at delivery (**p<0.0001**). No infants were vitamin B₁₂ deficient and 3.2% were vitamin B₁₂ insufficient at birth. **Neonatal serum** vitamin B₁₂ concentrations were significantly higher than maternal **serum** vitamin B₁₂ concentrations at mid-gestation and delivery (**p<0.0001**). Maternal and **neonatal serum** vitamin B₁₂ concentrations were significantly correlated at delivery (r=0.58, **p<0.0001**), but not at mid-gestation (r=0.26, p=0.09).

Placental Transcobalamin. TC mRNA expression ($\Delta\Delta Ct$) and TC protein abundance (TC: α -actin ratio) were non-normally distributed (right-skewed), but were normalized after logarithmic transformation. TC mRNA expression and protein abundance were not significantly correlated (r=0.04, p=0.77).

Findings from predictor analyses of TC mRNA expression and protein abundance are presented in **Table 3**. In multivariate analyses, later gestational age at delivery ($p=0.02$) and marital status (single vs. not single; $p=0.004$) were associated with higher TC mRNA expression, while increased prenatal supplement use (≥ 2 times/week; $p=0.007$) and greater placental weight ($p=0.04$) were associated with lower TC mRNA expression. Higher neonatal serum vitamin B₁₂ concentrations ($p=0.02$) and male infant sex ($p=0.001$) were associated with higher TC protein abundance.

Vitamin B₁₂ Concentrations and Placental Transcobalamin. Data on potential associations of placental TC mRNA expression and protein abundance with maternal and neonatal serum vitamin B₁₂ concentrations are presented in **Table 4**. In multivariate analyses, maternal vitamin B₁₂ concentrations at mid-gestation ($p>0.05$) or delivery ($p>0.05$) were not significantly associated with placental TC mRNA expression. Maternal vitamin B₁₂ concentrations at mid-gestation and delivery were not associated with placental TC protein abundance ($p>0.05$). Maternal dietary vitamin B₁₂ intake during pregnancy was not associated with neonatal serum vitamin B₁₂ concentrations, placental TC mRNA expression, or placental TC protein abundance ($p>0.05$). The association between TC protein abundance and neonatal serum vitamin B₁₂ concentrations is presented in **Figure 2**. In multivariate analyses, higher placental TC protein abundance was associated with higher neonatal serum vitamin B₁₂ concentrations ($p=0.003$).

DISCUSSION

In this study, maternal vitamin B₁₂ concentrations during gestation or at delivery were not significantly associated with placental TC mRNA expression or protein abundance. However, higher placental TC protein abundance was associated with higher neonatal serum vitamin B₁₂ concentrations.

Placental transcobalamin mRNA expression and protein abundance were not significantly correlated in this study. This lack of a correlation is in contrast to *a priori* hypotheses, but a recent review noted that only 40% of variation in protein concentrations can be explained by mRNA expression [25]. Post-translation modifications, protein degradation regulation, and protein conservation mechanisms may explain the lack of correlation between mRNA expression and protein abundance. Additionally, the complex structure of the placenta is comprised of multiple tissue types and contains proteins synthesized from the placenta, mother, and fetus, which may affect mRNA expression, protein synthesis, and post-translational modifications [15, 26]. However, these mechanisms have not been established in vitamin B₁₂ placental transport in animal or human studies.

To our knowledge, this is the first study to investigate vitamin B₁₂ and placental transcobalamin in pregnant adolescents. Findings suggest that maternal serum vitamin B₁₂ concentrations during pregnancy and delivery were not significantly associated with placental TC mRNA expression or protein abundance. However, higher placental transcobalamin protein abundance was associated with higher neonatal serum vitamin B₁₂ concentrations. Previous research on vitamin B₁₂ transport in perfused placentas have identified transcobalamin as the primary transporter (>70%) of vitamin B₁₂ across the placenta [12]. Research on the role of

regulation of placental transporters in relation to maternal nutriture is conflicting, and no previous studies have measured the associations between maternal serum vitamin B₁₂ concentrations during pregnancy and placental TC expression to compare with our findings. The placental nutrient sensing model suggests that placental transport proteins down-regulate in response to inadequate maternal nutritional status [27, 28], and up-regulate transporters in response to abundant maternal supply [29]. In contrast, the fetal demand model suggests that the fetus can up-regulate placental transporter expression to compensate for limited nutrient availability from the mother [27, 30]. The placenta may up-regulate TC transporters in women with higher serum vitamin B₁₂ concentrations to maximize transport to the fetus.

Findings from predictor analyses suggest that maternal factors may be associated with TC mRNA expression and vitamin B₁₂ transport. Longer gestation, single marital status, and lower placental weight were associated with higher TC mRNA expression. Fetal nutrient requirements vary over gestation, typically with the greatest demand during the final trimester. In the absence of time-integrated data on placental TC mRNA expression during pregnancy in human studies, it is not known how TC mRNA expression and protein abundance change during pregnancy. Contradictory to the up-regulatory role of maternal vitamin B₁₂ on TC mRNA expression, greater prenatal supplement use was associated with lower TC mRNA expression. However, maternal dietary vitamin B₁₂ intake during pregnancy was not associated with neonatal serum vitamin B₁₂ concentrations, placental TC mRNA expression, or placental TC protein abundance. Prenatal supplement intake may alter cobalamin availability and TC mRNA expression, but the potential mechanisms involved are unknown [27, 29].

In predictor analyses, male infant sex was associated with increased placental TC protein abundance. Variation in placental cytokine expression and insulin-like growth factor pathways

by sex has been reported [31]; higher placental TC protein abundance in male offspring may reflect a similar sexual dimorphism in nutrient transport.

This study had several limitations. Neonatal serum vitamin B₁₂ concentrations were measured from cord blood, and prospective maternal serum vitamin B₁₂ concentrations were available on a sub-set of participants; this constrains our ability to establish temporal associations between maternal and neonatal serum vitamin B₁₂ concentrations and placental transcobalamin. Additionally, TC mRNA expression and protein abundance were evaluated from placental tissue homogenates, and membrane fractions were not purified, which constrains our ability to elucidate mechanisms involved in the transfer dynamics between the maternal, placental, and fetal compartments. Further, there is no universally accepted method to account for variability of placental mRNA expression and protein abundance across different sampling sites; we combined multiple placental samples in an attempt to limit interplacental variability. We also did not analyze placental expression of CD320, a known transcobalamin receptor in the placenta [32], which may be a rate-limiting factor in placental vitamin B₁₂ transport [33]. The low prevalence of vitamin B₁₂ deficiency in this population is consistent with previous studies in North America [34, 35]; however, the low burden of vitamin B₁₂ deficiency and narrow distribution of TC mRNA expression and protein abundance may limit the generalizability of findings to other populations. Vitamin B₁₂ status assessment was also limited to a single biomarker of total vitamin B₁₂. Inclusion of holo-transcobalamin (i.e., holoTC, transcobalamin bound to cobalamin), a circulating vitamin B₁₂ biomarker [36] and a functional vitamin B₁₂ biomarker (methylmalonic acid) [15] would improve vitamin B₁₂ assessment [36]. Previous studies in adult women have noted that holo-transcobalamin concentrations remain relatively constant during throughout pregnancy [36], and may provide insights into the links between maternal, placental,

and fetal vitamin B₁₂ status than total vitamin B₁₂ [36-38]. However, no studies to date have measured holoTC as it relates to placental transcobalamin, and there is no data to date on holoTC or placental CD320 expression in adolescent populations. Assessment of holoTC and placental CD320 expression are important areas of future research in vitamin B₁₂ transport during pregnancy.

In summary, in a cohort of pregnant adolescents, maternal serum vitamin B₁₂ concentrations during pregnancy and at delivery were not significantly associated with placental TC mRNA expression or TC protein abundance. However, higher placental TC protein abundance was associated with higher neonatal serum vitamin B₁₂ concentrations.

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AUTHOR CONTRIBUTIONS

KOO, EKP, and EMC designed and conducted the studies; KOO, JLF, and AJL designed the TC sub-study. AJL conducted the laboratory analyses and wrote the initial draft of the manuscript; JLF had primary responsibility for final content. All authors contributed to the interpretation of data and in the development of this manuscript, and read and approved the final version. None of the authors had any conflicts of interest to disclose.

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The funders of the study had no role in the study design, data collection, analysis, data interpretation, or writing of the manuscript.

FIGURE LEGENDS

Figure 1.

Western blot analysis of TC protein abundance in human placenta. A) TC protein abundance in positive and negative control tissues, respectively. B) TC protein abundance in human placenta. PC⁺ represents positive control placental tissue.

Figure 2.

Associations Between Transcobalamin Protein Abundance and Neonatal Vitamin B₁₂ Cord Blood Concentrations. Vitamin B₁₂ concentrations and TC protein abundance were **natural logarithmically-transformed** to ensure normality before analysis.

TABLES

Table 1. Characteristics of the Study Population

Variables ¹	Entire Cohort (n=177)	TC Sub-study (n=63)
Maternal		
Age at enrollment (y)	17.2 ± 1.1	17.2 ± 1.2
Gestational age, mid-gestation blood draw (wks)	26.1 ± 3.6	26.4 ± 3.5
Gestational age at delivery (wks)	39.8 ± 1.3	39.8 ± 1.4
Parity ≥1, % (n)	20.9 (37)	17.5 (11)
Smoking at enrollment ² , % (n)	6.9 (12)	4.8 (3)
Marital status ³ , % (n)	14.9 (26)	12.9 (8)
Self-reported prenatal supplement use ⁴ , % (n)	69.7 (122)	71.0 (44)
Dietary folate (µg/day)	717.4 ± 415.5	708.4 ± 360.4
Dietary vitamin B ₁₂ (µg/day)	5.1 ± 3.3	5.4 ± 4.4
Pre-pregnancy BMI (kg/m ²)	25.0 ± 5.6	24.8 ± 5.9
≥25 kg/m ² , % (n)	38.1 (67)	36.5 (33)
≥30 kg/m ² , % (n)	20.5 (36)	19.1 (12)
Anemic ⁵ at mid-gestation, % (n)	11.2 (10)	8.6 (3)
Anemic ⁵ at delivery, % (n)	15.7 (26)	22.0 (13)
Gestational weight gain (kg)	17.3 ± 7.7	17.3 ± 7.0
Race, % (n)		
Caucasian	29.9 (53)	31.8 (20)
African-American	69.5 (123)	66.7 (42)
Native American	0.6 (1)	1.6 (1)
Ethnicity, % (n)		
Hispanic	26.6 (47)	31.8 (20)
Non-Hispanic	73.5 (130)	68.3 (43)
Placental weight (g)	605.7 ± 155.4	606.2 ± 105.4
Infant		
Birth weight (g)	3265.9 ± 442.1	3276.0 ± 405.3
Small-for-gestational-age ⁶ , % (n)	15.8 (28)	12.7 (8)
Birth length (cm)	51.1 ± 2.5	51.6 ± 2.4
Ponderal index (g/cm ³ *100)	2.5 ± 0.3	2.4 ± 0.3
Male sex, % (n)	49.2 (87)	42.9 (27)

¹ Values are mean ± SD and % (n)

² Current smokers vs. non-smokers

³ Married or in a relationship vs. single

⁴ Reported intake of prenatal supplements ≥2 times per week

⁵ Anemia: Hb<12.0 g/dL, anemia cut-off was adjusted for race and trimester [25]

⁶ Small-for-gestational-age: <10th percentile for birth weight by gestational age

Table 2. Maternal and Infant Vitamin B₁₂ Status			
Variable¹	Maternal Mid-Gestation	Maternal Delivery²	Infant Cord Blood
n	41	44	63
Serum vitamin B ₁₂ (pmol/L)	348.3 ± 120.6	230.9 ± 95.2 ^a	662.1 ± 363.1 ^{a,b,3}
Deficient (<148 pmol/L), % (n)	0.0 (0)	18.2 (8)	0.0 (0)
Insufficient (<221 pmol/L), % (n)	19.5 (8)	54.6 (24) ^a	3.2 (2) ^{a,b}

¹ Values are mean ± SD and % (n)

² There were n=22 participants with vitamin B₁₂ concentrations assessed at both mid-gestation and delivery for comparisons.

³ Maternal vitamin B₁₂ concentrations at mid-gestation (β(SE)=0.344 (0.201), p=0.093) and delivery (β(SE)=0.828 (0.180), p<0.0001) were associated with infant vitamin B₁₂ concentrations.

^a Significantly different than maternal mid-gestation vitamin B₁₂ concentrations (p<0.05)

^b Significantly different from maternal delivery vitamin B₁₂ concentrations (p<0.0001)

Table 3. Predictors of Placental Transcobalamin mRNA Expression and Protein Abundance

Variables	TC mRNA Expression ($\Delta\Delta Ct$)					TC Protein Abundance (TC: α -actin)				
	n	Univariate (p<0.20)		Multivariate (p<0.05)		n	Univariate (p<0.20)		Multivariate (p<0.05)	
		β (SE)	P-value	β (SE)	P-value		β (SE)	P-value	β (SE)	P-value
<i>Maternal</i>										
Age at enrollment (y)	49	-0.08 (0.05)	0.15	-	-	-	-	-	-	-
Gestational age at delivery (wks)	49	0.11 (0.04)	0.01	0.09 (0.04)	0.02	-	-	-	-	-
Prenatal supplement use ≥ 2 /wk ¹	48	-0.24 (0.14)	0.08	-0.33 (0.11)	0.007	60	0.18 (0.10)	0.08	-	-
Pre-pregnancy BMI (kg/m ²)	49	0.02 (0.01)	0.12	-	-	-	-	-	-	-
Single marital status ²	49	0.58 (0.18)	0.002	0.58 (0.19)	0.004	-	-	-	-	-
Non-Caucasian ³	-	-	-	-	-	61	0.16 (0.01)	0.12	-	-
Parity ≥ 1	49	-0.47 (0.15)	0.003	-	-	61	0.16 (0.12)	0.19	-	-
Delivery vitamin B ₁₂ (pmol/L)	37	0.38 (0.17)	0.03	-	-	-	-	-	-	-
Placental weight (g)	47	-0.001 (0.0006)	0.06	-0.001 (0.0005)	0.04	-	-	-	-	-
<i>Infant</i>										
Small-for-gestational-age ⁴	-	-	-	-	-	61	-0.23 (0.14)	0.11	-	-
Male sex	-	-	-	-	-	61	0.35 (0.09)	<0.001	0.29 (0.09)	0.001
Cord blood vitamin B ₁₂ (pmol/L)	-	-	-	-	-	61	0.26 (0.09)	0.005	0.21 (0.09)	0.02

Linear regression models were used to examine the predictors of TC mRNA expression and TC protein abundance. Variables for TC mRNA, TC protein abundance, and vitamin B₁₂ were natural logarithmically-transformed to achieve normality before analysis. Variables with univariate p-values less than 0.20 were included in multivariate models, eliminated using backward selection, and retained if their p-values were less than 0.05. Delivery and cord blood vitamin B₁₂ concentrations and placental weight were adjusted for timing of measurement (i.e., gestational age at delivery).

¹ Reported intake of prenatal supplements ≥ 2 times per week

² Single vs. married/in a relationship

³ Non-Caucasian vs. Caucasian

⁴ Small-for-gestational-age: <10th percentile for birth weight by gestational age

Table 4. Associations Between Maternal and Infant Vitamin B₁₂ Status And Placental TC mRNA Expression and Protein Abundance

		TC mRNA Expression ($\Delta\Delta Ct$)			
		Univariate		Multivariate ²	
Variables ¹	n	β (SE)	P-value	β (SE)	P-value
Maternal Vitamin B ₁₂ at Mid-gestation (pmol/L)	31	-0.22 (0.22)	0.33	-0.16 (0.19)	0.40
Maternal Vitamin B ₁₂ at Delivery (pmol/L)	37	0.38 (0.17)	0.03	0.30 (0.18)	0.10
		TC Protein Abundance (TC: α -actin)			
		Univariate		Multivariate ³	
Variables	n	β (SE)	P-value	β (SE)	P-value
Maternal Vitamin B ₁₂ at Mid-gestation (pmol/L)	40	-0.21 (0.17)	0.23	-0.17 (0.16)	0.30
Maternal Vitamin B ₁₂ at Delivery (pmol/L)	42	0.11 (0.15)	0.45	0.08 (0.14)	0.56
		Infant Cord Blood Vitamin B ₁₂ , pmol/L			
		Univariate		Multivariate ⁴	
Variables	n	β (SE)	P-value	β (SE)	P-value
TC mRNA Expression ($\Delta\Delta Ct$)	49	0.07 (0.16)	0.68	-0.11 (0.17)	0.53
TC Protein Abundance (TC: α -actin)	61	0.52 (0.16)	0.003	0.48 (0.15)	0.003

¹ Vitamin B₁₂ measurements, placental TC mRNA expression, and TC protein abundance were natural logarithmically transformed to achieve normality before analysis.

² Multivariate regression analyses for TC mRNA expression were adjusted for: marital status (single vs. married/in a relationship), prenatal supplement use (≥ 2 times per week), gestational age at delivery, and placental weight.

³ Multivariate regression analyses for TC protein abundance were adjusted for infant sex (male vs. female).

⁴ Multivariate regression analyses for infant cord blood vitamin B₁₂ were adjusted for maternal age, marital status (single vs. married/in a relationship), and gestational age at birth.

FIGURES

Figure 1. Transcobalamin Protein Abundance

Figure 2. Association Between Transcobalamin Protein Abundance and Neonatal Vitamin B₁₂ Concentrations