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Vitamin B_{12} 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 B_{12} to the fetus. However, few data currently exist on the ability of the placenta to modify vitamin B_{12} 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 B_{12} concentrations on placental transcobalamin (TC) expression in a cohort of healthy pregnant adolescents in the United States.
Design: Serum vitamin B_{12} 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 (ΔΔCt) and TC protein abundance (TC:α-actin) were evaluated. Linear and binomial regression models were used to examine the associations of maternal serum (mid-gestation, delivery) and cord blood vitamin B_{12} concentrations with placental TC mRNA expression and protein abundance ($n = 63$).
Results: Maternal serum vitamin B_{12} 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 increased cord blood vitamin B_{12} concentrations ($p = 0.003$).
Conclusions: Higher placental TC protein abundance was associated with higher cord blood vitamin B_{12} concentrations, suggesting a potential role in vitamin B_{12} transport to the fetus.

1. Introduction

Vitamin B_{12} deficiency (<148 pmol/L) is a major public health problem worldwide [1,2]. Maternal vitamin B_{12} deficiency has been associated with increased risk of common pregnancy complications [3] and impaired growth and brain development in offspring [4,5].

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

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

Few data exist on the ability of the placenta to modify vitamin

Abbreviations used: 95% CI, 95% confidence interval; BMI, body mass index; HoloTC, holotranscobalamin; MMA, methylmalonic acid; NTD, neural tube defect; qPCR, quantitative real-time 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.

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B_{12} transport in response to maternal vitamin B_{12} status. Pregnant adolescents, which represent over 5% of the U.S. obstetric population [17], are at an even greater risk for micronutrient deficiencies and pregnancy complications [18,19]. No data currently exist on the role of placental vitamin B_{12} transporters and vitamin B_{12} status in adolescent populations. We conducted this study to examine the associations of maternal serum and infant cord blood vitamin B_{12} concentrations and placental transcobalamin expression in a cohort of healthy pregnant adolescents. We hypothesized that 1) lower maternal serum vitamin B_{12} concentrations at mid-gestation and delivery would be associated with higher placenta transcobalamin expression, to maximize vitamin B_{12} transport to the fetus; and 2) higher placental transcobalamin expression would be associated with higher vitamin B_{12} concentrations in cord blood.

2. Subjects and methods

2.1. 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, and 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.

2.2. 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-h 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.

2.3. 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 ≤ -80 °C until analysis. Maternal serum and cord blood vitamin B_{12} 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 ≤ -80 °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 ≤ -80 °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 μmol/L forward primer, 0.35 μmol/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 (ΔCt = # cycles for TC - # cycles for β-actin) and compared to control placental samples using the $2-\Delta\Delta Ct$ method ($2-\Delta\Delta Ct = 2^{(\Delta Ct\ sample\ -\Delta Ct\ control\ placenta)}$) [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 13,000*g* for 20 min at 4 °C. Following centrifugation, the pellet was discarded and the supernatant was used for protein quantification or stored ≤ -80 °C. Protein concentrations were quantified using the Bio-Rad assay (Bio-Rad, Hercules, CA, USA). Lysates were diluted in SDS sample buffer and stored ≤ -80 °C until analysis.

Placental samples were separated on a 10% SDS-PAGE gel and transferred to a polyvinylidene difluoride 156 fluorescence 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 (Fig. 1).

2.4. Statistical analyses

Linear regression and Spearman correlations were used to examine the associations of maternal serum and infant cord blood vitamin B_{12} concentrations with placental TC mRNA expression (ΔΔCt) and protein abundance (TC:α-actin). Linear regression was