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Longitudinal Study of the Effect of Pregnancy on Maternal and Fetal Cobalamin Status in Healthy Women and Their Offspring[1]

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Abstract

Compromised cobalamin status during pregnancy may put both mother and child at risk of deficiency during lactation and subsequent development. We investigated whether changes in cobalamin status during pregnancy are associated with impaired status in the mother and the cord. Plasma cobalamin, methylmalonic acid (MMA), and holotranscobalamin (holoTC) concentrations were determined in 92 women at preconception, 8, 20, and 32 wk of pregnancy, at labor, and in the cord. All variables [geometric mean percentiles 10, 90 (P_{10}, P_{90})] were significantly reduced from preconception [cobalamin: 293 (155, 535) pmol/L; holoTC: 63 (38,98) pmol/L; MMA: 0.12 (0.09, 0.17) μmol/L] by 20 wk of pregnancy [cobalamin: 230 (123, 432) pmol/L; holoTC: 48 (34,78) pmol/L; MMA: 0.11 (0.08, 0.15) μmol/L $P < 0.001$]. Plasma cobalamin and holoTC remained lower throughout the remainder of pregnancy [32 wk: 198 (107, 339); labor: 224 (117, 444); $P < 0.001$] and [32 wk: 45 (26,82); labor: 40 (23,79); $P < 0.05$], respectively. By 32 wk, MMA was greater than preconception [0.14 (0.09, 0.20) μmol/L; $P < 0.01$]. Plasma holoTC at 32 wk and at labor was negatively correlated with cord MMA ($r = -0.51$, $P < 0.001$ and $r = -0.40$, $P < 0.01$, respectively). Women with lower holoTC at preconception had greater increases in MMA at 32 wk and at labor. Maternal MMA at 32 wk and at labor was significantly and independently associated with cord MMA only in women with lower holoTC at preconception (regression models: $R^2 = 0.707$, 0.682, respectively; $P < 0.01$). The moderate increases observed in the cobalamin biomarker, MMA, during pregnancy may indicate a functional depletion in intracellular cobalamin status. J. Nutr. 137: 1863–1867, 2007.

Introduction

Inadequate cobalamin status has been associated with adverse pregnancy outcomes such as neural tube defects (1–3), preterm birth (4), intrauterine growth-retardation (5,6) and recurrent miscarriage in one (7) but not in another study (8).

A number of cross-sectional, case-control and longitudinal studies reported that blood concentration of cobalamin drops during normal pregnancy (9–12). Hemodilution, altered renal function, hormonal changes, changes in the concentration of cobalamin-binding proteins, and materno-fetal cobalamin transfer are normal physiological consequences of pregnancy that affect plasma cobalamin concentrations in the mother, but it is unclear to what degree maternal cobalamin status becomes compromised as a result. Clinically significant cobalamin deficiency meriting intervention during pregnancy has only been reported in pregnant women with traditionally vegetarian diets (13,14).

The use of nonpregnant reference values may not be appropriate for assessing cobalamin status during pregnancy (9,12). The usefulness of functional indicators of cobalamin status in assessing pregnancy cobalamin status is unclear from previous studies. Elevated methylmalonic acid (MMA)[7] is a sensitive functional marker of low tissue cobalamin reserves, with ~95% of cobalamin-deficient patients having elevated serum MMA (15). However, the reliability of MMA as a marker of cobalamin status during pregnancy has been questioned by several studies reporting the sporadic occurrence of elevated MMA (9,11,16) that might not be linked to low cobalamin status. Holotranscobalamin (holoTC), the fraction of cobalamin bound to transcobalamin II (TCII) and available for uptake into tissues, is thought to be a more sensitive marker of early changes in cobalamin status than the total cobalamin level (17). A recent longitudinal study examined cobalamin-binding protein fractions during pregnancy and found a reduced percentage saturation of

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[7] Abbreviations used: holoTC, holotranscobalamin; MMA, methylmalonic acid; P_{10}, P_{90}, percentiles 10 and 90.

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these proteins, but it was not related to metabolic markers of cobalamin status (12).

The aim of this study was to investigate longitudinal changes in cobalamin status from preconception throughout normal pregnancy, correcting for hemodilution and renal function, and to assess whether these changes are associated with evidence of impaired cobalamin status, using the functional markers plasma MMA and holoTC.

Materials and Methods

The design and background to this study have been reported in detail previously (18,19). Briefly, this was a longitudinal study performed by the Units of Preventive Medicine and Public Health, Faculty of Medicine and Health Sciences, Rovira i Virgili University, and Obstetrics and Gynecology, Hospital St. Joan, Reus. It formed part of a study on the evolution of women's nutritional status from preconception throughout pregnancy. The hospital's ethics committee approval was obtained for the study, and all volunteers provided their signed informed consent in accordance with the declaration of Helsinki (20). Healthy women ($n = 92$), aged between 18 and 35 y, with the intention of becoming pregnant, participated in the study from preconception throughout pregnancy.

Sample collection and analysis. Women attended a medical checkup and interview before becoming pregnant and were instructed to contact the study team on the first day of their subsequent menstrual period. Sample collection was programmed between d 7 and d 12 of that cycle, in which they intended to become pregnant. If they had not become pregnant during the 2 subsequent menstrual cycles, they were rescheduled for another preconception blood sample. On missing their menstrual period, they were instructed to contact the team immediately and programmed for an ultrasound scan at 7 wk of pregnancy. On confirmation of a viable embryo, they were scheduled for the first pregnancy blood sample at 7.5–8 wk of pregnancy and subsequently at 20 and 32 wk, on admission to hospital with labor, and from the cord. Blood samples were drawn from the antecubital vein in potassium EDTA-treated vacutainer tubes at preconception (2–10 wk before conception, between d 7 and d 12 of the menstrual cycle), and at wk 8, 20, and 32 of pregnancy, as well as on admission to hospital with confirmed labor. Thus, the last blood sampling from the pregnant woman was performed prior to delivery in all cases. However, the length of time between labor onset and delivery varied among patients. Immediately after delivery, a final blood sample was taken from the umbilical cord vein. Plasma was stored at −20°C. Plasma cobalamin was determined by microbiological assay using *Lactobacillus leichmannii* (21); plasma MMA by GC-MS with isopropylchloroformate derivatization (22), and plasma holoTC by immunoassay (AxSym) (23). Intra and interassay CV were 7.1 and 10.7% for plasma cobalamin, 4.9 and 9.8% for plasma holoTC, and 4.0 and 3.0% for plasma MMA.

Vitamin supplementation. Specific information on vitamin supplement use and dosage was recorded in 7-d food records the week before each programmed blood sample. Women were also specifically questioned on vitamin use and dosage at each antenatal checkup, which they attended during the same week as blood sample collection. Briefly, no women took any vitamin supplements during either the preconception period or the first trimester of pregnancy, and 67 of 92 women did not take cobalamin supplements throughout the entire study. At the time of the study (1992–1996), periconception supplementation with folic acid was not common practice in Spain. Currently, women are recommended by their obstetrician to take folic acid supplements until the end of the first trimester of pregnancy. On initiation of iron supplementation, 1 woman reported taking cobalamin-containing supplements on a regular basis from 20 wk of pregnancy, and 24 women took supplements from 32 wk. This corresponds with a daily dose of 5–10 μg/d of cobalamin for these subjects.

Statistical analysis. All statistical analyses were performed using the SPSS software, version 12.0. Variables were log-transformed to approach normality when required for a statistical test. ANOVA repeated measures analysis was used to test the significance of differences in plasma concentrations between pregnancy time points and preconception. We determined Spearman's correlation coefficients between plasma cobalamin fractions (cobalamin and holoTC) at each time point with labor and cord MMA. Values in the text are means ± SD or geometric means (P_{10}, P_{90}).

When the women were subdivided into 2 groups, above and below the preconception plasma holoTC median (67 pmol/L), we calculated the percentage of change from baseline (preconception) in plasma MMA at each time point of pregnancy and correcting for hemodilution. We introduced a correction factor for hemodilution by multiplying the plasma MMA concentration at a given time of pregnancy by the ratio of preconception hematocrit:hematocrit at that time of pregnancy. Differences in mean percentage changes between consecutive time points were compared using ANOVA repeated measures analysis. Multiple linear regression analysis was used to determine the association between maternal plasma MMA at 32 wk of pregnancy and at labor with cord MMA. The dependent variable, cord MMA, was natural log-transformed with the resulting antilog of the β-coefficient value giving the percentage of change in cord MMA per 25% mean increase in mean maternal MMA. The model was adjusted for serum creatinine, fetal sex, birth weight, and gestational age, multivitamin use, and smoking. Post hoc analysis by Bonferroni correction of P-values was applied for multiple comparisons in both ANOVA repeated measures analyses. Significance was determined at $P < 0.05$.

Results

The age of the women at preconception was 29.5 ± 2.7 y. Only 43 of them reported using oral contraceptives during the 6 mo prior to preconception. Fifty-nine women were primiparous. Gestational age at labor was 280 ± 11 d. The number of women who smoked and the numbers of cigarettes smoked daily throughout the study were: preconception, 29 women (9.6 ± 6.1); 8 wk, 16 women (3.5 ± 2.8); 20 wk, 11 women (4.5 ± −3.3); and 32 wk, 10 women (5.2 ± −3.5). Plasma cobalamin, holoTC, and MMA concentrations did not differ between the groups of reported multivitamin supplement users ($n = 25$) and nonusers ($n = 67$) at 32 wk of pregnancy or at labor. Excluding supplement users from the analysis did not affect any of the results or conclusions. However, one subject was excluded due to inconsistent reports on supplement use and for having a plasma holoTC 6 times greater than the mean of the group at labor. Sufficient plasma volume for holoTC analysis only remained for between 50 and 62 mothers, depending on the time point of the study, and for 23 cords.

Plasma cobalamin was significantly lower than at preconception from 20 wk throughout the remainder of pregnancy. Plasma holoTC was significantly lower from 8 wk to 32 wk of pregnancy than at preconception. Plasma MMA was significantly lower at 8 wk and 20 wk, but was significantly higher by 32 wk of pregnancy and at labor, than at preconception. Despite the significant changes in indices of cobalamin status, the women's plasma cobalamin and MMA concentrations did not indicate cobalamin deficiency (<150 pmol/L cobalamin and >0.28 μmol/L MMA), at any time during pregnancy (**Table 1**).

Preconception plasma cobalamin and holoTC were not correlated with MMA at labor or in the cord. At 8 wk of pregnancy, significant negative associations began to emerge between both cobalamin measures (plasma cobalamin and holoTC) and MMA at labor ($r = -0.27$, and -0.33, respectively, $P < 0.05$). The strength and significance of these relationships were consistent throughout pregnancy only in the case of holoTC. The inverse correlation between maternal plasma holoTC at 32 wk of pregnancy and at labor with cord MMA was significant ($r = -0.51$,

TABLE 1 Changes in women's plasma cobalamin, holoTC, and MMA concentrations from preconception throughout normal pregnancy and in cord blood[1]

		Pregnancy, *wk*				
	Preconception	8	20	32	Labor	Cord
Cobalamin, *pmol/L*	293 (155, 535) [89]	267 (144, 449) [88]	230 (123, 432)[a] [90]	198 (107, 339)[b] [90]	224 (117, 444)[a] [84]	325 (146, 641) [82]
Holo TC, *pmol/L*	63 (38, 98) [56]	47 (31, 74)[a] [58]	48 (34, 78)[a] [61]	45 (26, 82)[b] [60]	40 (23, 79) [49]	92 (45, 214) [23]
MMA, μ*mol/L*	0.12 (0.09, 0.17) [88]	0.11 (0.09, 0.17)[a] [87]	0.11 (0.08, 0.15)[a] [89]	0.14 (0.09, 0.20)[b] [90]	0.14 (0.09, 0.21)[a] [83]	0.24 (0.13, 0.40) [72]

[1] Values are geometric means (P_{10}, P_{90}) [n]. Letters indicate different from preconception: [a]$P < 0.001$; [b]$P < 0.05$.

$P < 0.001$, and $r = -0.40$, $P < 0.01$, respectively). Only maternal plasma cobalamin at labor was significantly inversely correlated with cord MMA ($r = -0.3$, $P < 0.01$). Maternal MMA was significantly correlated with cord MMA from preconception throughout pregnancy (preconception through 20 wk, $r = 0.3$, $P < 0.05$; 32 wk and labor, $r = 0.4$, $P < 0.01$) (**Table 2**).

Because MMA was increased in situations of low cobalamin status, we divided the women into 2 groups, one above and one below the preconception holoTC median (67 pmol/L). This enabled us to investigate the effect of pregnancy on cobalamin status in the absence of the possible overriding effect of individuals with higher cobalamin status. Because real changes in cobalamin status during pregnancy may be masked or exaggerated by hemodilution, we calculated the mean percentage changes in plasma MMA from preconception using a correction factor based on changes in hematocrit from preconception. The corrected data (**Fig. 1**) showed that plasma MMA, whereas initially reduced at 8 wk of pregnancy, gradually increased from 20 wk throughout pregnancy and that the increase was greater in the group of women with lower preconception holoTC. MMA [geometric mean (P_{10}, P_{90})] at labor [0.15 μmol/L (0.09, 0.28)] was also significantly higher than at preconception [0.13 μmol/L (0.08, 0.18)], $P < 0.05$, in this group. The association between maternal MMA (at both 32 wk of pregnancy and at labor) and cord MMA according to preconception holoTC status was investigated using multiple linear regression analysis in separate models. Cord MMA was the dependent variable for each model. The models were adjusted for serum creatinine, fetal sex, birth weight, gestational age, multivitamin use, and smoking. The models in the low preconception holoTC group showed that a 25% increase in maternal MMA at 32 wk (R^2: 0.707, $n = 25$, $P < 0.01$) and at labor (R^2:0.682, $n = 26$, $P < 0.01$) was associated with a mean increase in cord MMA of 5.3 and 8.0%, respectively ($P < 0.05$). In the group with higher preconception holoTC, neither model was significant nor was there a significant independent association between maternal and cord MMA (data not shown).

TABLE 2 Correlations between women's plasma cobalamin, holoTC, and MMA fractions before and during pregnancy with MMA at labor and in the cord blood[1]

		MMA	
		Labor *r* (*n*)	Cord *r* (*n*)
Preconception	Cobalamin	−0.16 (80)	−0.05 (69)
	HoloTC	−0.14 (54)	0.08 (44)
	MMA		0.30[a] (69)
8 wk	Cobalamin	−0.27[a] (80)	−0.14 (69)
	HoloTC	−0.33[a] (53)	−0.07 (47)
	MMA		0.29[a] (68)
20 wk	Cobalamin	−0.08 (81)	−0.12 (71)
	HoloTC	−0.29[b] (55)	−0.09 (47)
	MMA		0.28[a] (70)
32 wk	Cobalamin	−0.08 (81)	−0.14 (70)
	HoloTC	−0.28[a] (54)	−0.51[c] (47)
	MMA		0.40[b] (70)
Labor	Cobalamin	−0.18 (83)	−0.34[b] (69)
	HoloTC	−0.14 (49)	−0.40[b] (41)
	MMA		0.36[b] (68)

[1] Values are Spearman's correlation coefficients [n]; Letters indicate significance: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

Discussion

To the best of our knowledge, this is the first study to examine markers of cobalamin status during pregnancy in which preconception samples have allowed women to act as their own nonpregnant controls. Furthermore, we were able to consider the impact of pregnancy-related confounders such as hemodilution, renal function, and supplement use. We observed reductions in plasma cobalamin throughout the course of normal pregnancy, as previously reported (10–12,24). The mean cobalamin intake in this previous group of women did not differ significantly from preconception throughout pregnancy (25). Thus, the biochemical changes in cobalamin status were not due to changes in dietary intake. All of the cobalamin status parameters were higher in cord blood than in maternal plasma at all time points of the study. This agrees with the findings of mother-cord pairs (26,27) as well as the magnitude of the differences between cord and maternal measurements. Mean cord plasma cobalamin was ~1.5 times, holoTC was 2.3 times, and MMA was 1.7 times greater than maternal means at labor. We

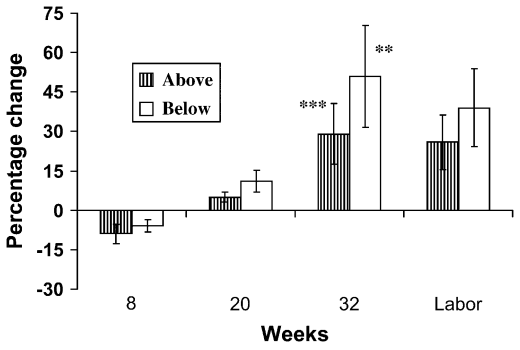

FIGURE 1 Percentage changes in plasma MMA concentration from preconception throughout pregnancy in women above and below the preconception holoTC median (67 pmol/L), corrected for hemodilution. Values are means ± SEM, $n = 28$. Asterisks indicate different from the preceding timepoint: ***$P < 0.001$, **$P < 0.01$.

also found changes in MMA toward the latter stages of pregnancy were consistent with deteriorating maternal cobalamin status. All of the mothers in our study entered pregnancy with normal cobalamin status, and changes in MMA were modest. However, increases in MMA were greater in mothers with holoTC below the median (67 pmol/L) at preconception. There was no biochemical evidence of compromised cobalamin status at preconception in this group [geometric mean (P_{10}, P_{90}) plasma cobalamin: 286 (169, 560) pmol/L; MMA: 0.13 (0.08, 0.18) μmol/L; and holoTC: 48 (32, 62) pmol/L)]. These findings may have important implications both for maternal status during lactation and consequent neonatal stores in children born to mothers who consume low cobalamin diets before and during pregnancy.

Koebnick et al. (12) reported a gradual reduction in plasma cobalamin from the first through the third trimester of pregnancy in a longitudinal study of 39 women. Our longer longitudinal study complements and extends this information. We found that there was already a tendency for a reduction in plasma cobalamin compared with preconception by 8 wk of pregnancy. It reached its lowest point by 32 wk of pregnancy but had started to rise again before delivery. We also demonstrated a reduction in holoTC concentration, which may be consistent with the reduction in holo-haptocorrin and increase in unsaturated binding capacity observed by these workers (12).

Bartels et al. (24) compared longitudinal measurements of serum cobalamin during pregnancy in a group of 35 women with 65 apparently healthy nonpregnant women. The mean serum cobalamin concentration was ~44 and 53% lower at 12 and 20 wk of pregnancy and 57% lower at both 28 and 36 wk of pregnancy compared with the mean concentrations recorded in the nonpregnant controls. Our healthy group of women, with good baseline cobalamin status and acting as their own baseline controls, had more modest, although significant, reductions in plasma cobalamin from preconception throughout pregnancy.

We also investigated changes in holoTC as a marker of the biologically active fraction of cobalamin in plasma during pregnancy. In the group as a whole, holoTC was reduced from preconception during early pregnancy and was gradually decreased throughout pregnancy. Fernandez-Costa and Metz (28) compared holoTC measured in different pregnant women in each trimester of pregnancy with measurements from nonpregnant controls. They reported a sharp increase in holoTC during the third trimester of pregnancy to values ~33% higher than nonpregnant controls. Our longitudinal data do not support this. We observed that, after the initial reduction in plasma holoTC during early pregnancy, it reached a plateau and remained at this lower level for the remainder of the study. This may suggest the presence of compensatory mechanisms to ensure sufficient available cobalamin to meet the enhanced cobalamin requirements caused by both maternal and fetal needs during pregnancy.

HoloTC was reported to be affected by synthetic hormone use in the form of oral contraceptives (29) and so may be affected by hormonal changes during pregnancy. However, holo TC was consistently negatively correlated with plasma MMA in our pregnancy study. The correlation was stronger than that observed with plasma cobalamin, as recently reported in an elderly cohort (30), suggesting that holo TC reflects cobalamin status during pregnancy.

On the basis of plasma MMA values being within the normal range for nonpregnant subjects [<0.280 μmol/L (31); <0.260 μmol/L (16)] by the end of pregnancy in our group of healthy pregnant women as a whole, it may appear that the changes in cobalamin status parameters do not indicate functional cobalamin deficiency, which agrees with previous reports (9–11). However, MMA increased from preconception. The reduction in MMA observed throughout the first 20 wk of pregnancy indicates that it undergoes the same hemodilution and hormonal effects as the other plasma variables investigated. Correcting for hemodilution, it was evident that MMA had increased with respect to preconception, irrespective of baseline holoTC status. The increase by the end of pregnancy was greater in the lower holoTC status group. This suggests that status had worsened compared with baseline status on entering pregnancy. Conceivably, depletion of maternal intracellular cobalamin stores may explain this moderate increase in MMA.

In contrast to a recent report (26), we also observed that maternal plasma cobalamin, and especially holoTC at labor, were negatively correlated with cord MMA. Fetuses of mothers with low cobalamin status during pregnancy have reduced cobalamin stores and therefore could be at an increased risk of further decline in cobalamin status during lactation (32). Our data suggest that the initial signs of compromised cobalamin status are present in pregnant women with normal cobalamin status. Thus, we can expect mothers and fetuses with suboptimal cobalamin status to have an even higher risk of deficiency during pregnancy.

Metz et al. (9) reported that serum MMA was similar in pregnant subjects with normal and subnormal serum cobalamin concentrations. They found no correlation between serum cobalamin and MMA concentrations despite MMA levels being elevated compared with nonpregnant reference values in one-third of pregnant patients, regardless of whether they had normal or low cobalamin status, based on serum cobalamin measurements. They proposed that this was due to altered renal function or flora of propionic-producing bacteria and concluded that the reduction in serum cobalamin during pregnancy is physiological. However, they only had one time point from pregnant subjects and it varied depending on the timing of their referral for suspected cobalamin deficiency. Because we observed that MMA increased in both groups of women, regardless of their starting holoTC status, it would appear that an increase in MMA is normal during pregnancy. The mechanisms proposed by Metz et al. (10) may contribute to this. However, in our study, MMA was inversely correlated with plasma cobalamin and especially holoTC. Also, maternal MMA at 32 wk and at labor was associated with cord MMA in mothers with holoTC below the preconception median. This analysis included an adjustment for serum creatinine, a marker of renal function.

We observed changes in functional indicators of cobalamin status in women who started pregnancy with plasma cobalamin within the normal range and went on to have normal pregnancies. When they were subdivided into groups based on their starting holoTC status, the increase in MMA by the end of pregnancy was greater in the women with lower preconception holoTC, and their MMA had increased by the end of pregnancy. Our interpretation of these observations is that there is a strain on cobalamin status during pregnancy.

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