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The distribution of total vitamin B12, holotranscobalamin, and the active vitamin B12 fraction in the first 5 weeks postpartum

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Abstract

Introduction: Total vitamin B12 levels decrease significantly during pregnancy and recover to normal values within 8-week postpartum. Holotranscobalamin (holoTC) reflects the active part of vitamin B12 and has been shown to remain constant during pregnancy and postpartum. A mechanism of redistribution of vitamin B12 is suggested, with a shift toward holoTC if there is insufficient total vitamin B12 available. Our objective was to examine vitamin B12 deficiency and the active vitamin B12 fraction in postpartum women.

Methods: Total vitamin B12 and holoTC were measured in 171 women within 48 hours (T0) and at 5 weeks (T5) postpartum. Vitamin B12 deficiency was defined as total vitamin B12 < 180 pmol/L or holoTC <32 pmol/L. The active vitamin B12 fraction was defined as holoTC/total vitamin B12.

Results: Without intervention, vitamin B12 deficiency based on both serum total vitamin B12 and holoTC changed from 75% and 60%, to respectively 10% and 6% at T5. The fraction of active vitamin B12 was significant higher in vitamin B12 deficient women at both time points and across time ($P < .0001$ and $P = .002$). A high fraction of active vitamin B12 was only present in women with total vitamin B12 deficiency at T0. At T5, no high vitamin B12 fraction was found.

Conclusion: The changes in total vitamin B12 levels seem to be based on a physiological changes rather than vitamin B12 deficiency. The results of this study confirm the hypothesis that a shift toward the metabolic active vitamin B12 (holoTC) occurs in women with insufficient available total vitamin B12.

KEYWORDS
cobalamin, holotranscobalamin, postpartum, vitamin B12, vitamin B12 fraction

1 | INTRODUCTION

Serum vitamin B12 (cobalamin) concentrations show a significant decrease in each trimester during pregnancy to marginal or nonpregnant deficient values in healthy pregnant women.[1-4] Postpartum, vitamin B12 concentrations spontaneously recover to preconceptional values.[3,5,6] However, serum vitamin B12 measurement has a low positive predictive value, meaning that a low serum vitamin B12 level does not always indicate an actual vitamin B12 deficiency at tissue level.[7] Therefore, supplementation of vitamin B12 in the absence of manifestations of deficiency is a matter of debate in the current literature.[7,8]

Several studies conclude that the observed change of serum vitamin B12 during pregnancy and postpartum is physiologic.[2,3] Despite depressed vitamin B12 concentrations, homocysteine, and methylmalonic acid levels were not elevated as would be expected in vitamin B12 deficiency.[1-3,5,6,9] Also, a concomitant increase in erythrocyte cobalamin was observed.[2] Explanations for the cobalamin reduction are hemodilution, transportation to the fetus, and redistribution of cobalamin. However, serum vitamin B12 concentrations during pregnancy

decline more than can be accounted for by hemodilution.[9] Also, the total requirement of the fetus during pregnancy is estimated to be 50 μg, while maternal stores in women with mixed diet are estimated at >1000 μg.[10] Therefore, in well-nourished women, body stores of vitamin B12 are adequate to meet fetal needs during gestation.[11] The combination of a decrease in total serum vitamin B12, the increase in erythrocyte cobalamin, and a decrease in saturation of cobalamin-binding serum proteins suggests a redistribution of cobalamin.[2]

Only 20% of serum vitamin B12 is bound to transcobalamin (holoTC), which is the active part of vitamin B12 available for tissue uptake.[12] The remaining 80% is bound to haptocorrin (holohaptocorrin, holoHC) and has no known biological function.[6] Several studies showed that holoTC remains constant during pregnancy and postpartum,[3,5,6] while holoHC decreases during pregnancy.[2] Our hypothesis is that a shift occurs toward holoTC in women with insufficient available total vitamin B12. Therefore, the fraction of active vitamin B12 (holoTC/total vitamin B12) will be higher in women with vitamin B12 deficiency. Our objectives were to examine the prevalence of vitamin B12 deficiency based on total vitamin B12 and holoTC directly and at 5 weeks postpartum, and to compare the fraction of active vitamin B12 between vitamin B12 deficient and not deficient women during the first weeks postpartum.

2 | MATERIALS AND METHODS

2.1 | Population

The source population consisted of women who delivered in a large teaching hospital in Tilburg, in the southern part of the Netherlands. The study was approved by the local ethics committee. All women received oral and written information about the study and provided oral and written informed consent. Women were eligible for inclusion if they were ≥18 years old, thoroughly understood the Dutch language, and had indications for blood sampling within 48 hours after delivery, including estimated blood loss >500 mL, delivery by cesarean section, manual removal of the placenta, and clinical symptoms of anemia. Women were excluded if: their hemoglobin was <6.4 g/d (because the hospital protocol indicates the need for packed red cell transfusion); they were addicted to alcohol or drugs; they had hematological disease, they had chronic inflammatory disease; or they were being treated with methotrexate.

2.2 | Laboratory parameters

Maternal venous blood was collected within 48 hours after delivery (T0) in the hospital and at 5 weeks postpartum (T5) in the outpatient clinic. The women were not informed of their vitamin B12 levels. None of the women received vitamin B12 supplementation. If they used multivitamins, they were allowed to continue them.

Total vitamin B12 was measured on an Advia Centaur immunoassay system (Siemens Healthcare Diagnostics, Breda, the Netherlands). HoloTC was measured in each serum sample using an AxSYM immunoassay analyzer (Abbott Diagnostics, Hoofddorp, the Netherlands). All parameters were measured in control and analyzed in the normal diagnostic practice of the laboratory. For the vitamin B12 and holoTC, the respective between-run coefficients of variation were 3.1% (target value 180 pmol/L) and 4.3% (target value 30 pmol/L).

2.3 | Cutoff values for vitamin B12 and holoTC

Vitamin B12 deficiency was defined as total vitamin B12 < 180 pmol/L or holoTC <32 pmol/L.[13] The fraction of active vitamin B12 was defined as holoTC/total vitamin B12.[14]

2.4 | Statistical analyzes

Statistical analyzes were performed using IBM SPSS Statistics 20.0. Continuous variables at baseline were compared by independent sample *t* tests and nominal variables by Chi-square tests, except holoTC. The McNemar test was used to determine the distribution of holoTC deficient and not deficient women between the vitamin B12 deficient and not deficient women at baseline. Also, the McNemar test was used to determine the distribution of vitamin B12 and holoTC deficiency from T0 to T5. The independent sample *t* test was used to determine the difference in fraction of active vitamin B12 between the vitamin B12 deficient and not deficient women at T0 and T5. Analysis of variance for repeated measures used to compare the change of mean fraction of active vitamin B12 over time between vitamin B12-deficient and not deficient women at T0. All results were reported as the mean ± standard deviation (SD), or percentage (%), as appropriate.

3 | RESULTS

A total of 171 women were included in the study. The demographic and clinical characteristics of the participants at baseline are shown in Table 1.

Table 2 shows the significant differences in distribution of total vitamin B12 deficiency and holoTC deficiency from T0 to T5 ($P < .0001$). Vitamin B12 deficiency at T0 was prevalent in 75% (n = 129) of the women based on total vitamin B12 values, and in 60% (n = 102) of the women based on holoTC. At T5, vitamin B12 deficiency remained in 9.9% (n = 17) based on total vitamin B12 values, and in 5.8% (n = 10) based on holoTC. All women with normal vitamin B12 (n = 42, 24.6%) and holoTC values (n = 69, 40.4%) on T0, continued to have normal values on T5.

Total vitamin B12 deficient women had a significant higher fraction of active vitamin B12 compared with not deficient women at both T0 (Figure 1A, $P < .0001$, mean 0.25 ± SD 0.12, and mean 0.19 ± SD 0.07) and T5 (Figure 1B, $P < .0001$, mean 0.28 ± SD 0.08, and mean 0.21 ± SD 0.06). A high fraction of active vitamin B 12 (>0.40) was only present in women with total vitamin B12 deficiency at T0 (n = 13, Figure 1A). Of these, 85% (n = 11) had a normal holoTC level. At T5, no high vitamin B12 fraction was found (Figure 1B). Women with total vitamin B12 deficiency at T0 had a significant higher fraction of active vitamin B12 over time compared to women without vitamin B12 deficiency at T0 ($P = .002$).

TABLE 1 Patient demographic and clinical characteristics at baseline (T0)

	Vitamin B12 < 180 pmol/L (n = 129)	Vitamin B12 ≥ 180 pmol/L (n = 42)
Age at entry (years)	30.5 ± 4.6	31.3 ± 3.8
BMI before pregnancy (kg/m^2)	25.7 ± 5.8	24.8 ± 4.9
Twin pregnancies	2 (1.6)	1 (2.4)
Ethnicity		
Caucasian	117 (90.7)	39 (92.9)
Turkish	4 (3.1)	0 (0.0)
African	4 (3.1)	1 (2.4)
Asian	2 (1.6)	1 (2.4)
South American	2 (1.6)	1 (2.4)
Smoking	13 (10.1)	3 (7.1)
Diet		
Omnivore	78 (60.5)	27 (64.3)
Vegetarian	4 (3.1)	0 (0.0)
Unknown	47 (36.4)	15 (35.7)
Multivitamin use[a]	43/101 (42.6)	21/36 (58.3)
Parity at baseline	1.5 ± 0.7	1.3 ± 0.6
Gestational age at delivery (weeks)	39.9 ± 1.5	39.5 ± 1.5
Delivery method		
Vaginal	53 (41.4)	13 (31.0)
Caesarean section	76 (58.6)	29 (69.0)
Infant birthweight (gram)	3510.5 ± 553.5	3435.1 ± 493.2
Infant feeding		
Breastfeeding	78 (60.5)	26 (61.9)
Bottle (formula) feeding	51 (39.5)	16 (38.1)

Numbers are mean ± SD or number (percentage).
[a]Unknown multivitamin use, n = 34.

4 | DISCUSSION

The results of the present study show a spontaneous recovery of vitamin B12 deficiency based on both total vitamin B12 and holoTC from delivery to 5 weeks postpartum. The fraction of active vitamin B12 was significant higher in vitamin B12-deficient women at both time points and across time. A high fraction of active vitamin B12 was only present in women with total vitamin B12 deficiency at T0. At T5, no high vitamin B12 fraction was found. These results confirm the hypothesis that a shift toward the metabolic active vitamin B12 (holoTC) occurs, if there is insufficient total vitamin B12 available. Therefore, the observed changes of total vitamin B12 during pregnancy and postpartum should be considered physiological. Earlier studies confirm that conclusion, as homocysteine and methylmalonic acid levels were not elevated as would be expected in vitamin B12 deficiency, and a concomitant increase in erythrocyte cobalamin was observed.[1-3,5,6,9]

To our knowledge, this is the first study reporting the course of serum vitamin B12, holoTC, and fraction of active vitamin B12 in the first weeks postpartum. Based on serum total vitamin B12, the prevalence of vitamin B12 deficiency in the present study was higher within 48 hours postpartum compared with the third trimester of pregnancy in the current literature (75% vs 35%-43%).[2,3] Therefore, it seems that total vitamin B12 levels even decrease from the third trimester of pregnancy to immediately postpartum. The prevalence of vitamin B12 deficiency at 5 weeks postpartum in the present study is considerably higher compared with the prevalence as reported in a previous study on vitamin B12 deficiency at 8 weeks postpartum (10% vs 3%).[3] This could be explained by the difference in time of measurement postpartum. It is expected that the serum vitamin B12 can spontaneously improve from 5 to 8 weeks postpartum. The present study showed a significant increase of mean holoTC from T0 to T5, which does not correspond to earlier studies which found that holoTC remains constant during pregnancy and postpartum.[3,5,6] However, these studies measured holoTC at the third trimester of pregnancy and at 8 weeks postpartum, and we measured holoTC in the first postpartum weeks. An explanation could be that holoTC decreases from the third trimester of

TABLE 2 Distribution of vitamin B12 and holotranscobalamin (holoTC) deficiency within 48 h postpartum (T0) and at 5 wk postpartum (T5)

		Vitamin B12 T5		
		<180 pmol/L	≥180 pmol/L	Total
Vitamin B12 T0	<180 pmol/L	17 (9.9)	112 (65.5)	129 (75.4)
	≥180 pmol/L	0 (0.0)	42 (24.6)	42 (24.6)
	Total	17 (9.9)	154 (90.1)	171 (100.0)
		HoloTC T5		
		<32 pmol/L	≥32 pmol/L	Total
HoloTC T0	<32 pmol/L	10 (5.8)	92 (53.8)	102 (59.6)
	≥32 pmol/L	0 (0.0)	69 (40.4)	69 (40.4)
	Total	10 (5.8)	161 (94.2)	171 (100.0)

Numbers (percentage).
McNemar test: $P < .0001$.

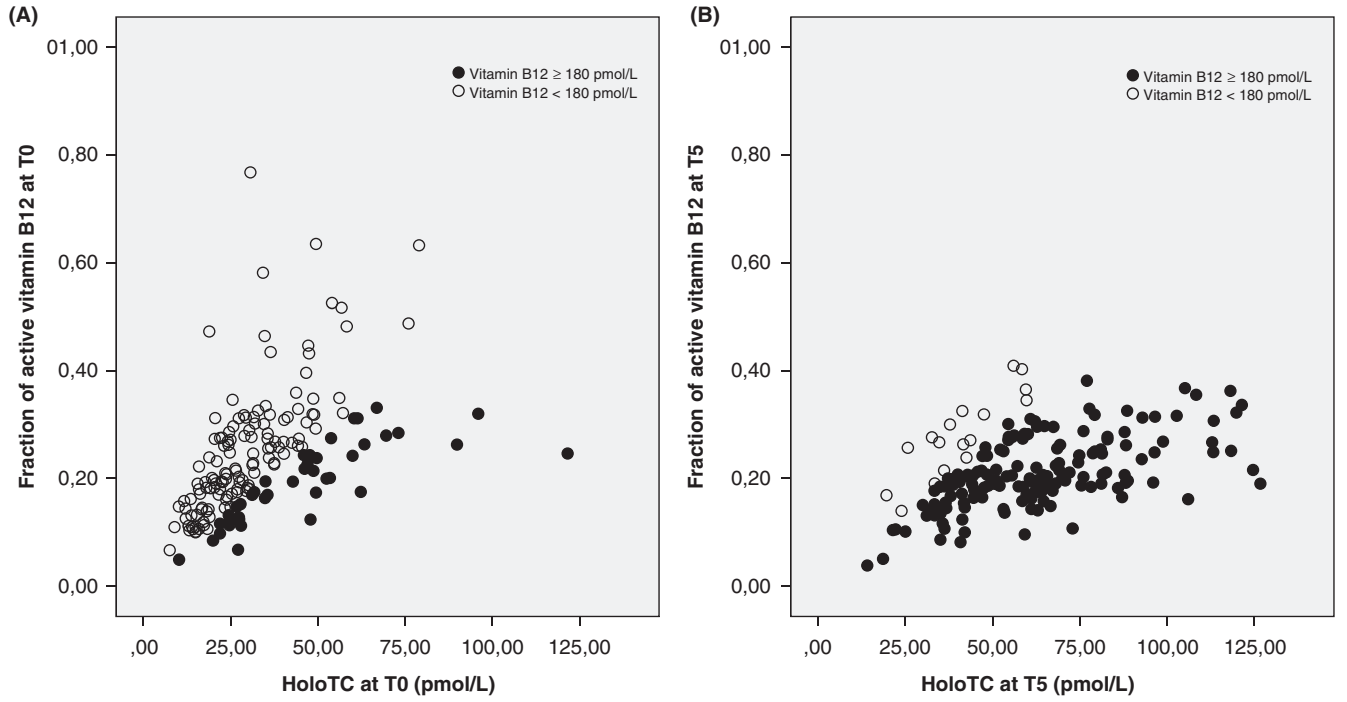

FIGURE 1 (A) Fraction of active vitamin B12 and holotranscobalamin (holoTC) between vitamin B12-deficient and not deficient women within 48 h postpartum (T0). (B) Fraction of active vitamin B12 and holotranscobalamin (holoTC) between vitamin B12-deficient and not deficient women at 5 wk postpartum (T5)

pregnancy to directly postpartum and then increases again to normal values at 8 weeks postpartum.

4.1 | Strengths and weaknesses

The present study is limited by selection bias. All included women had indication for blood sampling based on potential anemia. However, earlier studies found no association of hemoglobin level and vitamin B12 status during pregnancy and postpartum.[2,3] Homocysteine and methylmalonic acid levels were not measured to define vitamin B12 deficiency in the present study. However, earlier studies found no significant correlation between homocysteine or methylmalonic acid and vitamin B12 deficiency, both during pregnancy and postpartum.[3,5,6] Another limitation of the study is that in 36% of the included women the diet is unknown. Women who are vegetarian or vegan are at increased risk of vitamin B12 deficiency.[7] Yet, we believe that this has not affected our results. The 4 included vegetarian women in the present study were vitamin B12 deficient within 48 hours postpartum. Without the use of multivitamins, they all reached normal total vitamin B12 and holoTC values at 5 weeks postpartum (data not shown).

5 | CONCLUSION

The results of the present study confirm the hypothesis that vitamin B12 changes postpartum are physiological and that a shift toward the metabolic active vitamin B12 (holoTC) occurs in women with insufficient available total vitamin B12.

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DISCLOSURE

Assays for holoTC were kindly provided by Abbott, Hoofddorp, the Netherlands. However, they have no influence on the results. The analyzes were performed by others.

AUTHOR CONTRIBUTION

DW, EW, and JP designed the study. DW and EW performed the study. DW, EW, and JV analyzed the data. DW and EW wrote the manuscript.

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