Holotranscobalamin remains unchanged during pregnancy. Longitudinal changes of cobalamins and their binding proteins during pregnancy and postpartum

We confirm a decrease in cobalamins during pregnancy, and report that the active part of cobalamins (holotranscobalamin, holoTC) remains unchanged. The decrease in cobalamins is explained by a decreased holohaptocorrin (holoHC), suggesting that holoTC rather than cobalamins should be used as a marker of vitamin B12 deficiency during pregnancy.

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We studied the interrelations between cobalamins, TC and HC (holo and total) during pregnancy in a follow-up study of pregnant women previously described in detail.[1] The participants were healthy pregnant Danish Caucasian women >18 years of age, who had blood samples collected for preparation of serum and plasma at 18th, 32nd, 39th gestation week and 8 weeks postpartum. Plasma cobalamins[2] were measured on the Microparticle Enzyme Immunoassay technology (Abbott Diagnostics, IL, USA). ELISA assays were used to measure TC and HC (holo and total) in serum.[3, 4] HoloTC and holoHC were quantified by measuring the amount of TC (HC) not removed after treatment with vitamin B_{12}-covered beads.[5] Total CV was <10% for all analytes measured. Total homocysteine (tHcy) and methylmalonic acid (MMA) have been previously published.[1]

Statistical analysis using repeated measure ANOVA was performed using Stata 9.2 (StataCorp LP, Texas, USA). The distribution of holoTC, MMA, tHcy, total HC and holoHC were log transformed to obtain normal distribution.

In agreement with previous studies,[1, 6] we found an almost 50% decline in the concentration of cobalamins during late pregnancy (Table 1). A small increase in MMA was observed during pregnancy and post partum (Table 1) but we question whether this is caused by an increased metabolic rate during pregnancy and lactation rather than a sign of vitamin B12 deficiency. Only two women (holoTC: 33 - 43 pmol/L and 39 - 48 pmol/L) had a MMA above the upper limit of the reference intervals (>0.28 μmol/L) at all four time points during pregnancy and post partum. None of the women had a MMA >0.75 μmol/L, the diagnostic level for vitamin B12 deficiency,[7] at any time during pregnancy and post partum. These results suggest that the vitamin B12 status during pregnancy remains unchanged and that a sufficient amount of cobalamin was available for transportation into the cells despite the decreased level of total plasma cobalamins.

Interestingly, the decline in cobalamins during pregnancy was not reflected in holoTC (Table 1). The concentration of holoTC observed during pregnancy was comparable to the concentrations seen for healthy non-pregnant women[5] (Table 1), and none of the women had holoTC concentrations below the lower limit of the reference interval for non-pregnant women at any time point during pregnancy. These observations have two implications. Firstly, healthy pregnant Danish women do not seem to suffer from an inadequate supply of vitamin B12. Secondly, it suggests that holoTC, in contrast to cobalamins, can be used as a marker for vitamin B12 deficiency during pregnancy.

The decrease in cobalamins observed during pregnan-

Table 1. Median (range) of transcobalamin (holo and total TC), haptocorrin (holo and total HC), cobalamins, calculated holoHC bound analogues (anal), methylmalonic acid (MMA) and total homocysteine (tHcy) during pregnancy (18th, 32nd and 39th gestation week (gw)) and 8 weeks postpartum (8pp), n=141. Reference intervals are included (where appropriate reference intervals for younger females are given). Repeated measures ANOVA is used for statistical analysis of changes during pregnancy (18th, 32nd and 39th gw).

	95% reference interval	*18th gw*	*32nd gw*	*39th gw*	*8 pp*	*p-value*
Total TC, pmol/L	610-1400	850 (440-1520)	930 (540-1710)	950 (480-1680)	810 (460- 1690)	$p<0.0001$
Total HC, pmol/L	250-760	510 (240-1110)	470 (170-1280)	450 (190-1190)	450 (200-1410)	$p<0.0001$
Cobalamins, pmol/La	200-600	230 (60-620)	170 (50-360)	170 (60-680)	310 (110-670)	$p<0.0001$
HoloTC, pmol/L	40-150	79 (35-260)	76 (35-190)	79 (30-260)	93 (19-460)	n.s.
HoloHC, pmol/L	220-590 (140-500)	310 (120-520)	270 (130-420)	260 (180-940)	380	$p<0.0001$
Calculated holoHC bound analc, pmol/L	–	150 (-160-380)	170 (-130-440)	170 (-40-400)	150 (-90-710)	$p<0.0001$
MMA,μmol/L	0.08-0.28	0.11 (0.04-0.42)	0.14 (0.04-0.66)	0.14 (0.04-0.62)	0.16 (0.06-0.57)	$p<0.0001$
tHcy, μmol/L	4.5-8.1	6.6 (2.0-21)	7.0 (2.9-13)	7.7 (3.6-20)	11 (4.8-48)	$p<0.0001$

[a]140 women had cobalamins measured at 32nd, 39th gestation week and 8 pp. [b]Calculated as holoHC + holoTC – cobalamins. This calculation assumes equimolar measurement of holoHC, holoTC and cobalamins.

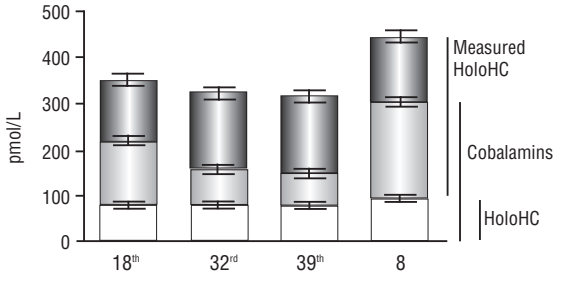

Figure 1. Comparison of holoTC, cobalamins, and holoHC in pregnant women at 18th, 32nd, 39th gestational week (gw) and 8 weeks post partum (8 pp) (n=141). Means and 95% CI (calculated from the normal distrubuted log transformed data) for holoTC (white), cobalamins (white + grey shaded) and measured holoHC (grey + black shaded) are shown. The grey shaded area indicates holoHC saturated with true cobalamins (cobalamins - holoTC) and the black shaded area indicates holoHC saturated with analogues (measured holoHC + holoTC - cobalamins).

cy was explained by a decrease in HC saturated with true cobalamins (total plasma cobalamins minus holoTC, calculated holoHC) (Figure 1). The difference between the measured and the calculated concentration of holoHC increased during pregnancy and decreased post partum. Our results showed that measured holoHC was approximately twice as high as calculated holoHC. This supports the presence of analogues on HC[8] and suggests that the amount of analogues bound to HC increased during pregnancy. At present, we have no explanation to offer for this observation.

The samples used in the present study were stored for more than 12 years prior to analysis. We do not believe that this influenced the results obtained since they were well in line with concentrations obtained at birth[9] and 3 weeks post partum[10] in samples analyzed a maximum of 2 years after collection.

In conclusion, we report that the decline in cobalamins during pregnancy is caused by alteration in cobalamins attached to HC rather than in alterations in holoTC. Our data suggests that, holoTC, rather than cobalamins can be used as a marker for vitamin B12 deficiency during pregnancy.

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References

1. Milman N, Bergholt T, Eriksen L, Byg KE, Graudal N, Pedersen P, Hertz J. Iron prophylaxis during pregnancy -- how much iron is needed? A randomized dose-response study of 20-80 mg ferrous iron daily in pregnant women. Acta Obstet Gynecol Scand 2005;84:238-47.
2. Milman N, Byg KE, Bergholt T, Eriksen L, Hvas AM. Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. Eur J Haematol 2006;76:521-5.
3. Nexø E, Christensen A-L, Petersen TE. Measurement of transcobalamin by ELISA. Clin Chem 2000;46:1643-9.
4. Mørkbak AL, Pedersen JF, Nexø E. Glycosylation independent measurement of the cobalamin binding protein haptocorrin. Clin Chim Acta 2005;356:184-90.
5. Nexø E, Christensen AL, Hvas AM, Petersen TE, Fedosov SN. Quantification of holo-transcobalamin, a marker of vitamin B12 deficiency. Clin Chem 2002:48;561-2.
6. Koebnick C, Heins UA, Dagnelie PC, Wickramasinghe SN, Ratnayaka ID, Hothorn T, et al. Longitudinal concentrations of vitamin B12 and vitamin B12-binding proteins during uncomplicated pregnancy. Clin Chem 2002;48:928-33.
7. Hvas Am, Nexo E. Holotranscobalamin-a first choice assay for diagnosing early vitamin B deficiency? J Intern Med 2005;257:289-98.
8. Kolhouse JF, Kondo H, Allen NC, Podell E, Allen RH. Cobalamin analogues are present in human plasma and can mask cobalamin deficiency because current radioisotope dilution assays are not specific for true cobalamin. N Engl J Med 1978;299:785-92.
9. Obeid R, Morkbak AL, Munz W, Nexo E, Herrmann W. The cobalamin-binding proteins transcobalamin and haptocorrin in maternal and cord blood sera at birth. Clin Chem 2006;52:263-9.
10. Morkbak AL, Ramlau-Hansen CH, Moller UK, Henriksen TB, Moller J, Nexo E. A longitudinal study of serum cobalamins and its binding proteins in lactating women. Eur J Clin Nutr 2007;61:184-9.