The Cobalamin-Binding Proteins Transcobalamin and Haptocorrin in Maternal and Cord Blood Sera at Birth

Rima Obeid,1 Anne L. Morkbak,2 Winfried Munz,3 Ebba Nexo,2 and Wolfgang Herrmann1*

Background: Two proteins carry vitamin B12 in plasma. Transcobalamin (TC) carries ~25% of total plasma vitamin B12 and is 6% to 20% saturated with cobalamin. Haptocorrin (HC) binds ~80% of total cobalamin and is largely saturated with cobalamin.

Methods: We investigated the distribution and the relationship between concentrations of cobalamin, total and holo forms of TC, and HC in blood samples from pregnant women just before delivery (n = 92) and in cord blood samples from their newborn babies. We also investigated the relationship between these proteins and concentrations of methylmalonic acid (MMA), the functional marker of vitamin B12 status.

Results: Concentrations of total serum cobalamin, total HC, holoHC, and percentage of HC saturation were higher in cord blood than in the maternal blood (mean cobalamin, 268 vs 188 pmol/L; total HC, 648 vs 538 pmol/L; holoHC, 441 vs 237 pmol/L; HC saturation, 70% vs 47%). Moreover, total TC was low in cord blood, whereas both holoTC and TC saturation were higher in cord blood than in the maternal blood (mean total TC, 654 vs 1002 pmol/L; holoTC, 118 vs 53 pmol/L; TC saturation, 19.8% vs 5.4%). Higher maternal serum cobalamin was associated with higher cord blood holoTC and TC saturation (P <0.05). Gestational age was also a significant determinant of baby total TC, TC saturation, total HC, and holoHC.

Conclusion: The close correlation between the amounts of holoTC present in cord blood and in maternal serum supports the importance of maternal cobalamin status for ensuring a sufficient supply to the baby.

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Vitamin B12 (cobalamin) circulates in plasma bound to 2 carrier proteins, transcobalamin (TC)4 and haptocorrin (HC). TC is a 43-kDa nonglycoprotein that transfers B12 from the intestine into the blood stream and then into all the cells of the body (1, 2). Cobalamin-saturated transcobalamin, also called holotranscobalamin (holoTC), constitutes between 6% and 20% of total plasma vitamin B12. The unsaturated TC is called apotranscobalamin (apoTC), and this portion constitutes the major part of TC (~90%) (3, 4). Transcobalamin is synthesized by the enterocytes; however, several other organs can synthesize this protein (5–7).

HC, also called “R-binder”, constitutes a group of immunologic cross-reacting proteins (TCI and TCIII). HC is synthesized in many cells of the body, including leukocytes, and it binds between 80% and 94% of total cobalamin in plasma (6). Unlike TC, HC is a glycoprotein and is largely saturated with cobalamin (6). No functions are known for HC other than its ability to bind metabolically inert forms of vitamin B12. Congenital HC deficiency is not associated with apparent symptoms or metabolic disorders other than a marked and benign decrease in total serum cobalamin (8, 9). This is in sharp contrast to patients with congenital TC deficiency, who usually suffer from severe neurologic manifestations in addition to marked increases in homocysteine (Hcy) and
methylamnionic acid (MMA) concentrations in serum and urine (10–12).

The transport and distribution of cobalamin via biological membranes take place at several major sites in humans. First, cobalamin is transferred from intrinsic factor to TC in the enterocytes and then enters the blood stream bound to this carrier protein. Second, vitamin B\textsubscript{12} bound to TC is reabsorbed in the proximal tubule and enters the blood stream most likely bound to newly synthesized TC (13). Third, a particularly interesting site of vitamin B\textsubscript{12} exchange in the human is the placenta. The placenta produces TC. Moreover, human placenta produces large amounts of TC receptor (14), which mediates the uptake of cobalamin from the mother to the baby (15).

Assessments of total TC and HC, as well as the cobalamin-saturated part of the proteins, holoTC, and holoHC, have been a challenge for many years. Specific isolation of each of the proteins is crucial for the assessment of the proteins themselves or their cobalamin moiety. Previous studies investigated concentrations of the unsaturated proteins by measuring their capacity to bind radiolabeled vitamin B\textsubscript{12} (16). This was followed by indirect calculations of the holo- or the total form of the proteins. More recently, specific and reproducible assays have become available for quantification of holoTC (RIA) (17), total TC (ELISA) (18), and HC and holoHC (ELISA) (19). We studied the distribution of the cobalamin-binding proteins (TC and HC) in maternal blood and in placental vein blood from their corresponding newborns. In addition, we investigated the relationship between cobalamin binders and metabolic markers of cobalamin in maternal and cord blood.

Materials and Methods

Participants and Methods

The study included 92 pregnant women (mean age, 29.8 years) who were randomly recruited among consecutive deliveries at the Department of Obstetrics and Gynecology at the University Hospital of Saarland during the period of April 2004 to September 2004. All women were older than 17 years of age and free of chronic diseases. Mothers expecting babies with any kind of congenital malformations were not eligible for the study. Complicated pregnancies, premature birth, or intrauterine growth retardation (IUGR) were not exclusion criteria in this study. Both vaginal and cesarean births were included.

Maternal anthropologic measures and data on weight increase, smoking, and vitamin usage during pregnancy; parity; and gravidity were obtained. Seventeen women received folate, but none of the participants reported taking vitamin B\textsubscript{12} supplements during pregnancy. The gestational age was defined based on the last menstrual date and ultrasound examination. Clinical parameters of the newborns were also documented (weight, length, head circumference, blood gases, and venous and arterial blood pH).

The study was approved by the local Ethics Committee at the University Hospital of Saarland, and all participants gave their informed consent to the study.

Blood Sampling and Biochemical Measurements

Nonfasting peripheral venous blood samples were obtained from the mothers 1 to 12 h before birth. A blood sample was collected from the umbilical vein immediately after expulsion of the placenta. Maternal and cord blood samples were collected in tubes without anticoagulant, left to clot for ≤45 min, and centrifuged at 2000g and +4 °C. Serum was directly separated and stored at −70 °C until analysis. All biochemical analyses were performed within 6 months of blood collection.

Concentrations of tHcy and MMA were measured by gas chromatography–mass spectrometry as described elsewhere (20). The imprecision (as CVs) for the tHcy and MMA assays were 5% and 6%, respectively. The concentrations of cobalamin were determined by a chemiluminescence immunoassay (ADVIA Centaur System), and the CV for this assay was <5%. The concentration of holoTC was measured by RIA (Axis-Shield) (17). The CVs for the holoTC assay were 6% and 8% at 37 and 95 pmol/L, respectively. We measured total TC, total HC, and holoHC at the Department of Clinical Biochemistry. We used ELISA to quantify total TC, with a calibration curve constructed with recombinant TC. The TC assay was based on an immobilized antibody that captures the TC and a biotinylated detection antibody, which reacts with horseradish peroxidase-avidin, producing a color that is proportional to the concentration of TC (4). The CV for total TC was 4% to 6%, and the reference interval for females (n = 40) was 560–1400 pmol/L. Total HC was also measured by ELISA based on an immobilized anti-HC and a secondary antibody for detection. Before ELISA, HC was deglycosylated to ensure equimolar measurement (19). HoloHC was quantified in the same way after removal of unsaturated HC by treatment of samples with vitamin B\textsubscript{12}-covered beads (19). The CVs were 5% for the total and 10% for the holoHC. Reference intervals for females (n = 71) for total HC and holoHC were 250–760 pmol/L and 220–590 pmol/L, respectively. TC and HC saturation were calculated: TC saturation = holoTC/total TC; and HC saturation = holoHC/total HC. The reference interval for females for HC saturation was 0.60–1.00 (unpublished data).

Statistical Analysis

We performed data analyses using SPSS (Ver. 13). All variables were skewed and were therefore log-transformed to approach gaussian distribution before use of tests that would propose such a distribution of the data. Data are presented as the mean (range). We investigated possible differences in means of variables between maternal and cord sera, using the paired Student t-test. Intragroups multiple comparisons were performed with one-way ANOVA. The post hoc Tamhane test was performed.
Babies (n/H11005/100 (7.6%) had IUGR (weight before 37 weeks of gestation (preterm), and 7 babies appropriate birth weights. Nine babies (9.8%) were born
ty-six women (82.6%) gave birth to term babies with
their corresponding babies included in this study. Seven-
Table 1 shows the main characteristics of mothers and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mother</th>
<th>Between-subject CV,%</th>
<th>Baby</th>
<th>Between-subject CV,%</th>
<th>Mean baby/mother ratio</th>
<th>P (t-test) after log-transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy, μmol/L</td>
<td>5.7 (2.6–10.5)</td>
<td>28</td>
<td>5.5 (2.3–10.2)</td>
<td>31</td>
<td>1.0</td>
<td>0.242</td>
</tr>
<tr>
<td>MMA, μmol/L</td>
<td>240 (71–563)</td>
<td>38</td>
<td>370 (138–848)</td>
<td>35</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cobalamin, pmol/L</td>
<td>188 (81–770)</td>
<td>46</td>
<td>268 (88–1018)</td>
<td>50</td>
<td>1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total TC, pmol/L</td>
<td>1002 (544–1584)</td>
<td>22</td>
<td>654 (190–1350)</td>
<td>30</td>
<td>0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HoloTC, pmol/L</td>
<td>53 (24–180)</td>
<td>55</td>
<td>118 (34–483)</td>
<td>61</td>
<td>2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC saturation, fraction</td>
<td>0.054 (0.02–0.15)</td>
<td>49</td>
<td>0.20 (0.45–1.00)</td>
<td>80</td>
<td>3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total HC, pmol/L</td>
<td>538 (228–1236)</td>
<td>33</td>
<td>648 (250–1403)</td>
<td>36</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HoloHC, pmol/L</td>
<td>237 (119–391)</td>
<td>24</td>
<td>441 (205–818)</td>
<td>29</td>
<td>1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HC saturation, fraction</td>
<td>0.47 (0.22–0.83)</td>
<td>29</td>
<td>0.70 (0.43–0.93)</td>
<td>16</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Data are mean (range).
* Between-subject CV (%) = 100 × (SD/mean).

to identify the significantly different group means when the ANOVA test was significant. Correlations between different variables were examined by the Pearson test applied on the log-transformed data. All tests were 2-sided, and P values <0.05 were considered statistically significant.

Results
Table 1 shows the main characteristics of mothers and their corresponding babies included in this study. Seventy-six women (82.6%) gave birth to term babies with appropriate birth weights. Nine babies (9.8%) were born before 37 weeks of gestation (preterm), and 7 babies (7.6%) had IUGR (weight <10th percentile compared with age- and sex-matched babies in the general population, according to the German standards). Eight women had complications during pregnancy, and 4 of them had poor birth outcome (1 premature and 3 IUGR babies).

Table 2 summarizes concentrations of cobalamin and its binding proteins, TC and HC, in addition to tHcy and MMA in the maternal and cord blood samples. The mean concentration of MMA was higher in cord blood compared with that in the mother, whereas the mean concentration of tHcy did not differ significantly between maternal and cord blood samples (Table 2). Serum concentrations of cobalamin were significantly higher in cord blood compared with maternal blood (mean, 268 vs 188 pmol/L; P <0.001). HoloTC concentrations were significantly higher in cord blood compared with the corresponding maternal blood (mean, 118 vs 53 pmol/L). In contrast, the concentrations of total TC were significantly lower in cord blood compared with the maternal blood (mean, 654 vs 1002 pmol/L). The concentrations of total HC were higher in cord blood than in the maternal blood (mean, 648 vs 538 pmol/L), and so was holoHC (mean, 441 vs 237 pmol/L; Table 2). Furthermore, both TC and HC saturation were higher in cord blood than in maternal blood (mean TC saturation, 0.19 vs 0.05; mean HC saturation, 0.7 vs 0.47, respectively). Mean cord blood/maternal ratio of TC saturation and that of HC saturation were 3.6 and 1.6, respectively. TC saturation in cord blood samples showed very wide between-subject variation (80%; Table 2). Significant negative correlations were found between cord blood TC saturation and gestational age (r = −0.405; P <0.001) on one hand, and birth weight (r = −0.438; P <0.001) on the other hand. Multiple regression analysis revealed that 75% (adjusted R²) of the variations in TC saturation between cord blood samples were predicted by variations in cord blood cobalamin (B = 1.264), cord blood holoHC (B = −0.765), cord blood tHcy (B = −0.245), and gestational age (B = −0.031). Only 2 babies showed a TC saturation of ~1.0; interestingly, both were UUGR babies. The lowest total TC values (<400 pmol/L) were found in 6 babies (3 IUGR, 1 preterm, and 2 term).

Fig. 1 shows concentrations of cobalamin-binding pro-
teins and their saturation according to gestational age. Increased gestational age was associated with higher cord blood total TC, and this led to lower TC saturation because holoTC did not differ with increased gestational age. By contrast, cord blood total HC and holoHC increased, and HC saturation remained unchanged with advanced gestational age (Fig. 1). Maternal blood showed a slightly lower holoTC and a significantly lower TC saturation with advanced gestation. Gestational age ranged in our study from 27 to 42 weeks, with 76 women (83%) delivering at or after 37 weeks of gestation. Results similar to those described above were found when only term deliveries were considered (n = 76; data not shown).

Fig. 2 illustrates the relationship between concentrations of maternal serum cobalamin, TC, and HC and the cobalamin saturation of the 2 proteins. Concentrations of holoTC were higher in cord blood when the mothers had higher total cobalamins. In addition, higher cord blood TC saturation was related to higher maternal cobalamin (Fig. 2). Neither total TC nor total HC was related to maternal cobalamin (Fig. 2). Fig. 3 shows the relationship between cord blood and maternal serum concentrations of holoTC.

Table 3 shows correlations between concentrations of cobalamin, its binding proteins, and those of the metabolic markers in the maternal and cord blood samples from the corresponding babies. A strong positive correlation was observed between maternal and cord blood concentrations of holoTC ($r = 0.68; P < 0.001$; Fig. 3). There was a weak but significant inverse correlation between parity and baby holoTC (Pearson correlation $r = -0.25; P = 0.021$). Mothers who were nulliparous or monoparous (n = 51) had slightly higher holoTC compared with mothers with 2 or more previous babies (n = 41; mean, 52 vs 44 pmol/L; $P = 0.058$). Moreover, babies from multiparous mothers (≥2 pregnancies) had slightly lower holoTC compared with babies from nulliparous and monoparous women (mean, 91 vs 113 pmol/L; $P = 0.073$). No differences in any other variable were found with increased parity (data not shown).

**Discussion**

We studied the relationship between maternal and cord blood concentrations of cobalamin, its binding proteins, and its metabolic markers. The new findings in this study are that total TC is lower, whereas total HC and both holoTC and holoHC are higher in cord blood at birth compared with the mother’s blood. In addition to gestational age, maternal concentration of cobalamin was a major predictor of the concentrations of holoTC and TC saturation in cord blood. Maternal serum cobalamin was...
not a significant predictor of total TC, holoHC, total HC, and HC saturation in cord blood.

Cobalamin and its binding proteins have previously been evaluated in a longitudinal study on healthy German women during pregnancy (16). Serum concentrations of cobalamin were found to decrease during pregnancy (16). This was associated with a slight, but significant, decrease of TC saturation (16). In line with this report, we found a decrease in maternal TC saturation with increasing gestational age (Fig. 1). Gestational age was also a determinant for cord blood holoHC, total HC, total TC, and TC saturation (Fig. 1). This suggests that rapid changes in TC and HC concentrations occur even after 37 weeks of gestation because the major part of our population delivered after 37 weeks. Protein synthesis increases in general in the fetus at a later gestational age, and this may explain the increased total TC and total HC in babies born at later gestational age compared with those born at earlier ages of gestation (Fig. 1). Moreover, concentrations of holoTC did not differ, whereas those of holoHC were higher in babies born at later gestational age, and both remained higher than concentrations detected in the mothers. Lower maternal TC saturation at late gestation was caused by a slight increase in maternal total TC combined with a decrease in holoTC (Fig. 1).
Lower maternal TC saturation could be related to accelerated transport of cobalamin (as holoTC) into the baby, which may imply increased synthesis of TC in the “depleted” mothers.

As in the current study, higher serum concentrations of cobalamin have previously been reported in babies at birth compared with maternal concentrations (21, 22). The placenta accumulates numerous micronutrients, including cobalamin (23). Higher concentrations of the vitamin have been detected in placenta tissues compared with the circulating concentrations in the fetuses and the mothers (23). Additionally, we know that vitamin B₁₂ injections to pregnant animals produce a rapid accumulation of the vitamin in the placenta and a dose-dependent transfer of the vitamin to fetal tissues (24). Fetal tissues accumulate more cobalamin than do the maternal tissues (25). Therefore, cobalamin is thought to be actively transported from the mother into the fetus.

Higher cord blood concentrations of holoTC and a high TC saturation were associated with higher maternal concentrations of cobalamin (Fig. 2). This implies that maternal cobalamin is a strong predictor of holoTC and TC saturation in cord blood. Conversely, cord blood concentrations of total HC and holoHC as well as HC saturation did not significantly relate to maternal cobalamin concentrations (Fig. 2 and Table 3). These results suggest that neither HC nor its holo form is transferred from the mother into the baby. Moreover, there could be a redistribution of cobalamin between TC and HC in the baby before birth. Accordingly, cobalamin probably becomes more bound to HC rather than to TC in cord blood samples from babies at advanced gestation.

Unlike total HC, total TC was lower in the cord blood compared with the maternal blood (Table 2 and Fig. 2). This was associated with a higher cobalamin saturation of TC and HC in the cord blood compared with the maternal blood (mean TC saturation, 0.19 vs 0.05; mean HC saturation, 0.70 vs 0.47; Table 2). These findings are very important because they display some similarities to the case of individuals receiving vitamin B₁₂ supplementation (26, 27). A slight, dose-dependent decrease (5% to 16%) in serum concentrations of total TC has been reported in individuals receiving vitamin B₁₂ (26, 27). On the other hand, TC saturation increases in vitamin-treated individuals and decreases in cases of vitamin depletion (26, 27). Patients treated with 0.4 mg of vitamin B₁₂ per day showed a substantial increase in TC saturation (from 0.14 to 0.28), but TC saturation did not exceed 0.60 in any individual in this study. Mean TC saturation in our mothers was lower than that reported elsewhere (26), and this seems consistent with a reduction of TC saturation known to take place during pregnancy (16). Moreover, the TC saturation in our cord blood was high even compared with vitamin-treated individuals from previous studies (26).

High concentrations of cobalamin and holoTC in cord blood were not consistent with the concentrations of tHcy or MMA, which are considered to be functional markers for vitamin B₁₂ status in adults (Table 2). We found that fetal concentrations of MMA were higher than those of the mothers (Table 2) but unrelated to the concentrations of cobalamin attached to each of the 2 binders (holoTC and holoHC) as well as the saturation of these proteins (Table 3). Therefore, other factors, such as a lower rate of elimination or increased production of MMA, may be involved in increased concentrations of this compound in cord blood. An analytical error may occur when serum samples are used for measuring concentrations of tHcy. However, there is no evidence that tHcy may increase in samples left at room temperature for 20–45 min, especially at the low concentrations of tHcy used in our study. Moreover, our results seem comparable to (28) or even lower than those reported by similar studies (22). This may not support an artificial increase in concentrations of tHcy in our study.

In general, the concentration of holoHC correlated significantly to that of total serum cobalamin. However, the holoHC concentration was higher than that of total cobalamin (Table 2). The same was found in another, different group of blood samples (19). The explanation for these findings could be that we measured holoHC fraction based on antibodies against the HC protein. Therefore, the holoHC fraction may be higher because cobalamin analogs can bind holoHC. In the assay for total cobalamin, we used intrinsic factor capable of binding only cobalamin.

A possible physiologic role for HC, especially during pregnancy, has not been clarified. Unlike TC, HC does not

### Table 3. Correlations between different maternal and cord blood markers.∗

<table>
<thead>
<tr>
<th>Cord blood</th>
<th>MMA</th>
<th>Vitamin B₁₂</th>
<th>Total TC</th>
<th>HoloTC</th>
<th>Total HC</th>
<th>HoloHC</th>
<th>Cord blood</th>
<th>MMA</th>
<th>Vitamin B₁₂</th>
<th>Total TC</th>
<th>HoloTC</th>
<th>Total HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HoloHC</td>
<td>0.21</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
<td>0.34</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total HC</td>
<td>0.64</td>
<td>0.68</td>
<td>0.22</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td>0.54</td>
<td>-0.132</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total TC</td>
<td>0.24</td>
<td>0.23</td>
<td>0.54</td>
<td>-0.27</td>
<td>0.43</td>
<td></td>
<td></td>
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<tr>
<td>Total vitamin B₁₂</td>
<td>0.65</td>
<td>0.27</td>
<td>0.29</td>
<td>-0.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MMA</td>
<td>0.57</td>
<td>-0.27</td>
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</tbody>
</table>

∗Correlations were assessed by Pearson test. Only significant correlations are shown (P <0.05).
facilitate the transfer of cobalamin into the fetus (29). Inconsistent results about changes of HC during pregnancy have been reported (16,30). These contradicting results could be related to analytical variations in estimating this binder. A sharp decrease in HC saturation from the first to the third trimester of pregnancy has been reported (16). We also found that HC saturation was markedly lower than the reference interval of 0.60–1.00 (median, 0.87; Table 2). The low HC saturation is explained by a combination of an increase in total HC and a decrease in holoHC in pregnant women (i.e., median holoHC, 240 pmol/L; median total HC, 540 pmol/L; Table 2) compared with nonpregnant women (median holoHC, 340 pmol/L; median total HC, 410 pmol/L) (19).

In conclusion, our results indicate a different pattern of cobalamin distribution in newborn babies compared with the distribution in their corresponding mothers. Compared with maternal blood, cord blood had higher concentrations of circulating cobalamin and higher concentrations of the holo-forms of cobalamin binders and higher binder saturation. The distribution of cobalamin binders in the babies was related to maternal vitamin B12 status and to the age of gestation, but that distribution remained significantly different from values found in the mothers.

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References