OBSTETRICS

Maternal homocysteine and related B vitamins as risk factors for low birthweight

Marije Hogeveen, MD; Henk J. Blom, PhD; Elisabeth H. van der Heijden, Ben A. Semmekrot, PhD; Jan M. Sporken, MD; Per M. Ueland, PhD; Martin den Heijer, PhD

OBJECTIVE: We designed a large prospective study to explore the relationship between maternal homocysteine concentrations and related B vitamins and birthweight.

STUDY DESIGN: Blood was sampled from pregnant women at 30-34 weeks of gestation and their newborn infants (n = 366).

RESULTS: Concentrations of all analytes were higher in umbilical cord compared with maternal samples. Birthweight was related negatively to maternal homocysteine (r = −0.12) but not related to maternal cobalamin, methylmalonic acid, and folate (r = 0.02, r = 0.06, and r = 0.04, respectively). Regression analysis revealed smoking (β = –313; 95% confidence interval [CI], −479 to −149), gestational age (β = 150; 95% CI, 118–182), female sex (β = –146; 95% CI, –256 to –35), and parity (β = 104; 95% CI, 37–171) as strong determinants of birthweight. Maternal homocysteine, cobalamin, methylmalonic acid, and folate were not determinants of birthweight in multivariate analysis.

CONCLUSION: Maternal homocysteine and B vitamins are not related to birthweight in a multivariate model that was adjusted for potential confounders.

Key words: birthweight, cobalamin, folate, homocysteine, methylmalonic acid

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Homocysteine and methionine and related B vitamins like cobalamin and folate have been shown to play a critical role in fetal nutrition, growth, and development.[1-5] Several authors have described relationships among total homocysteine (tHcy), B vitamins, and pregnancy complications or adverse pregnancy outcomes. Elevated tHcy and low B vitamin concentrations are associated with increased risk of neural tube defects and pregnancy complications, such as preeclampsia, recurrent early pregnancy loss, and abruptio placentae.[6-9]

Revealing possible and treatable factors for low birthweight (LBW) is of the utmost importance because LBW increases the risk of morbidity and death in infancy.[10] Associations between LBW and chronic disease in adulthood, such as hypertension, coronary heart disease, stroke, or diabetes mellitus, have been described.[11-13] Several studies demonstrated an association between high maternal tHcy concentrations and risk of having LBW offspring[8,14-19] and negative correlations between maternal homocysteine and birthweight.[3,15-17] In contrast, others have found either no differences or even a positive association between maternal tHcy and birthweight.[20-23] However, some of these studies are relatively small.[15-18] Others use birthweight <2500 g as a cut-off point instead of birthweight related to or corrected for gestational age.[8,20] Also, the different timing of maternal blood sampling might contribute to inconsistent results.[8,14,16,22,23]

Several studies determined cobalamin and/or folate concentrations together with tHcy concentrations in relation to birthweight.[3,14,15,19-21,24] However, none of these studies determined methylmalonic acid (MMA) concentration, which is a sensitive marker of intracellular cobalamin, deficiency.

We collected plasma samples from pregnant women and their offspring and prospectively explored whether maternal tHcy, folate, cobalamin, and MMA are risk factors for LBW.

Subjects and Methods

Participants

In the Netherlands, pregnant women visit either a midwife or a gynecologist, depending on medical and obstetric history or complications during pregnancy. Delivery can take place at home or in the hospital supported by the midwife (uncomplicated pregnancies and deliveries) or in the hospital supported by a gynecologist. In the Netherlands, approximately 70% of pregnant women deliver in the hospital.

From the Departments of Pediatrics (Dr Hogeveen), Epidemiology and Biostatistics (Ms van der Heijden and Dr den Heijer), and Endocrinology (Dr den Heijer), Radboud University Nijmegen Medical Center; the Department of Gynecology and Obstetrics, Canisius Wilhelmina Hospital (Dr Sporken); and the Department of Pediatrics, Canisius Wilhelmina Hospital (Dr Semmekrot), Nijmegen, and the Metabolic Unit, Department of Clinical Chemistry, VU University Medical Center, Amsterdam (Dr Blom), the Netherlands; and the Section for Pharmacology, Institute of Medicine, University of Bergen, and Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway (Dr Ueland).

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TABLE 1
Population characteristics of 366 maternal and umbilical samples

Characteristic	Mother	Child
Age at delivery, y[a]	33.3 (30.7–35.5)	—
Gestational age, wk[a]	—	39.7 (38.4–40.7)
Birthweight, g[a]	—	3425 (3075–3855)
Total homocysteine, μmol/L[a]	5.5 (4.5–6.7)	5.8 (4.6–7.2)[b]
Methylmalonic acid, μmol/L[a]	0.16 (0.13–0.22)	0.28 (0.24–0.37)[b]
Folate, nmol/L[a]	9.1 (6.1–16.4)	30 (21.7–45.9)[b]
Cobalamin, pmol/L[a]	179 (134–219)	208 (156–307)[b]
Creatinine, μmol/L[a]	45 (40–50)	54 (48–61)[b]
Primiparity, n/N	172/358 (48%)	
Smoking, n/N	50/350 (14%)	
Use of folic acid, n/N	198/347 (57%)	
Male sex, n/N	—	159/363 (44%)

[a] Data are presented as median and interquartile range; [b] Probability value < .01, comparing maternal and umbilical data with the use of the 2-sample Wilcoxon rank-sum test.

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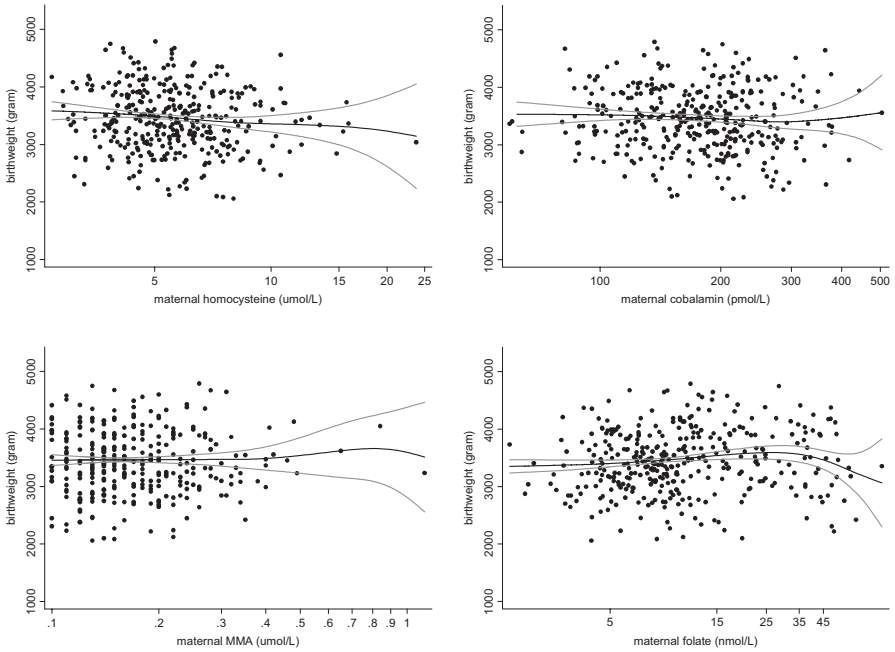
FIGURE
Curves represent the association of maternal concentrations of total homocysteine, MMA, cobalamin, and folate



Solid lines represent mean values; *thin lines* represent 95% CI. Curves were fitted by Gaussian generalized additive regression models.

CI, confidence interval; *MMA*, methylmalonic acid.

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Patients were included in our study when they visited the outpatient clinic of the department of Obstetrics and Gynecology at the Canisius Wilhelmina Hospital in Nijmegen, the Netherlands, from 2002-2004. All women who attended the clinic were potentially eligible. Our aim was to study the relationship between maternal homocysteine concentrations in the general population; therefore, we have included pregnant women from this large regional hospital. Women with high-risk pregnancies or serious illnesses themselves were referred to a tertiary hospital, mostly at <30 weeks of gestation. They were asked to participate when the time schedule of the clinician, the nurses, and/or the administrative worker allowed this. Approximately 25% of all women who delivered in this hospital during the study period were included. To check whether the included women were representative for the entire population that visited this hospital, we randomly selected 500 additional women from the same period and found comparable results for maternal age, gestational age, birthweight, and newborn infant sex.

In the Netherlands, women are evaluated routinely for irregular antibodies between 30 and 34 weeks of gestation. After written informed consent had been obtained, 478 maternal blood samples were drawn during routine blood sampling of women between 30 and 34 weeks of gestation. Only 8 mothers refused participation after being asked. Of mothers with blood samples, 386 women visited the hospital for the delivery of their child and delivered a total of 406 babies from whom venous umbilical cord blood was drawn (including 20 twin pairs). We decided to exclude twin pregnancies because of a higher risk of lower birthweight in twins, which left 366 mother-baby pairs.

All mothers were asked to fill out a questionnaire. The study protocol was approved by the local medical ethics committee.

Blood sampling

Maternal venous blood was collected in tubes that contained 3.5 mL ethylenediaminetetraacetic acid (EDTA). Immedi-

TABLE 2
Different analyses to study determinants of birthweight in our population

	β coefficient (95% CI)[a]		
Possible determinant	**Model 1[b]**	**Model 2[c]**	**Model 3[d]**
Gestational age, wk	150 (118–182)	—	—
Maternal age at delivery, y	–3.5 (–17 to 10)	–3.6 (–16 to 8.7)	–8.3 (–22 to 5.2)
Parity, 0-7	56 (–8 to 121)	60 (1.9–119)	104 (37–171)
Smoking, no/yes	–286 (–450 to –122)	–261 (–410 to –112)	–313 (–479 to –149)
Use of folic acid, no/yes	11 (–107 to 129)	13.6 (–94 to 121)	–64 (–186 to 56)
Female sex, no/yes	–150 (–263 to –36)	–149 (–252 to –46)	–146 (–256 to –35)
Total homocysteine, 1 SD = 2.3 μmol/L[e]	–65 (–122 to –7.4)	–56 (–108 to –4.5)	–12 (–82 to 58)
Cobalamin, 1 SD = 69 pmol/L[e]	–33 (–90 to 24)	–29 (–81 to 23)	–37 (–100 to 29)
Methylmalonic acid, 1 SD = 0.09 μmol/L[e]	9.5 (–54 to 73)	12 (–45 to 69)	9.2 (–51 to 70)
Folate, 1 SD = 13 nmol/L[e]	45 (–12 to 102)	55 (3.5–107)	28 (–78 to 133)
Creatinine, 1 SD = 7.5 μmol/L[e]	–55 (–112 to 2.9)	–45 (–98 to 7.1)	–44 (–104 to 17)

CI, confidence interval; *SD*, standard deviation.

[a] The coefficients give the increase/decrease in birthweight (g) for several variables; [b] Univariate analysis of possible determinants on birthweight; [c] Univariate analysis of possible determinants on standardized birthweights; [d] Multivariate analysis of possible determinants on standardized birthweights; [e] Coefficients that are presented for maternal concentrations were calculated with calculated Z scores of these metabolites and now represent the increase or decrease in birthweight (g) after a 1 SD increase in maternal analytes.

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ately after birth, the umbilical cord was clamped, and venous cord blood was collected into a vacuum tube that contained 3.5 mL EDTA. Samples from mothers and cords were immediately placed on ice. After centrifugation (within 2 hours after sampling), plasma was separated, and EDTA plasma and blood cells were stored separately at –20°C until analysis.

Biochemical analyses

tHcy, folate, cobalamin, and MMA acid concentrations were determined in both maternal and umbilical cord EDTA plasma. Plasma tHcy was determined by liquid chromatography-mass spectrometry-mass spectrometry. Deuterated homocysteine was added as an internal standard, and samples were treated with dithioerythritol followed by acid precipitation. Supernatants were analyzed by reversed-phase chromatography. The between-day coefficient of variation was approximately 5%. The method has been validated against existing methods according to the protocol described by Nexo et al[25] and shows excellent correlation with a method based on high performance liquid chromatography and fluorescence detection.[26] Plasma folate concentrations were determined by a *Lactobacillus casei* microbiologic assay,[27] and plasma cobalamin concentrations were determined by a *L rhamnosus* microbiologic assay.[28] Both the folate and the cobalamin assays were adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz AG, Bonaduz, Switzerland).[29] Plasma MMA values were measured with a liquid chromatography tandem mass spectrometry method.[30]

Statistical analyses

Results are presented as median values with the interquartile range, except when otherwise stated. After logarithmic transformation, Z scores for variables were calculated before further analysis. Linear regression was used to determine predictors of birthweight. Because birthweight and gestational age were strongly related, we calculated birthweights that were standardized for gestational age. Again, linear regression analyses were performed with the standardized birthweights as dependent variables. To reveal nonlinear relations, association curves were constructed to show associations between maternal concentrations and birthweight, with the use of Gaussian generalized additive regression models. Correlations between variables were calculated by Pearson correlation after logarithmic transformation of data. STATA statistical software (version 10.0; Stata Corporation, College Station, TX) was used for all statistical analyses.

RESULTS

Population characteristics

Population characteristics that include blood indices are presented in Table 1. Babies were born at or near term. All maternal analytes were lower, compared with umbilical analyte concentrations.

Maternal tHcy concentrations, related B vitamins, and birthweight

Possible relations between maternal tHcy, cobalamin, MMA, and folate concentrations with birthweight are shown as regression model curves (Figure). There were no significant correlations between birthweight and maternal MMA ($r = 0.02$), cobalamin ($r = -0.06$), or folate ($r = 0.04$; Pearson correlation coefficients, all probability values $> .15$). Maternal tHcy showed a slight negative association with birthweight ($r = -0.12$; $P = .03$).

Table 2 shows the association between several possible determinants and birthweight. Univariate analysis revealed

TABLE 3
Odds ratios for low birthweight, according to maternal plasma indices

Variable	Odds ratio (95% CI)
Total homocysteine >6.71 μmol/L	1.17 (0.70–1.95)
Cobalamin <134 pmol/L	0.70 (0.44–1.11)
Methylmalonic acid >0.22 μmol/L	0.93 (0.48–1.79)
Folate <6.11 nmol/L	1.35 (0.93–1.96)
Creatinine >49.7 μmol/L	1.34 (0.89–2.01)

The risk of birthweight being in the lowest quartile that was determined in our study (<3075 g) was determined for maternal concentrations of total homocysteine, methylmalonic acid, and creatinine in the highest quartile and folate and cobalamin in the lowest quartile, compared with the other 3 quartiles together.
CI, confidence interval.

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smoking to be the strongest predictor of birthweight; babies of smoking mothers had birthweights approximately 300 g lower than those of nonsmoking mothers. Gestational age and newborn infant sex were weaker determinants of birthweight. An increase in maternal tHcy level of 1 SD (2.3 μmol/L) resulted in a decrease in birthweight of 65 g. With the use of birthweights that had been standardized for gestational age, smoking and sex remained the strongest determinants in univariate and multiple linear regression analysis. The relationship between tHcy and standardized birthweight disappeared after the introduction of smoking into the model. There was no difference in results between women with or without folic acid use, considering the relation between maternal homocysteine and birthweight (data not shown). Maternal folate, cobalamin, and MMA were not associated significantly with LBW. Analysis of umbilical cord blood analytes and birthweight did not reveal a clear relation in univariate or multiple regression analysis (data not shown).

We also analyzed whether high maternal concentrations of tHcy, MMA, and creatinine or low concentrations of cobalamin or folate were predictors of LBW. However, we did not find significant odds ratios (Table 3).

Comment

We prospectively studied the potential relationship between maternal tHcy, related B vitamins, and risk of LBW in a large Dutch study population of pregnant women. We focused on maternal concentrations because our aim was to explore possible treatable risk factors for LBW. Maternal tHcy, folate, cobalamin, and MMA concentrations were not associated significantly with birthweight. Gestational age, smoking, and sex were all strong determinants of birthweight, as previously observed by others.[31-35]

Study population and design

There are limitations of the present study. First, we collected samples from 1 hospital. Although it is a large regional hospital in the Netherlands, one cannot extrapolate these results directly for all pregnant women. Furthermore, data on length and weight of the infant and blood pressure of the mother or other vascular complications before or during pregnancy were not known. These traits may be related to tHcy and B vitamins and hence be potential confounders. Despite this, our study population seems to be comparable with study populations of others, with respect to gestational age, maternal age, and birthweight.[24,36] *LBW* is often defined as weight <2500 g. Because birthweight and gestational age are correlated strongly, we used birthweights that were standardized for gestational age in our analyses. To study maternal cobalamin status more accurately, we also determined MMA concentrations, which reflects intracellular cobalamin status. We have studied maternal variables in blood drawn between 30-34 weeks of gestation, which can be considered a narrow window. Milman et al[37] describe concentrations of tHcy, cobalamin, MMA, and folate in 434 Danish women at 32 weeks of gestation. They measured concentrations that are comparable to our data (Table I; birthweight was not studied). Several other authors have addressed maternal homocysteine concentrations throughout pregnancy (Murphy et al,[17,38] Andersson et al,[39] Walker et al[40]). They observed that maternal concentrations are correlated throughout pregnancy, may decrease at first, seem to plateau between 20 and 32 weeks of gestation, and may increase at labor.[17,38-40] This suggests that taking maternal samples between 30 and 34 weeks of gestation should be a period with stable homocysteine concentrations.

Determinants of birthweight

Univariate analysis showed that gestational age, smoking, and sex were the strongest determinants of birthweight. In normal pregnancy, there is a strong positive correlation between birthweight and gestational age.[31] Boys normally have a slightly higher birthweight than girls.[31,35] Smoking is a well-known strong and dose-dependent risk factor for LBW, as confirmed in our study.[33,34,41] Parity, which has been shown to be related to birthweight,[32,35] was also found by us to be a determinant next to smoking and sex. The risk of LBW increases again with the fourth and subsequent children.[35,35,42]

Observational studies have demonstrated a positive correlation between maternal serum or red blood cell folate and infant birthweight or higher birthweight in folic acid–supplemented mothers.[24,43-46] Others, however, have described no differences in birthweight between infants born to mothers who receive folic acid supplements, compared with nonsupplemented mothers.[18,46] In line with these results, neither the use of folic acid in early pregnancy nor maternal folate concentrations influenced birthweight in our study.

Univariate analyses show an inverse association between maternal tHcy and birthweight, which disappeared after adjustment for smoking and in multivariate analysis. Smoking is associated with lower birthweight and with higher tHcy concentrations and is a possible confounder, which is a good explanation for the loss of association described earlier.[33,34,41,47,48]

In this well-nourished and partly supplemented Dutch population, maternal tHcy and related B vitamins are not as-

sociated with birthweight. The question remains whether birthweight is influenced by these analytes in other populations with no widespread use of folic acid supplement during pregnancy.

Analyte concentrations

We observed higher concentrations of tHcy, cobalamin, MMA, and folate in umbilical cord blood, compared with maternal blood samples. Some studies reported lower or similar tHcy concentrations in umbilical cord samples than in maternal samples.[3,14,16,23,49] In most studies, maternal blood was drawn at or immediately after delivery; in our study, maternal samples were drawn between 30 and 34 weeks of gestation. Murphy et al[17] showed lower maternal tHcy concentrations at 32 weeks, compared with maternal tHcy at delivery and in fetal cord samples. Differences in study design and time of sampling may be responsible for differences in results. The higher umbilical cord blood concentrations of folate and cobalamin could result from active placental transport and the ability to concentrate vitamins in the fetus.[50,51] Umbilical MMA concentrations are also higher than maternal concentrations, which confirms the results from Obeid and Hermann.[52]

In conclusion, our relatively large study demonstrated that maternal tHcy and related B vitamins have a weak or even no association with birthweight. ■

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