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Maternal Homocysteine before Conception and throughout Pregnancy Predicts Fetal Homocysteine and Birth Weight

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Background: **Increased homocysteine has been associated with pregnancy complications.**

Methods: **We investigated prospectively the effect of maternal homocysteine on normal pregnancy outcome. The study included 93 women and their offspring; 39 of the women took folic acid during the second and/or third trimesters of pregnancy. We measured homocysteine at preconception; at weeks 8, 20, and 32 of pregnancy; during labor; and in the fetal cord; we also recorded birth weight.**

Results: **Geometric mean (SE) maternal total homocysteine (tHcy) increased between 32 weeks of pregnancy and labor [7.98 (1.05) μmol/L in unsupplemented women and 6.26 (1.07) μmol/L in supplemented women; $P < 0.0001$ for both]. Fetal tHcy was lower than maternal tHcy [6.39 (1.06) μmol/L in unsupplemented pregnancies ($P < 0.0001$), and 5.18 (1.06) μmol/L in supplemented pregnancies ($P < 0.05$)]. Maternal tHcy was correlated from preconception throughout pregnancy (8 weeks, $r = 0.708$; 20 weeks, $r = 0.637$; 32 weeks, $r = 0.537$; labor, $r = 0.502$; $P < 0.0001$ for all time points) and with fetal tHcy [preconception, $r = 0.255$ ($P < 0.05$); 8 weeks, $r = 0.321$ ($P < 0.01$); 20 weeks, $r = 0.469$; 32 weeks, $r = 0.550$; labor, $r = 0.624$ ($P < 0.0001$)]. Mothers in the highest tHcy tertile at 8 weeks of pregnancy were three times [odds ratio, 3.26 (95% confidence interval, 1.05–10.13); $P < 0.05$] and at labor were four times [3.65 (1.15–11.56); $P < 0.05$] more likely to give birth to a neonate in the lowest birth weight tertile. Neonates of mothers in the highest tHcy tertile at labor weighed, on average, 227.98 g less than those of mothers in the low and medium tertiles ($P = 0.014$).**

Conclusions: **Supplemented mothers had lower tHcy at labor than unsupplemented mothers, as did their neonates. Maternal and fetal tHcy was significantly correlated throughout the study. Neonates of mothers in the highest tertile of homocysteine weighed less.**

Several case–control studies have reported an association between increased maternal plasma total homocysteine (tHcy)[4] and pregnancy complications *(1–6)*. Such studies have been repeatedly quoted as demonstrating an association between increased maternal tHcy and pregnancy complications. However, many of them have concluded this by relating a tHcy measurement in the nonpregnant state with a previous pregnancy complication *(1, 2, 4)*. To the best of our knowledge, no studies have investigated the effect of tHcy measured longitudinally at preconception and during each trimester of pregnancy on outcome. It is not clear whether increased tHcy is the cause or consequence of these complications. It is possible that underlying mechanisms that occur when tHcy is increased preconceptionally are expressed during pregnancy. This is difficult to investigate because, typically, the first blood sample available for analysis is at the end of the first trimester of pregnancy and because tHcy is not routinely analyzed. tHcy is significantly decreased as a physiologic response to pregnancy and is decreased even further in mothers who use folic acid supplements *(7)*. The aim of this study was to investigate the effect of maternal tHcy, preconceptionally and throughout preg-

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[4] Nonstandard abbreviations: tHcy, total homocysteine; BMI, body mass index, 95% CI, 95% confidence interval; and OR, odds ratio.

nancy, on outcome in uncomplicated pregnancies. We had previously examined variations in tHcy in uncomplicated pregnancies in the same group of women from preconception through weeks 8, 20, and 32 of pregnancy *(7)*. Here we report how these previously reported tHcy concentrations relate to maternal concentrations at delivery and, more importantly, how they relate to the fetal concentration as determined in the cord blood as well as to pregnancy outcome.

Materials and Methods

The design and background to this study have been explained in detail previously *(7)*. Briefly, this was a longitudinal study performed in the Unit of Preventive Medicine and Public Health, Faculty of Medicine and Health Sciences, Rovira i Virgili University in collaboration with the Unit of Obstetrics and Gynecology of the St. Joan Hospital, Reus. It formed part of a study on the evolution of women's nutritional status preconceptionally and throughout pregnancy. The study was performed with the approval of the hospital's ethics committee, and signed informed consent in accordance with the Declaration of Helsinki *(8)* was obtained from all participating volunteers. Ninety-three healthy women (age range, 18–35 years) who were planning on becoming pregnant participated in the study from the preconception period throughout pregnancy.

sample collection and analysis

Blood samples were drawn from the antecubital vein in potassium EDTA-containing Vacutainer Tubes at preconception (2–10 weeks before conception, between days 7 and 12 of the menstrual cycle); at 8, 20, and 32 weeks of pregnancy; on admission to hospital with confirmed labor (the length of time that passed between this sample and birth varied in function of length of labor, but sample collection was always before birth); and from the fetal cord at birth. All blood samples were fasting with the exception of those taken at labor and birth. Blood samples were refrigerated before plasma separation by centrifugation. Cord blood and maternal bloods were refrigerated immediately after collection, which stabilized their tHcy concentrations *(9)*. Plasma was then separated within a maximum of 12 h and stored at −20 °C. tHcy was determined by the IMx homocysteine immunoassay (Abbott GmbH). Volunteers attended a medical check-up during which they were questioned on the use of vitamin supplements and smoking habits on the same week as blood sample collection. Neonate birth weight was recorded.

folic acid supplementation

The decision on the use of folic acid supplements remained the joint responsibility of the woman and her obstetrician and has been described in detail previously *(7)*. At the time of the study (1992–1996), periconceptional supplementation with folic acid was not common practice in Spain. Briefly, no women took folic acid supplements during either the preconception period or the first trimester of pregnancy, and 54 women did not use folic acid supplements throughout the entire study. Thirty-nine women took folic acid supplements during the second and/or third trimesters of pregnancy. The majority of supplemented women took 0.5 mg of folic acid/day (34 during the second trimester and 33 during the third trimester). Of the remaining women, three took 0.75–2.0 g/day and two took 15.25–15.5 mg/day. The doses for these women were higher because they used different brands of folic acid supplements available over the counter, which varied considerably in dose. A wide variety of low-dose folic acid supplements was not available at the time of the study.

statistical analysis

All statistical analyses were performed with SPSS (Ver. 11.5) software (SPSS, Inc.). tHcy data were log-transformed to approach normalization when required for statistical tests, and back-transformed results are shown. A two-way repeated-measures ANOVA was initially used to explore the effect of supplement use (intersubject factor) on tHcy concentrations across time of pregnancy (intrasubject factor). Because the interaction term was significant, we analyzed the effect of time of pregnancy on tHcy concentrations within each group (nested model). A nonorthogonal repeated contrast was used to test the significance of differences in tHcy means between consecutive time points of pregnancy.

Pearson linear correlation coefficients were determined to assess associations between maternal preconception tHcy with tHcy throughout pregnancy and between maternal tHcy throughout the study and fetal cord tHcy. The Student unpaired *t*-test was used to compare mean birth weight of neonates born to mothers in the highest tertile of tHcy with that of those born to mothers in the low and medium tertiles combined. Logistic regression analysis was used to calculate the risk of having neonates in the lowest birth weight tertile of this study for mothers in the highest tHcy tertiles, adjusting for the following confounding factors: gestational age of fetus at birth, sex, maternal body mass index (BMI), age, and smoking. Multiple linear regression analysis was used to study the difference in mean birth weight observed in neonates of mothers in different tHcy tertiles when adjusted for the same confounding factors listed previously. All *P* values from post hoc analyses were Bonferroni-corrected. Significance for the two-tailed hypothesis was established at the level of 0.05.

Results

Geometric mean tHcy concentrations at preconception, throughout pregnancy, and during labor in mothers and from fetal cord at birth in the unsupplemented and folic acid-supplemented groups during the second and/or third trimesters of pregnancy are shown in Fig. 1. As we

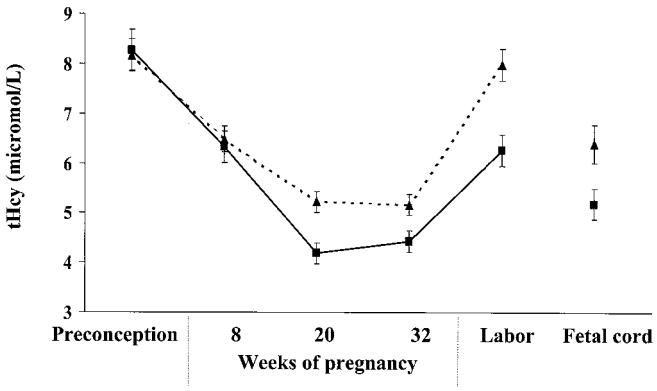

Fig. 1. Geometric mean (SE; *error bars*) maternal tHcy at preconception and during pregnancy, and fetal cord tHcy.

Unsupplemented group (▲): preconception and 8 and 20 weeks of pregnancy (n = 54); 32 weeks of pregnancy (n = 53); labor and fetal cord (n = 47). Group taking folic acid supplements during the second and/or third trimesters (■): preconception (n = 39); 8 and 20 weeks of pregnancy (n = 35); 32 weeks of pregnancy (n = 38); labor (n = 37); fetal cord (n = 36). The data shown at preconception and at 8, 20 and 32 weeks of pregnancy have been reported previously *(7)*. Two-way repeated-measures ANOVA (intrasubject factor, time of pregnancy; intersubject factor, supplement use). Significance of interaction between time of pregnancy and supplement use: $P < 0.001$. Post hoc analysis by repeated contrast with Bonferroni correction showed that tHcy was significantly lower than preconception concentrations at 8, 20, and 32 weeks of pregnancy in both groups and at labor in supplemented mothers ($P < 0.001$), and was significantly higher at labor than at 32 weeks in both groups ($P < 0.0001$). tHcy in the supplemented group was significantly lower than in the unsupplemented group at 20 and 32 weeks of pregnancy and during labor ($P < 0.05$). Comparing difference in mean fetal cord and maternal tHcy at labor: $P < 0.0001$, unsupplemented; $P < 0.05$, supplemented. ANOVA comparing difference in mean fetal cord tHcy between unsupplemented and supplemented groups, $P < 0.01$.

reported previously *(7)*, mean tHcy was significantly decreased during the first two trimesters of pregnancy in both groups (unsupplemented and folic acid-supplemented), reaching a plateau during the third trimester. The new observation that we report here is that by labor onset, tHcy had increased compared with 32 weeks of gestation in both groups. The tHcy concentration at labor was similar to the preconception concentration [mean preconception concentration in all women, 8.53 μmol/L; 95% confidence interval (95% CI), 8.00–9.06 μmol/L; median, 8.21 μmol/L; range, 4.30–22.35 μmol/L] in the unsupplemented group (mean concentration during labor, 8.56 μmol/L; 95% CI, 7.74–9.41 μmol/L; median, 8.43 μmol/L; range, 4.21–18.32 μmol/L), but it remained significantly lower than at preconception in the supplemented group (mean concentration during labor, 6.58 μmol/L; 95% CI, 5.85–7.31 μmol/L; median, 6.31 μmol/L; range, 1.42–10.99 μmol/L). Fetal cord tHcy was also significantly lower than maternal in both the unsupplemented (mean, 6.75 μmol/L; 95% CI, 5.95–7.55 μmol/L; median, 6.18 μmol/L; range, 3.28–14.05 μmol/L) and supplemented (mean, 5.40 μmol/L; 95% CI, 4.72–6.09 μmol/L; median, 5.19 μmol/L; range, 2.19–12.35 μmol/L) groups. Compared with the unsupplemented group, in the supplemented group tHcy was significantly lower throughout the supplementation period and fetal cord tHcy was also significantly lower.

The correlations between maternal tHcy before pregnancy with tHcy at each time point throughout pregnancy are shown in Table 1, as are the correlations between maternal tHcy throughout the study and fetal cord tHcy. Preconception tHcy was significantly correlated with pregnancy tHcy at each time point. Maternal tHcy at preconception and throughout pregnancy was significantly correlated with fetal cord tHcy.

Mean maternal and fetal tHcy was significantly lower in the group supplemented with folic acid from midpregnancy until the end. An important indicator of neonatal health is birth weight. To investigate whether there was an association between maternal tHcy and birth weight, we divided the women into two groups derived from tertiles of tHcy at each time point of the study: the low-medium and high tertiles. We investigated whether birth weight differed between neonates born to mothers in the low-medium tertiles and those born to mothers in the highest tertile of tHcy. The results are shown in Fig. 2. Neonates born to mothers in the highest tHcy tertile tended to have lower birth weights than those born to mothers in the low-medium tertiles throughout the study. The mean birth weight of neonates born to mothers in the highest tertile of tHcy at preconception, 8 weeks of pregnancy, and at labor was significantly lower than that of neonates born to mothers in the low-medium tertiles. However, these analyses were not adjusted for confounding influences on birth weight, such as gestational age, fetal sex, and maternal BMI, age, and smoking.

We therefore determined the odds ratios (ORs) of a

Table 1. Correlations[a] between maternal tHcy before conception and during pregnancy and between maternal tHcy throughout the study and fetal cord tHcy.

		Weeks of pregnancy			
	Preconception	8	20	32	Labor
Maternal preconception tHcy and tHcy during pregnancy		0.708[b] (n = 86)	0.637[b] (n = 88)	0.537[b] (n = 89)	0.502[b] (n = 81)
Maternal tHcy during study and fetal cord tHcy	0.255[c] (n = 80)	0.321[d] (n = 79)	0.469[b] (n = 81)	0.550[b] (n = 81)	0.624[b] (n = 79)

[a] Pearson linear correlation coefficient.
[b] $P < 0.0001$.
[c] $P < 0.05$.
[d] $P < 0.01$.

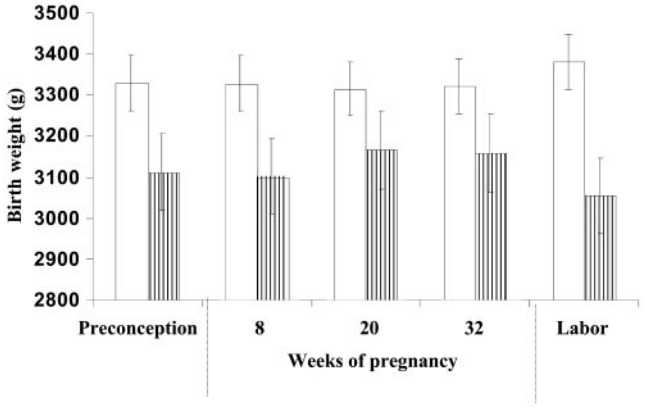

Fig. 2. Mean (SE) birth weight according to tertile of maternal tHcy.

□, low-medium tertiles. Preconception (n = 60); 8 weeks of pregnancy (n = 59); 20 and 32 weeks of pregnancy (n = 61); labor (n = 56). ▨, high tertile. Preconception and 8, 20, and 32 weeks of pregnancy (n = 30); labor (n = 28). By Student unpaired *t*-test, mean birth weight of neonates born to mothers in the high tHcy tertile at preconception, 8 weeks of pregnancy, and at labor was significantly lower than that of neonates born to mothers in the low-medium tertiles: preconception and 8 weeks of pregnancy, $P < 0.05$; during labor, $P < 0.01$.

neonate being in the lowest body weight tertile (<3120 g) of our study when maternal tHcy is in the highest tertile compared with the low-mid tHcy tertiles for each time point, using logistic regression analysis adjusting for gestational age, fetal sex, and maternal BMI, age, and smoking. The variables included in the regression analysis are described in Table 2, and the adjusted ORs are shown in Table 3. The ORs for lower birth weight were significantly higher for infants born to mothers in the high tHcy tertile at 8 and 20 weeks of pregnancy and at labor and for infants in the highest fetal cord tHcy tertile compared with infants born to mothers in the low-medium tHcy tertiles and in the lower tertiles for fetal cord tHcy.

We next investigated the relationship between maternal tHcy and birth weight (dependent variable), using multiple linear regression analysis adjusting for the known confounders listed above. The results are shown in Table 4. Being in the highest tHcy tertile compared with the low-medium tertiles was negatively associated with birth weight throughout the study. This effect was significant in the case of maternal tHcy measured during labor and in the case of fetal cord tHcy. The model predicts that neonates born to mothers in the highest tHcy tertile at labor would weigh, on average, 227.98 g less than infants born to mothers in the low-medium tertiles.

Table 2. Description of variables included in multiple linear and logistic regression analysis according to maternal tHcy tertile at labor.

	Low-Medium tertiles	High tertile
n	56	28
Birth weight, g		
Mean (95% CI)	3381 (3269–3492)	3055 (2870–3240)
Range	2560–4690	1950–3800
Maternal tHcy, μmol/L		
Mean (95% CI)	6.20 (5.80–6.60)	10.49 (9.64–11.34)
Range	1.42–8.43	8.44–18.32
Fetal cord tHcy, μmol/L		
Mean (95% CI)	5.17 (4.75–5.60)	8.08 (6.98–9.19)
Range	2.19–12.35	3.09–14.05
Gestational age, days		
Mean (95% CI)	281 (279–283)	277 (272–283)
Range	268–313	236–295
Fetal sex, % male	55	43
Preconception BMI, kg/m^2		
Mean (95% CI)	23.4 (22.6–24.2)	22.7 (21.8–23.6)
Range	19.7–34.4	19.0–27.4
Preconception age, years		
Mean (95% CI)	30 (29–30)	30 (29–31)
Range	24–36	24–35
Smokers,[a] %	9	11

[a] All smoked <5 cigarettes/day throughout pregnancy.

Discussion

The decrease in tHcy that occurs in early pregnancy may be attributable to a physiologic phenomenon resembling an extension of the luteal phase of the menstrual cycle. tHcy has been reported to be significantly lower in the luteal phase of the menstrual cycle than in the follicular phase *(10)*. We showed that the decrease in tHcy is significantly associated with the increase in estradiol concentrations that occur during pregnancy *(11)*. Increased tHcy in early pregnancy has been associated with pregnancy complications that typically end in spontaneous abortion *(1, 2)*. In vitro, homocysteine enhances spontaneous contractions of myometrium derived from pregnant women *(12)*. If the same applies in pregnancy, this may explain why high tHcy in early pregnancy leads to complications. However, the increase in tHcy late in uncomplicated pregnancies may suggest a physiologic role for homocysteine in the preparation for labor. Further investigation to establish at what point between 32 weeks of pregnancy and labor the increase in tHcy occurs could help determine whether homocysteine has such a role.

Although tHcy differed between mothers who took folic acid supplements during pregnancy and those who did not, the pattern of pregnancy-associated changes in tHcy was the same in both groups. That fetal cord tHcy was significantly lower than maternal tHcy at labor is in agreement with other studies reporting the same pattern with maternal tHcy determined just after birth *(13, 14)*. We were able to confirm this in women who did or did not take folic acid supplements and observed that supplemented mothers and their fetuses had significantly lower tHcy at labor than unsupplemented mothers and their fetuses. Apart from its role in the prevention of neural tube defects, other beneficial effects on pregnancy outcome of folic acid supplementation before conception or early in pregnancy have been reported. Folic acid supplementation has been associated with increased birth weight, longer gestational length, and a lower incidence

Table 3. ORs of neonate body weight being in the lowest tertile[a] when mothers and neonates were in the highest compared with low-medium tHcy tertiles.

		Weeks of pregnancy				
	Preconception	8	20	32	Labor	Fetal cord
OR[b]	1.67 (0.54–5.13)	3.26 (1.05–10.13)	2.82 (0.94–8.47)	1.20 (0.39–3.72)	3.65 (1.15–11.56)	4.46 (1.32–15.08)
n	90	89	91	91	84	83
P	0.372	0.041	0.064	0.751	0.028	0.016

[a] Lowest tertile of birth weight observed in our study.
[b] OR of birth weight being in the lowest tertile adjusted for gestational age, fetal sex, and maternal BMI, age, and smoking (95% CI in parentheses).

of low-birth-weight infants *(15)*. Scholl et al. *(16)* reported that lower serum folate concentrations at week 28 of pregnancy were associated with increased risk of low infant birth weight. Although the health benefits of lower tHcy concentrations, even within the reference interval, for adults have been reported *(17–19)*, we do not know whether reducing maternal tHcy, and consequently fetal tHcy, is of benefit to the neonate. All of the mothers in this study had normal pregnancy outcomes. If lowering tHcy during pregnancy is beneficial to the mother or to the neonate, it would be a reason to continue with folic acid supplementation throughout pregnancy rather than to stop at the end of the first trimester once the fetal neural tube has closed, as is common practice.

Our results show that preconception tHcy is significantly correlated with maternal tHcy throughout pregnancy and fetal tHcy at birth in a group of healthy women with uncomplicated pregnancies (Table 1). Maternal tHcy status before pregnancy thus is generally a good predictor of pregnancy tHcy status and of fetal tHcy status. Ideally speaking, women at risk of having a pregnancy/neonate affected by high tHcy could be identified preconceptionally so that measures to decrease tHcy (such as taking folic acid supplements) could be instituted before they become pregnant. Although it may be argued that folic acid supplementation throughout pregnancy may be necessary only for mothers with high risk of tHcy-associated pregnancy complications, we observed a beneficial effect on birth weight, in uncomplicated pregnancies, of having low maternal tHcy at 8 weeks of pregnancy and at labor. This trend was maintained at 20 and 32 weeks of pregnancy but was weaker and not significant. This may be for several reasons. Only singleton uncomplicated pregnancies were included in this study; therefore, none of the mothers had extremely high tHcy, even in the high tertile. At weeks 20 and 32 of pregnancy, tHcy was at its lowest concentrations (~40% lower than at preconception in the highest tertile), and the concentrations observed even in the high tertiles at these points were lower than those of the low-medium tertiles at preconception, which were shown not to have a negative effect on birth weight. Alternatively, other confounding physiologic changes that occur during pregnancy, uncontrolled in our analysis, may have masked the relationship between tHcy and birth weight at these points when tHcy was at its lowest.

Using multiple linear regression analysis, we confirmed that the tendency for a negative effect on birth weight of being in the highest maternal tHcy tertile compared with the low-medium tertiles was maintained throughout the study (Table 4). This was significant at labor, and the same effect was observed with respect to fetal cord tHcy and birth weight. This may be expected because maternal and fetal tHcy were highly correlated. Babies born to mothers in the high tHcy tertiles at labor weighed, on average, 227.98 g less than those born to mothers in the low-medium tertiles. This was independent of gestational age, fetal sex, and maternal BMI, age, and smoking, all of which affect birth weight. Malinow et al. *(14)* also reported a significant inverse correlation between both maternal and fetal cord tHcy and birth weight.

Various studies have investigated the association between maternal tHcy and birth weight. However because of fundamental differences in study designs, timing of maternal tHcy samples with respect to the pregnancy outcome in question, and definition of increased tHcy and low birth weight, the conclusions differ somewhat. Ronnenberg et al. *(20)* reported that preconception tHcy status was not associated with low birth weight but was significantly associated with preterm birth. Their study was a case–control study, and they reported an adjusted OR of 1.1 (95% CI, 0.3–3.7) for having a low-birth-weight (<2500 g) neonate when maternal tHcy at preconception was ≥12.4 μmol/L. Here we report an adjusted OR of 1.70

Table 4. Effect of maternal tHcy at each time point of the study on birth weight.

Model[a]	n	B coefficient[b] (SE)	*P*	Corrected R^2
Maternal tHcy				
At preconception	90	−116.78 (93.49)	0.215	0.267
At 8 weeks of pregnancy	89	−118.69 (93.64)	0.209	0.263
At 20 weeks of pregnancy	91	−109.14 (90.52)	0.231	0.274
At 32 weeks of pregnancy	91	−26.56 (93.08)	0.776	0.264
At labor	84	−227.98 (91.04)	0.014	0.313

[a] Adjusted for gestational age, fetal sex, and maternal BMI, age, and smoking.
[b] Difference in mean birth weight (in g) between children born to mothers in the highest tHcy tertile compared with low-medium tHcy tertiles.

(95% CI, 0.56–5.20) for having a neonate in the lowest body weight tertile (<3120 g) when maternal tHcy at preconception was in the highest tertile (≥9.12 μmol/L). In both studies the analysis was adjusted for the same confounding factors with the exception of analytic batch and hemoglobin concentration in our study. The ORs were not significant in either of the studies, but in ours the OR was higher. Our cutoff values for both increased tHcy and low birth weight were less extreme because they were based on tertiles of both variables measured in a group of women with uncomplicated pregnancies. In addition, in contrast to Ronnenberg et al. *(20)*, the timing of our preconception samples was rigidly controlled, and they were taken just before ovulation so that variation in tHcy attributable to menstrual cycle phase was kept to a minimum.

Vollset et al. *(21)* reported a strong association between low birth weight and tHcy measured either before or after pregnancy in women 40–42 years of age. The adjusted OR for very low infant birth weight (<1500 g) when maternal tHcy was in the upper tHcy quartile (≥10.7 μmol/L) compared with the lowest was 2.07. However, the time between tHcy measurement and pregnancy varied up to years. Cotter et al. *(3)* reported that infants born to preeclamptic women in the highest tHcy tertile (>7.8 μmol/L) at 16 weeks of pregnancy weighed significantly less than those born to the remaining preeclamptic group. This is consistent with our findings for women with tHcy ≥7.09 μmol/L at 8 weeks of pregnancy.

Infante-Rivard et al. *(22)* reported that maternal tHcy is protective against intrauterine growth restriction in a case–control study. They reported an adjusted OR of 0.37 (95% CI, 0.24–0.58) of intrauterine growth restriction for a 5 μmol/L difference in maternal tHcy measured up to 48 h postpartum. The mean (95% CI) tHcy for mothers of cases was 5.11 (4.95–5.26) μmol/L and of controls was 5.59 (5.41–5.76) μmol/L. On the assumption that it is valid to compare maternal tHcy ranges between the two studies, given that ours was determined before birth whereas theirs was after, they are quite similar: the maternal tHcy in our study was 1.42–18.32 μmol/L, whereas the range in their study *(22)* was 1.76–15.98 μmol/L. In addition, there is only a slight difference between the tHcy concentrations in our low-medium tertile group (Table 2) and their control group, based on means and 95% CIs. However, the variation in tHcy between cases and controls in their group was considerably narrower than the range we observed between the low-medium and high tertile groups at labor and between our high tertile group and their case group. A comparison of fetal cord tHcy between the two studies showed more or less the same as for the mothers. Our overall range was 2.19–14.05 μmol/L, whereas theirs was 1.03–17.94 μmol/L. There was virtually no variation between fetal tHcy in cases and controls in their study. One reason for this may be that we defined our groups on the basis of maternal tHcy, whereas they defined theirs on the basis of birth weight. With respect to the regression analysis from which the main conclusions are derived in each study, we investigated the effect of tHcy concentration, whereas they investigated the effect of increments in tHcy in absolute values on birth weight. The reason for the difference in conclusions between the two studies is unclear, but there are many differences in design.

The strength of our study is that it provides prospective tHcy data from 2–10 weeks before conception until the moment when labor is confirmed at hospital admission. This enabled prospective investigation of the association between maternal tHcy and birth weight in uncomplicated pregnancies, based on tHcy measurements from the pregnancy in question at time points that had a direct effect on birth weight. In addition, the fact that women in Spain did not take folic acid supplements before conception at the time of our study enabled us to investigate how tHcy fluctuates during uncomplicated pregnancies in the absence of folic acid supplementation. This is vital as a reference point for future studies of the role of tHcy in the development of pregnancy complications because periconceptional folic acid supplementation of pregnant women will inevitably affect tHcy. A group of women took folic acid supplements during the second and/or third trimesters of pregnancy as prescribed by their obstetricians, usually coinciding with the initiation of iron supplements. This was not an intervention study in which timing and dose of folic acid supplementation was rigidly controlled. Although this is a potential weakness of our study, our aim of gaining understanding of tHcy fluctuations during uncomplicated pregnancy in the presence and absence of folic acid supplementation was not impaired.

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References

1. Nelen WLDM, Blom HK, Steegers EPA, Den Heijer M, Thomas CMG, Eskes TKAB. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol 2000;95:519–24.
2. Goddijn-Wessel TAW, Wouters MGAJ, van de Molen EF, Spuijbroek MD, Steegers-Theunissen RP, Blom HJ, et al. Hyperhomocysteinemia: a risk factor for placental abruption or infarction. Eur J Obstet Gynecol Reprod Biol 1996;66:23–9.
3. Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: a risk factor for the development of nonsevere preeclampsia. Am J Obstet Gynecol 2003;189:391–6.
4. van der Molen EF, Verbruggen B, Novakova, Eskes TKAB, Monnens LAH, Blom HJ, et al. Hyperhomocysteinemia and other

thrombotic risk factors in women with placental vasculopathy. BJOG 2000;107:785–91.
5. Rajkovic A, Catalano PM, Malinow MR. Elevated homocysteine levels with preeclampsia. Obstet Gynecol 1997;90:168–71.
6. Powers RW, Evans RW, Majors AK, Ojimba JL, Ness RB, Crombleholme WR, et al. Plasma homocysteine concentration is increased in preeclampsia and is associated with evidence of endothelial activation. Am J Obstet Gynecol 1998;179:1605–11.
7. Murphy MM, Scott JM, McPartlin JM, Fernandez-Ballart JD. The pregnancy-related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study. Am J Clin Nutr 2002; 76:614–9.
8. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. JAMA 1997;277:925–6.
9. Hill DM, Johnson LJ, Burns PJ, Neale AM, Harmening DM, Kenney AC. Effects of temperature on stability of blood homocysteine in collection tubes containing 3-deazaadenosine. Clin Chem 2002; 48:2017–22.
10. Tallova J, Tomandl J, Bicikova M, Hill M. Changes of plasma total homocysteine levels during the menstrual cycle. Eur J Clin Invest 1999;29:1041–4.
11. Murphy MM, Scott JM, Molloy AM, Arija V, Fernandez-Ballart JD. Association between estradiol and homocysteine fluctuations during pregnancy. J Inherit Metab Dis 2003;26(Suppl):34.
12. Ayar A, Celik H, Ozcelik O, Kelestimur H. Homocysteine-induced enhancement of spontaneous contractions of myometrium isolated from pregnant women. Acta Obstet Gynecol Scand 2003; 82:789–93.
13. Molloy AM, Mills JL, McPartlin J, Kirke PN, Scott JM, Daly S. Maternal and fetal plasma homocysteine concentrations at birth: the influence of folate, vitamin B_{12}, and the 5,10-methylenetetrahydrofolate reductase 677C→T variant. Am J Obstet Gynecol 2002;186:499–503.
14. Malinow MR, Rajkovic A, Duell PB, Hess DL, Upson BM. The relationship between maternal and neonatal umbilical cord plasma homocysteine suggests a potential role for maternal homocysteine in fetal metabolism. Am J Obstet Gynecol 1998; 178:228–33.
15. Rolschau J, Kristoffersen K, Ulrich M, Grinsted P, Schaumberg E, Foged N. The influence of folic acid supplement on the outcome of pregnancies in the county of Funen in Denmark Part 1. Eur J Obstet Gynecol Reprod Biol 1999;87:105–10.
16. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr 1996;63:520–5.
17. Boushey CJ, Beresford SAA, Omen GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA 1995;274:1049–57.
18. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995;346: 1395–8.
19. Nygard O, Nprdrehang JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230–6.
20. Ronnenberg AG, Goldman MB, Chen D, Aitken IW, Willett WC, Selhub J, et al. Preconception homocysteine and B vitamin status and birth outcomes in Chinese women. Am J Clin Nutr 2002;76: 1385–91.
21. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. Am J Clin Nutr 2000;71:962–8.
22. Infante-Rivard C, Rivard GE, Gauthier R, Théorêt Y. Unexpected relationship between plasma homocysteine and intrauterine growth restriction. Clin Chem 2003;49:1476–82.