Plasma total cysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study[1–3]

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ABSTRACT

Background: Total homocysteine (tHcy) is associated with pregnancy complications and adverse pregnancy outcomes. The associations of plasma total cysteine (tCys) with such outcomes have not been investigated in large populations.

Objective: We investigated the association between plasma tCys and pregnancy complications, congenital malformations, and other adverse pregnancy outcomes.

Design: The plasma tCys concentrations of 5883 women aged 40–42 y that were measured in 1992–1993 during a cardiovascular health screening were compared with the outcomes and complications of 14 492 pregnancies in the same women that were registered in the Medical Birth Registry of Norway from 1967 to 1996.

Results: After adjustment for parity, mother's age, tHcy, total cholesterol, body mass index, smoking, and coffee drinking, high plasma tCys concentrations (above the 95th percentile) were associated with significantly higher risks of preeclampsia [n = 342; odds ratio (OR): 1.6; 95% CI: 1.1, 2.4; P = 0.03], premature delivery (n = 774; OR: 1.8; 95% CI: 1.3, 2.5; P = 0.001), and very low birth weight (n = 175; OR: 2.0; 95% CI: 1.1, 3.9; P = 0.03) than were lower plasma tCys concentrations. tCys was not associated with the risk of placental abruption. High tCys concentrations showed a weak association with congenital malformations and stillbirths with birth weight < 1500 g. The associations were independent of the tHcy concentrations.

Conclusion: High tCys concentrations were associated with risks of preeclampsia, premature delivery, and low birth weight. *Am J Clin Nutr* 2003;77:467–72.

KEY WORDS Plasma total cysteine, pregnancy complications, preeclampsia, pregnancy outcomes, total homocysteine, Hordaland Homocysteine Study, Norway, women

INTRODUCTION

High concentrations of plasma total homocysteine (tHcy) are associated with serious pregnancy complications, including pregnancy-induced hypertension, preeclampsia (1), and placental abruption (2), and with adverse pregnancy outcomes such as neural tube defects (3). Furthermore, an elevated plasma tHcy concentration is regarded as a risk factor for atherosclerosis (4, 5) and venous thrombosis (6).

The mechanism by which hyperhomocysteinemia confers increased risks of pregnancy complications and adverse outcomes is not yet known. Low dietary folate intakes and low circulating folate concentrations during pregnancy are associated with increased risks of preterm delivery, infant low birth weight, fetal growth retardation, and neural tube defects (7). One metabolic effect of folate deficiency is an elevated plasma tHcy concentration. It is not yet known whether an elevated tHcy concentration is harmful by itself through its vascular effect or is merely a reflection of the folate status. It was proposed that elevated tHcy causes endothelial dysfunction; therefore, women with hyperhomocysteinemia may be prone to endothelial dysfunction in the placental vasculature (8, 9). An elevated tHcy concentration and impaired folate status may also affect biological methylation and DNA synthesis (10) and thereby cell proliferation and normal fetal growth.

Cysteine is another sulfhydryl amino acid with structural and chemical properties similar to those of homocysteine (11). The concentration of total cysteine (tCys) in the serum or plasma of healthy subjects is ≈250 μmol/L, which is 20-fold that of plasma tHcy (12). Several studies found that tCys is associated with the risk of vascular disease in the coronary, cerebral, and peripheral arteries (13–17). However, the relation between tCys and pregnancy complications or adverse outcomes has received little attention. Raijmakers et al (18) reported that preeclamptic women had higher plasma cysteine and homocysteine concentrations during pregnancy than did women without preeclampsia.

The Hordaland Homocysteine Study included measurements of plasma tCys and tHcy concentrations in men and women aged 40–42 y and 65–67 y in 1992–1993. In this study, the major determinants of tCys concentration were body mass index (BMI; in kg/m^2), diastolic blood pressure, total cholesterol, and coffee drinking (19). Almost 93% of the 40–42-y-old women who participated in the Hordaland study were registered with ≥ 1 pregnancy in the Medical Birth Registry of Norway. tHcy was found to be associated with common pregnancy complications and adverse pregnancy outcomes in this population (20). In the present study, we investigated the relation between tCys concentrations measured in 1992–1993 and

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TABLE 1
Characteristics of the 5883 women in 1992–1993 by low or high total cysteine (tCys) concentration[1]

Characteristic	Percentage of total *n*	tCys concentration		*P*
		<304 μmol/L (*n* = 5594)	≥304 μmol/L (*n* = 289)	
	n (%)	*n* (%)		
>5 cups coffee/d	2275 (38.7)	2138 (38.2)	137 (47.4)	0.0018
Ever a smoker	3624 (61.7)	3450 (61.7)	174 (60.2)	0.6015
No use of vitamins[2]	971 (22.5)	922 (22.4)	49 (24.9)	0.4191
BMI (in kg/m^2) > 30	387 (6.6)	329 (5.9)	58 (20.1)	<0.0001
DBP > 90 mm Hg	542 (9.2)	492 (8.8)	50 (17.3)	<0.0001
tHcy > 12 μmol/L	817 (13.9)	726 (13.0)	91 (31.5)	<0.0001
Cholesterol > 6.5 mmol/L	685 (11.6)	634 (11.3)	51 (17.7)	0.0011

[1] *n* in brackets. DBP, diastolic blood pressure; tHcy, total homocysteine.
[2] Data on vitamin intakes were obtained from only 4311 women.

pregnancy complications and outcomes registered from 1967 to 1996.

SUBJECTS AND METHODS

Study population

In 1992–1993, baseline data for the Hordaland Homocysteine Study were collected by the National Health Screening Services in cooperation with the University of Bergen, Norway. A total of 18 043 men and woman aged 40–67 y participated in the cardiovascular health screening. The screening included measurements of height, weight, blood pressure, heart rate, serum total cholesterol, serum triacylglycerol, tHcy, and tCys. Information about lifestyle, diet, and reproductive history was collected with the use of self-administered questionnaires. Details on the data collection were published previously (21). Plasma tCys and tHcy concentrations were measured by using a fully automated assay featuring precolumn derivatization with monobromobimane followed by HPLC separation and fluorescence detection (22, 23).

Since 1967, the Medical Birth Registry has received notification of all births with a gestation > 16 wk. The notification included information about birth weight, length of gestation, medical conditions, complications during pregnancy and birth, congenital malformations, and obstetrical interventions. All women aged 40–42 y who participated in the Hordaland Homocysteine Study were linked with data from 1967 to 1996 filed by the Medical Birth Registry of Norway. Among the 5883 women, 14 492 pregnancies were reported to the registry during 1967–1996.

Statistical methods

The relations between tCys and pregnancy complications and adverse pregnancy outcomes were studied with logistic regression. With the use of PROC GENMOD of SAS (release 8.2 for HP-Unix; SAS Institute Inc, Cary, NC), the dependence of a given pregnancy outcome on other pregnancy outcomes in the same woman was taken into account by performing logistic regression analyses for clustered binary data with the use of generalized estimating equations (GEE) methodology. The analyses were performed both by using tCys quartiles and by using tCys as a binary variable with the 95th percentile as the cutoff value. Because no associations with outcomes were observed over quartiles, we report the results only with the latter representation of tCys. Hence, we report odds ratios (ORs) and 95% CIs of the comparison between tCys concentrations above and below the 95th percentile. The ORs are presented with adjustment for the mother's age at delivery, parity, tHcy, BMI, total cholesterol, smoking habits, and coffee drinking. All analyses were repeated separately for the years from 1980 to 1996, which were closer in time to the tCys measurements and hence more likely to show stronger associations between tCys and pregnancy complications and outcomes. However, there was no general tendency to strengthen the results; therefore, we mostly present results from 1967–1996.

The analyses were also performed for different combinations of low (< 250 μmol/L), medium (250–300 μmol/L), and high (≥ 300 μmol/L) tCys concentrations and low (< 12 μmol/L) and high (≥ 12 μmol/L) tHcy concentrations. Subjects with a low tCys concentration and a low tHcy concentration served as the reference category. Statistical analyses were performed with SAS statistical software (release 8.2 for HP-Unix; SAS Institute Inc).

RESULTS

Study population

The characteristics of the 5883 women who were 40–42 y old at the time of the tCys measurements in 1992–1993 are shown in **Table 1**. The mean (± SD) tCys concentration was 252.6 ± 29.5 μmol/L. The women with a tCys concentration above the 95th percentile drank significantly more coffee and had significantly higher BMI values, diastolic blood pressure, and cholesterol and tHcy concentrations than did those with a lower tCys concentration.

Total cysteine, pregnancy complications, and adverse pregnancy outcomes

The ORs for pregnancy complications and adverse pregnancy outcomes of the women with a baseline tCys concentration above the 95th percentile compared with those with a lower tCys concentration are shown in **Table 2**. The results were adjusted for parity and mother's age at delivery. Additional adjustments were performed for tHcy, BMI, cholesterol, smoking, and coffee intake. Additional adjustment for blood pressure slightly weakened the association between tCys and preeclampsia but had no effect on the other associations (data not shown).

High tCys concentrations were strongly associated with preeclampsia (Table 2). Preeclampsia was negatively associated with smoking and strongly and positively associated with BMI

TABLE 2
Odds ratios (ORs) for pregnancy complications and adverse pregnancy outcomes of women with a high (above the 95th percentile) total cysteine (tCys) concentration (≥304 mmol/L) compared with those with a lower tCys concentration (<304 μmol/L)

	Adjusted for parity and mother's age		Adjusted for multiple factors[2]	
Complication or outcome	OR[1]	*P*	OR[1]	*P*
Preeclampsia		0.0001		0.03
<304 μmol/L (*n* = 308)	1		1	
≥304 μmol/L (*n* = 34)	2.3 (1.6, 3.5)		1.6 (1.1, 2.4)	
Preeclampsia < 37 wk		0.09		0.11
<304 μmol/L (*n* = 46)	1		1	
≥304 μmol/L (*n* = 5)	2.6 (0.9, 7.8)		2.4 (0.8, 7.2)	
Placental abruption		0.8		0.7
<304 μmol/L (*n* = 70)	1		1	
≥304 μmol/L (*n* = 4)	0.8 (0.3, 2.7)		0.8 (0.3, 2.4)	
Premature delivery (<37 wk)		0.0008		0.001
<304 μmol/L (*n* = 712)	1		1	
≥304 μmol/L (*n* = 62)	1.7 (1.3, 2.4)		1.8 (1.3, 2.5)	
Premature delivery (<32 wk)		0.1		0.1
<304 μmol/L (*n* = 176)	1		1	
≥304 μmol/L (*n* = 15)	1.6 (0.8, 3.1)		1.6 (0.8, 3.2)	
Low birth weight (<2500 g)		0.08		0.07
<304 μmol/L (*n* = 664)	1		1	
≥304 μmol/L (*n* = 47)	1.4 (1.0, 2.0)		1.4 (1.0, 2.1)	
Very low birth weight (<1500 g)		0.04		0.03
<304 μmol/L (*n* = 159)	1		1	
≥304 μmol/L (*n* = 16)	1.9 (1.0, 3.6)		2.0 (1.1, 3.9)	
Stillbirth		0.3		0.3
<304 μmol/L (*n* = 167)	1		1	
≥304 μmol/L (*n* = 13)	1.4 (0.7, 2.8)		1.5 (0.7, 3.0)	
Stillbirth < 1500 g		0.09		0.07
<304 μmol/L (*n* = 104)	1		1	
≥304 μmol/L (*n* = 11)	2.0 (0.9, 4.2)		2.1 (0.9, 4.5)	
Neonatal mortality (first week)		0.2		0.4
<304 μmol/L (*n* = 53)	1		1	
≥304 μmol/L (*n* = 5)	1.8 (0.7, 4.5)		1.5 (0.6, 4.1)	

[1] 95% CI in parentheses.
[2] Parity, mother's age, total homocysteine, cholesterol, body mass index, smoking, and coffee drinking.

(data not shown). Adjustment for smoking and BMI slightly weakened the relation between tCys and preeclampsia (data not shown). There was no association when only births from 1980 to 1996 were considered (OR: 1.2; 95% CI: 0.6, 2.3; $P = 0.7$). The relation between tCys and preeclampsia was not significant when pregnancy was shorter than 37 wk although the point estimate was stronger (Table 2).

Placental abruption was reported in 0.5% of the pregnancies. High tCys concentrations were not associated with placental abruption.

There was a strong association between tCys concentrations and premature delivery defined as a gestation of < 37 wk. The association was stronger for the time period closest to the tCys measurement (ie, 1980–1996) (OR: 2.0; 95% CI: 1.1, 3.5; $P = 0.01$) than for the period of 1967–1996. However, the association between tCys concentrations and premature delivery was not significant when gestation was < 32 wk (Table 2).

There was a weak association between tCys concentrations and low birth weight, which was defined as < 2500 g. However, there was a much stronger and significant association between tCys concentrations and very low birth weight, which was defined as < 1500 g (Table 2).

There was a weak and nonsignificant association between tCys concentrations and stillbirths ($n = 180$). However, when birth weight was considered, tCys was strongly associated with stillbirths in which birth weight was < 1500 g. tCys was also weakly associated with neonatal mortality during the first week of life (Table 2).

Total cysteine and congenital malformations

Among the 14 492 pregnancies, ≥ 1 malformation was reported for 191 pregnancies. After adjustment for parity, mother's age, tHcy, cholesterol, BMI, smoking, and coffee drinking, there was a weak association between tCys and the risk of all malformations combined (OR: 1.7; 95% CI: 1.0, 2.8; $P = 0.06$). None of the relations between tCys and specific malformations were significant (data not shown).

Combinations of total cysteine and total homocysteine and the risk of pregnancy complications and adverse pregnancy outcomes

We investigated a combination variable of tCys and tHcy in relation to the risk of the outcomes studied (**Table 3**). The combination of low tCys and low tHcy was used as the reference category. Increased risks of preeclampsia, premature delivery, and low birth weight were seen in subjects having the combinations of high tCys and low tHcy or high tCys and high tHcy. Similar trends were observed for all the other pregnancy complications and adverse pregnancy outcomes that were investigated in this study. However, for placental abruption, an increased risk was seen in subjects having the combination of low tCys and high

TABLE 3
Odds ratios (ORs) for pregnancy complications and adverse pregnancy outcomes of various combinations of total cysteine (tCys) and total homocysteine (tHcy) concentrations[1]

	OR for preeclampsia[2]	OR for premature delivery (<37 wk)[2]	OR for low birth weight (<2500 g)[2]
Low tCys, low tHcy (*n* = 2577)	1 [172]	1 [342]	1 [295]
Medium tCys, low tHcy (*n* = 2207)	1.1 (0.8, 1.4) [164]	0.8 (0.7, 1.0) [245]	1.0 (0.8, 1.2) [258]
High tCys, low tHcy (*n* = 243)	1.7 (1.0, 2.7) [33]	1.4 (0.9, 2.1) [44]	1.4 (0.9, 2.2) [39]
Low tCys, high tHcy (*n* = 257)	0.8 (0.4, 1.8) [16]	0.7 (0.4, 1.1) [28]	0.7 (0.4, 1.1) [28]
Medium tCys, high tHcy (*n* = 491)	1.2 (0.7, 2.1) [48]	1.1 (0.8, 1.5) [81]	0.9 (0.6, 1.3) [67]
High tCys, high tHcy (*n* = 108)	1.5 (0.7, 3.0) [18]	2.3 (1.4, 3.8) [34]	1.6 (0.9, 3.0) [24]
P for heterogeneity	<0.3	<0.001	<0.02
P for interaction between tCys and tHcy	0.93	0.0015	0.23

[1] Adjusted for parity, mother's age, tHcy, cholesterol, body mass index, smoking, and coffee drinking. Concentration levels for tCys were defined as follows: low, <250 μmol/L; medium, 250–300 μmol/L; high, ≥300 μmol/L. Concentration levels for tHcy were defined as follows: low, <12 μmol/L; high, ≥12 μmol/L.

[2] 95% CI in parentheses; *n* in brackets.

tHcy (data not shown). The interaction between tCys and tHcy was also investigated by adding an interaction term to the model. The results showed a significant interaction between tCys and tHcy for premature delivery but not for preeclampsia or low birth weight (Table 3).

DISCUSSION

By linking data on 5883 women aged 40–42 y from the Hordaland Homocysteine Study with data from the Medical Birth Registry of Norway, we studied the relation between plasma tCys and pregnancy complications and adverse pregnancy outcomes. The results showed that high tCys concentrations are weakly associated with several complications and adverse outcomes, including preeclampsia, premature delivery, low birth weight, and stillbirth. Furthermore, tCys was weakly associated with congenital malformations. These associations persisted after adjustment for tHcy and other possible confounders.

In this study, concentrations of tCys above the 95th percentile were associated with preeclampsia. Raijmakers et al (18) reported higher tCys and tHcy concentrations during pregnancy in preeclamptic women than in women without preeclampsia. In agreement with this, an increased risk of preeclampsia was seen in subjects having the combination of high tCys and high tHcy in the present study (Table 3). Elevated concentrations of metabolites during preeclampsia have been explained by the reduction of plasma volume, which may be as much as 40% in severe cases (24, 25). This cannot explain our findings, however, because the tCys and tHcy concentrations were measured after rather than during preeclampsia.

The role of tCys in the pathogenesis of pregnancy complications is not yet known. It has been proposed that elevated tHcy concentrations cause endothelial dysfunction, and hyperhomocysteinemia may provoke placental vascular dysfunction (8, 9). Cysteine may affect the placental vasculature by a similar mechanism, but the possibility has not been investigated. Elevated tCys concentrations may reflect renal impairment, which has consistently been shown to cause increased concentrations of both tHcy and tCys (26). This, however, does not explain why elevated tCys concentrations are also a risk factor for pregnancy complications and adverse pregnancy outcomes at low tHcy concentrations (Table 3). In a previous study, which evaluated the relation between tCys and the risk of cardiovascular disease, low tCys concentrations were associated with the risk of cerebrovascular and peripheral vascular disease (17). We proposed that low cysteine concentrations may be a marker of low glutathione concentrations (which were recently associated with an increased risk of coronary artery disease) (27). However, in the present study, high tCys concentrations rather than low ones were associated with pregnancy complications and adverse pregnancy outcomes. Therefore, the possibility of glutathione involvement is not plausible in this case.

We considered the possibility that tCys may simply be a marker for other risk factors associated with placental disease. There is some evidence that adverse pregnancy outcomes are associated with both coffee drinking (28) and BMI (29–31), and we previously showed that both these factors are strong determinants of tCys (19). However, the relations between tCys and different complications and outcomes were upheld even after adjustment for several possible confounders. This indicates that tCys is not merely a marker for the risk factors evaluated in this study.

Various pregnancy complications caused by impaired function of the placental vascular bed are associated with both high tHcy and high tCys concentrations (18). This is in accordance with an increased risk of occlusive vascular disease conferred by high concentrations of these aminothiols (13–17). In the present study, we observed no significant association between tCys and various congenital abnormalities, including neural tube defects, which have been linked with hyperhomocysteinemia (32) and impaired folate status (33). The lack of a significant relation between tCys and congenital abnormalities may be explained by the fact that cysteine metabolism is independent of folate status (34). However, insufficient power to detect such a relation because of the low number of malformations in the subgroups with a high tCys concentration cannot be ruled out.

Most of the pregnancy complications and adverse pregnancy outcomes evaluated in this study are in fact interrelated. Pregnancies with very low birth weight (<1500 g) overlap with premature deliveries (gestation of <37 wk) to a significant extent. In fact, all the preeclamptic pregnancies having a duration of <37 wk were a subset of the premature deliveries (gestation of <37 wk). Such a strong interrelation between outcome measures prevents the disentanglement of the primary lesions caused by elevated tCys concentrations.

We exploited the possibility to retrospectively assess the relation between tCys and pregnancy complications by using data from a study that previously showed strong associations between tHcy and pregnancy complications and adverse pregnancy outcomes (20). In this previous study, the risk of preeclampsia, premature delivery, very low birth weight, stillbirth, and different congenital malformations increased over quartiles of tHcy concentration. tHcy was strongly associated with placental abruption only at very high tHcy concentrations (> 15 μmol/L) (20). In the present study, we found no associations with these pregnancy complications and adverse pregnancy outcomes over quartiles. Furthermore, we found no association between tCys and congenital malformations or between tCys and placental abruption even at tCys concentrations above the 95th percentile. Finally, the relations to tCys were not strengthened when the analyses were repeated for the time period closer to the tCys measurements. Thus, the relation between tCys and pregnancy complications and adverse pregnancy outcomes is much weaker than that found for tHcy. Nevertheless, when a combination variable of tCys and tHcy was investigated, the results (Table 3) showed that an elevated tHcy concentration is not a risk factor for preeclampsia, premature delivery, or low birth weight except when the tCys concentration is elevated. Thus, the possibility of interaction between tCys and tHcy in the pathogenesis of pregnancy complications and adverse pregnancy outcomes cannot be ruled out.

In conclusion, our data show that high tCys concentrations are associated with several pregnancy complications and adverse pregnancy outcomes, including preeclampsia, premature delivery, and low birth weight. The associations were observed at both high and low tHcy concentrations. This is the first large study to evaluate the role of tCys in pregnancy complications and outcomes. However, because tCys concentrations were measured after the outcomes had occurred, we cannot rule out the possibility that the pregnancy complications and adverse pregnancy outcomes were caused by another factor that also caused elevated tCys concentrations. Our results should be confirmed by prospective studies in which tCys concentrations are measured before the pregnancy.

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