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[1–3]

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ABSTRACT

Background: Fasting plasma total homocysteine (tHcy) decreases during pregnancy. Previous reports suggested that this is due to folic acid supplementation, hemodilution, or a decrease in albumin. However, these hypotheses have not been tested in a longitudinal study.

Objective: We investigated the relation between pregnancy-related physiologic changes and tHcy in a group of healthy women who were either unsupplemented or supplemented with folic acid.

Design: In a longitudinal study from preconception throughout pregnancy, we studied 54 unsupplemented women and 39 women who were supplemented with folic acid during the second or third trimester of pregnancy. tHcy, hematocrit, and serum albumin were determined preconceptionally and at 8, 20, and 32 wk of pregnancy.

Results: For the entire group, geometric mean tHcy concentrations at preconception (8.2 μmol/L) were significantly greater ($P < 0.001$) than those at 8 wk of pregnancy (6.4 μmol/L). When the unsupplemented and supplemented groups were regarded separately, geometric mean tHcy concentrations at preconception were significantly greater than those at 20 (5.22 and 4.18 μmol/L, respectively) and 32 (5.16 and 4.42 μmol/L, respectively) wk of pregnancy ($P < 0.001$ for both). Mean reductions from preconception concentrations at 8, 20, and 32 wk of pregnancy were significantly greater ($P < 0.001$) for tHcy (−11.5%, −25.5%, and −24.5%, respectively) than for hematocrit (−1.9%, −4.2%, and −4.3%, respectively) or serum albumin (−1.1%, −9.8%, and −13.4%, respectively). There was no correlation between changes in either hematocrit or serum albumin and changes in tHcy.

Conclusions: This study refutes the previous explanations for the reduction in plasma tHcy known to occur in pregnancy, namely, folic acid supplementation, hemodilution, and a decrease in serum albumin. We suggest that the changes may be endocrine-based. *Am J Clin Nutr* 2002;76:614–9.

KEY WORDS Homocysteine, pregnancy, preconception, folic acid, supplementation, albumin, hematocrit, longitudinal study

INTRODUCTION

An elevated concentration of fasting plasma total homocysteine (tHcy) is a risk factor for many diseases ranging from cardiovascular diseases (1) to pregnancy-related diseases (2, 3). Pregnancy-associated hyperhomocysteinemia is associated with adverse effects of pregnancy such as neural tube defects (4), abruptio placentae (5), and preeclampsia (6). However, only 3 studies to date have addressed the subject of changes in maternal tHcy concentrations during normal pregnancy (7–9). These cross-sectional studies compared the tHcy concentrations in women at different stages of pregnancy with those in nonpregnant women. tHcy concentrations were 29–60% lower in pregnant women than in nonpregnant women and reached their lowest values during the second trimester of pregnancy (8, 9). Nonfasting tHcy concentrations during preconception and throughout pregnancy were reported in a longitudinal study (10). However, of the 102 women studied, tHcy concentration values were provided for only 16 women. The authors reported a slight reduction in tHcy concentrations during the first trimester of pregnancy, with tHcy concentrations remaining constant thereafter. Various hypotheses explaining the decrease in tHcy concentrations during pregnancy have been proposed. Among these are hormonal influences on homocysteine metabolism (7, 10), maternal dietary protein intake during pregnancy (7), pregnancy-associated hemodilution (8–10), a decrease in albumin (9), folic acid supplementation (9), or fetal utilization (8, 10, 11). However, Andersson et al (8) rejected the possibility that the reduction in tHcy concentrations during pregnancy may be endocrine-based.

Ours is the first longitudinal study to provide reference values for tHcy concentrations in normal singleton pregnancies and to investigate changes in tHcy, hematocrit, and serum albumin from preconception through each trimester of pregnancy. Our early time point of 7.5–8 wk of pregnancy enabled us to determine changes in tHcy concentration at the earliest time during pregnancy so far reported. This was before the effect of hemodilution. Another

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[2] Supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT:ALI 89-0388), Spain; Fondo de Investigación Sanitaria (FIS:00/0954), Spain; EU Demonstration Project BMH 4983549; and Abbott GmbH, Weisbaden-Delkenheim, Germany.

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Received May 2, 2001.

Accepted for publication November 2, 2001.

important factor in our study is that none of the women took folic acid supplements either preconceptionally or before the second trimester of pregnancy. At the time of the study (1992–1996), folic acid supplementation during preconception and early pregnancy was not common practice in Spain. Fortification of cereal-based products with folic acid has also not been introduced in Spain. Furthermore, the proportion of the Spanish population who normally eat commercial breakfast cereals is low compared with that in other countries (Consumer Research Department, Kellogg's Europe, unpublished observation, 2000). This longitudinal study enabled us to determine the physiologic changes in tHcy concentrations produced by pregnancy alone without the masking effect of prophylactic folic acid during early pregnancy. The study also allowed us to compare the effects of folic acid supplementation or no supplementation during the second or third trimester of pregnancy.

SUBJECTS AND METHODS

Subjects

Reus is a city of 90 993 inhabitants (12) that is 10-km inland from the Mediterranean coast of Catalonia in northeastern Spain. This longitudinal study was performed in the Unit of Preventive Medicine, Faculty of Medicine, Rovira i Virgili University in collaboration with the Unit of Obstetrics and Gynecology of the St Joan Hospital, Reus. The study 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 (13) was obtained from all participating volunteers. Volunteers were recruited by advertisement through the local media and town hall. Healthy, nonpregnant women aged 18–35 y who were planning to become pregnant were sought to participate in the study during preconception and throughout pregnancy. Exclusion criteria were the presence of chronic illnesses that affect a woman's nutritional status or that require specific dietetic or nutritional intervention, the use of medications (such as folate antagonists) that may affect homocysteine metabolism, a failure to conceive after 18 menstrual cycles, and sterility. Of the 139 women who volunteered to participate in the study, 1 became pregnant with twins and was thus excluded. Of the remaining women, 93 remained in the study until completing successful pregnancies, 23 dropped out of the study before becoming pregnant, 6 failed to conceive within the first 18 mo of trying, 7 abandoned the study during pregnancy, 7 miscarried, 1 moved, and 1 was excluded because her supplementation pattern did not conform to the design of the study.

Study design

Throughout the study, blood samples were collected from the subjects after they had fasted overnight. If subjects did not become pregnant within 3 menstrual periods after the first preconception blood sample, another sample was taken. Similarly, blood samples were taken every 3 menstrual periods until subjects conceived. Data from the last preconception visit, PreC, which was ≈2–10 wk before conception, were used. Subjects were instructed to perform a pregnancy test and to inform the investigating team immediately after missing their first menstrual period. Pregnancy was confirmed by ultrasound examination. Pregnant subjects were called for the first-trimester blood sample between 53 and 59 d after the first day of their last menstrual period (ie, at ≈7.5–8 wk of pregnancy). The second-trimester blood sample was taken at 20 wk of pregnancy, and the third-trimester blood sample was taken at 32 wk of pregnancy.

Folic acid supplementation

The decision regarding the use of folic acid supplements remained the joint responsibility of the subject and her obstetrician. None of the women took folic acid supplements during either the preconception period or the first trimester of pregnancy, and 54 women did not take folic acid supplements throughout the entire study. Thirty-nine women took folic acid supplements during the second or third trimester of pregnancy. During the second trimester, 34 women took 500 μg folic acid/d, 1 took 750 μg folic acid/d, 1 took 1 mg folic acid/d, and 1 took 15.250 μg folic acid/d. During the third trimester, 33 women took 500 μg folic acid/d, 1 took 750 mg folic acid/d, 1 took 1 mg folic acid/d, 1 took 2 mg folic acid/d, 1 took 15 mg folic acid/d, and 1 took 15.500 mg folic acid/d. The supplemented group was not subdivided according to folic acid dose because analyses showed that the conclusions were unchanged by including all supplement users in the same group.

Interview

On the week of each blood sample collection, subjects were interviewed regarding their current use of medications and vitamin supplements, and anthropometric and blood pressure measurements were taken. On the first of these occasions, a detailed medical history was also recorded. During these interviews, subjects were questioned about the structured diaries in which they had recorded in detail their use of medications or vitamin supplements during the previous 7 d. Subjects who had used medications or supplements were questioned about the name of the preparation, the dose, and the length of treatment. Subjects' answers to these questions were corroborated against the content of their diaries. Those subjects who confirmed the use of supplements in both the interview and the diary were categorized as supplement users. Smokers and nonsmokers were not separated for analytic purposes because smoking during pregnancy does not affect maternal tHcy concentrations (14).

Sample collection and analysis

Fasting venous blood samples were drawn from the antecubital vein into potassium EDTA–treated evacuated tubes for whole blood and plasma analyses and into untreated evacuated tubes for serum preparation. Blood samples were refrigerated immediately, and plasma was separated by centrifugation at 1000 × g for 15 min at 4 °C within 2 h of sample collection. Plasma was stored at −20 °C before tHcy analysis. tHcy concentrations were determined by using the IMx homocysteine immunoassay (Abbott Laboratories Diagnostics Division, Abbott Park, IL). Hematocrit was determined with the use of a Coulter STKS hematology analyzer (Beckman Coulter, Miami). Serum albumin concentrations were determined by using the Boehringer Mannheim Albumin Colorimetric Assay (Boehringer Mannheim Laboratories, Mannheim, Germany).

Statistical analysis

All statistical analyses were performed by using the SPSS version 10.0 program (SPSS Inc, Chicago). tHcy data were log transformed to approach normalization. Mean percentage changes between preconception and pregnancy were also determined for log-

TABLE 1
Characteristics of the study population preconceptionally and smoking habits during pregnancy in the folic acid–unsupplemented and –supplemented groups[1]

	Unsupplemented (n = 54)	Supplemented (n = 39)
Age (y)[2]	29.7 ± 2.6	29.1 ± 3.0
Primiparous (%)	66.7	50.0
Oral contraceptive use (%)	81.5	71.8
Hemoglobin (g/L)[2]	133.0 ± 7.5	133.0 ± 8.4
BMI (kg/m^2)[2]	23.4 ± 2.7	22.8 ± 3.2
Smoking during pregnancy		
Until week 10		
(%)	37.4	20.0
Cigarettes/d[2]	4.43 ± 7.04	3.76 ± 5.80
Until term		
(%)	8.3	12.0
Cigarettes/d[2]	1.68 ± 3.52	0.74 ± 1.91

[1] There were no significant differences between the characteristics of the unsupplemented and supplemented groups.
[2] $\bar{x}$ ± SD.

transformed hematocrit and albumin data. A two-way repeated-measures analysis of variance was initially used to explore the effect of supplement use (intersubject factor) on tHcy concentrations throughout pregnancy (intrasubject factor). Because the interaction term was significant, we analyzed the effect of time during pregnancy on tHcy concentrations within each group (nested model). A nonorthogonal repeated contrast was used to test the significance of differences in tHcy mean concentrations between consecutive time points during pregnancy.

To investigate whether PreC tHcy concentration was a confounder in the previously explored relation, we expanded the model by fitting a three-way repeated-measures analysis of variance with 1 intrasubject factor (time during pregnancy) and 2 intersubject factors (supplement use and PreC tHcy concentration tertile). Given the significance of both two-way interactions (time during pregnancy × supplement use and time during pregnancy × PreC tHcy concentration tertile), we tested the effect of time during pregnancy on tHcy concentrations within each supplement group and PreC tHcy concentration tertile (nested model). Simple contrast was used to test the significance of differences in tHcy mean concentrations between each time point during pregnancy and the PreC period (reference value). Student's paired t test was used to compare the mean percentage differences in tHcy, hematocrit, and serum albumin between preconception and pregnancy. All P values from post hoc analyses were Bonferroni corrected. Pearson's linear correlation coefficients were determined to assess associations between pregnancy-induced changes in hematocrit, serum albumin, and tHcy. Significance for the two-tailed hypothesis was established at $P = 0.05$.

RESULTS

As shown in **Table 1**, the subjects were divided into 2 groups on the basis of whether they received folic acid supplementation. For analytic purposes to differentiate between the physiologic effects of pregnancy and the pharmacologic effect of folic acid supplements on tHcy concentrations, the 2 groups were analyzed separately. The preconception characteristics and smoking habits during pregnancy of the 2 groups are shown in Table 1. Age, parity, previous oral contraceptive use, hemoglobin concentration, body mass index, and the percentage of smokers were not significantly different between the 2 groups.

The geometric mean tHcy concentrations before and during pregnancy for both groups are shown in **Table 2**. In both groups there was a significant ($P < 0.001$) reduction in tHcy concentration during pregnancy, which was evident by 8 wk ($P < 0.001$). There was a further significant reduction at 20 wk ($P < 0.001$) in both the unsupplemented and supplemented groups. Although there was no significant difference in tHcy concentrations between 32 and 20 wk in either group, tHcy concentrations at 32 wk were significantly lower than those before conception ($P < 0.001$). There was a significantly greater reduction in tHcy concentration at 20 ($P < 0.0005$) and 32 ($P = 0.015$) wk in the group supplemented with folic acid than in the unsupplemented group.

The trend in tHcy concentration reduction during pregnancy according to PreC tHcy concentration tertile is shown in **Figure 1**. Neither the interaction between time, supplement use, and PreC tHcy concentration tertile nor the interaction between supplement use and PreC tHcy concentration tertile were significant. A similar decreasing trend in tHcy concentration throughout pregnancy is evident in each tertile with or without the use of folic acid supplements. In each tertile, tHcy concentrations significantly decreased from preconception throughout pregnancy ($P < 0.05$). There were no significant differences between the unsupplemented and supplemented groups in the magnitude of the decrease in tHcy concentration from preconception to any time point during pregnancy. In both the unsupplemented and supplemented groups, tHcy concentrations reached a trough between 20 and 32 wk of pregnancy, after which there was no further reduction.

TABLE 2
tHcy concentrations in the folic acid–unsupplemented and –supplemented groups before and during pregnancy[1]

		Pregnancy			
Group	Preconception	8 wk	20 wk	32 wk	P[2]
	μmol/L		*μmol/L*		
Unsupplemented	8.17 ± 1.28[3] [54]	6.48 ± 1.30 [54]	5.22 ± 1.29[3] [54]	5.16 ± 1.32 [53]	<0.001
Supplemented[4]	8.27 ± 1.35[3] [39]	6.32 ± 1.34 [35]	4.18 ± 1.32[3,5] [35]	4.42 ± 1.37[6] [38]	<0.001

[1] Geometric $\bar{x}$ ± SD; n in brackets.
[2] Two-way repeated-measures ANOVA (intrasubject factor: time of pregnancy; intersubject factor: supplement use). Interaction term between time of pregnancy and supplement use: $P < 0.001$.
[3] Significantly different from 8 wk, $P < 0.001$ (post hoc analysis by repeated contrast with Bonferroni correction).
[4] Supplemented with folic acid during the second or third (or both) trimester of pregnancy.
[5,6] Significantly different from unsupplemented: [5] $P < 0.0005$, [6] $P = 0.015$.

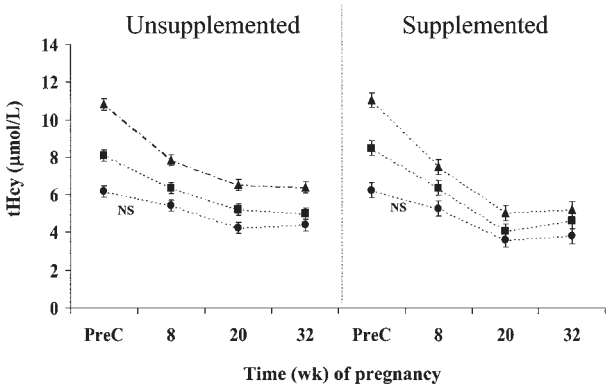

FIGURE 1. Geometric mean (±SEM) homocysteine (tHcy) concentrations before conception and during pregnancy according to preconception (PreC, 2–10 wk before conception) tHcy concentration tertile (▲, high; ■, medium; ●, low) and use of folic acid supplements. The unsupplemented group (n = 54) did not take folic acid supplements during the entire study; the supplemented group (n = 39) took folic acid during the second or third trimester. None of the women received supplements before week 20 of the pregnancy. Tertiles were based on geometric mean PreC concentrations of tHcy. Three-way repeated-measures ANOVA, in which the intrasubject factor was time during pregnancy (wk) and the intersubject factors were supplement use and PreC tHcy concentration tertile, showed significant effects of time during pregnancy ($P < 0.001$), supplement use ($P = 0.019$), and PreC tHcy concentration tertile ($P < 0.001$). Two-way interaction terms between time during pregnancy and supplement use and between time during pregnancy and PreC tHcy concentration tertile were significant ($P < 0.001$ and $P = 0.024$, respectively). The two-way interaction term between supplement use and PreC tHcy concentration tertile and the three-way interaction term were not significant ($P = 0.816$ and 0.786, respectively). Post hoc analysis by simple contrast with Bonferroni correction showed that tHcy concentrations at any time during pregnancy were significantly lower than those before conception ($P < 0.05$), except when indicated with NS. There were no significant differences between the unsupplemented and supplemented groups in the magnitude of the decrease in tHcy concentration from PreC to any time point during pregnancy.

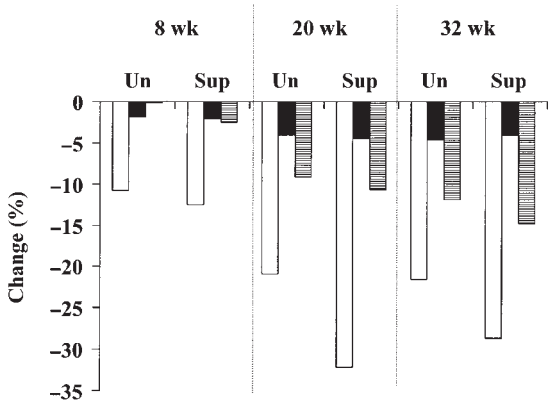

FIGURE 2. Mean percentage changes in log-transformed tHcy concentration (□), hematocrit (■), and serum albumin concentration (≣) between the preconception period and pregnancy in the unsupplemented (Un; $n = 54$ subjects who did not take folic acid supplements during the entire study) and the supplemented (Sup; $n = 39$ subjects who took folic acid during the second or third trimester) groups. None of the women were supplemented preconceptionally. Two-way repeated-measures ANOVA to test differences between preconception and pregnancy time points and between the supplemented and unsupplemented groups for each of the 3 variables tHcy concentration, hematocrit, and albumin concentration showed a significant effect of time during pregnancy for each of the 3 variables ($P < 0.001$) and a significant supplement use effect for tHcy concentration ($P < 0.001$) and albumin ($P = 0.039$). The two-way interaction terms between time during pregnancy and supplement use were nonsignificant for each of the 3 variables. A paired t test with Bonferroni correction to test the differences between the reductions in the 3 variables showed that the reduction in tHcy concentration was significantly greater than the reductions in hematocrit and albumin in all cases ($P < 0.001$).

To examine the progression of hemodilution, of the decrease in serum albumin concentration, and of the decrease in tHcy concentration during pregnancy, we plotted the percentage changes in tHcy, hematocrit, and albumin throughout pregnancy (**Figure 2**). The largest percentage reduction from preconception was for tHcy concentration. By 8 wk of pregnancy, the mean decreases in tHcy concentration were 10.8% and 12.5% in the unsupplemented and supplemented groups, respectively, despite decreases of only 1.8% and 2.1%, respectively, in hematocrit and of only 0.1% and 2.5%, respectively, in serum albumin. By 20 wk of pregnancy, the mean decreases in tHcy concentration from preconception were 20.9% and 32.2% in the unsupplemented and supplemented groups, respectively, despite decreases of only 3.9% and 4.5%, respectively, in hematocrit and of only 9.2% and 10.7%, respectively, in serum albumin. At 32 wk of pregnancy, the mean decreases in tHcy concentration (21.6% and 28.7% in the unsupplemented and supplemented groups, respectively) were also greater than those in hematocrit (4.6% and 4.0%, respectively) and serum albumin concentration (11.9% and 14.8%, respectively). Thus, at each time point during pregnancy, the mean percentage reduction in tHcy concentration was significantly greater ($P < 0.0005$) than the reductions in hematocrit and serum albumin in both the unsupplemented and supplemented groups.

There was no significant correlation between the change in hematocrit and the change in tHcy concentration at any time point during pregnancy (8 wk: $r = -0.053$, $P = 0.653$; 20 wk: $r = 0.112$, $P = 0.339$; 32 wk: $r = -0.164$, $P = 0.179$). Neither was there any significant correlation between the change in serum albumin concentration and the change in tHcy concentration (8 wk: $r = 0.029$, $P = 0.797$; 20 wk: $r = -0.158$, $P = 0.147$; 32 wk: $r = 0.042$, $P = 0.699$).

DISCUSSION

To our knowledge, this is the first study to report a significant reduction in tHcy concentration as early as 7.5–8 wk of pregnancy. Because the women in our study did not take folic acid supplements preconceptionally or during early pregnancy, our results show that the decrease in tHcy concentration at this time point is a physiologic effect of pregnancy and is independent of folic acid supplementation. Although it was evident that tHcy concentrations were significantly lower in folic acid supplement users than in their unsupplemented counterparts at 20 and 32 wk of pregnancy, it was also evident that tHcy concentrations decreased significantly in the unsupplemented group. Thus, there is a physiologically induced reduction in tHcy concentration during pregnancy. The results from the supplemented group show that folic acid enhanced the physiologic reduction in tHcy concentration due to pregnancy.

Intervention studies showed that subjects with high baseline tHcy concentrations have greater reductions in tHcy concentration after treatment with homocysteine-lowering vitamins than do those with low baseline tHcy concentrations (15). However, the tHcy response to pregnancy in this study was not driven by baseline tHcy concentration.

The results in Table 2 and Figure 1 show that tHcy concentrations decreased gradually during early and midpregnancy and reached a trough in later pregnancy, with no further decreases. In a longitudinal study, Cikot et al (10) reported only a slight reduction in tHcy concentration during early pregnancy, with no further decrease throughout mid-to-late pregnancy. This finding is clearly at odds with the results in the literature cited previously and with the observations reported in this article. The small size of the Dutch sample and the fact that tHcy concentrations were determined in nonfasting blood samples are 2 critical factors in the study design that complicate comparison of the results. Observations similar to ours of decreased tHcy concentrations in pregnancy were reported by other authors in cross-sectional studies (8, 9).

tHcy concentrations during pregnancy may be endocrine induced, and hormones may exert a stronger influence than that of vitamins in reducing tHcy concentration. Thus, a minimum tHcy concentration below which further reduction cannot occur despite increasing estrogen concentrations may be reached in pregnancy, as shown in the trough effect of this and other studies. Alternatively, other hormonal changes that occur during later pregnancy may reduce the initial tHcy-reducing effect. It has been suggested by Steegers-Theunissen et al (16) that alterations in the methionine requirement might explain the reduction in tHcy concentrations in pregnancy. It is difficult, however, to see how increased utilization of methionine by the fetus at 8 wk of pregnancy would decrease maternal tHcy concentrations in early pregnancy. Homocysteine arising from such fetal methionine metabolism would, if at all, be reflected in a simultaneous increase in maternal tHcy concentration. The initial decrease and subsequent trough in tHcy concentration may, conversely, explain reduced methionine requirements in pregnancy. Even if fetal uptake of tHcy occurred in mid-to-late pregnancy, it is an unlikely explanation for the reduction in maternal tHcy concentration at 8 wk of pregnancy.

tHcy concentrations might be expected to decrease during pregnancy due to hemodilution. In normal pregnancy, maternal plasma volume begins to increase at ≈10 wk gestation. The mean increase in plasma volume by 30–34 wk is 50% (17). In this case the reduction in tHcy concentration that we observed at 8 wk of pregnancy would have been independent of hemodilution. We used hematocrit changes, before and during pregnancy, as a marker of the evolution of hemodilution. This longitudinal study showed that the pregnancy-induced reduction in tHcy concentration was significantly greater in terms of a percentage decrease than that in hematocrit even in the advanced stages of pregnancy during which hemodilution would have been well established. This effect was observed in the unsupplemented group as well as in the group supplemented with folic acid. We found that in both groups, as well as in the combined study population, the changes in tHcy concentration were due to the time during pregnancy and were not related to a reduction in serum albumin concentration.

Furthermore, there was no significant correlation between the change in either hematocrit or albumin concentration and the change in tHcy concentration during pregnancy. If the correlation had been significant, we would have been able to quantify the contribution of these physiologic variables to the decrease in tHcy concentration by using multiple regression analysis. Because the correlations were not significant, we cannot reject the possibility that the variation in tHcy concentration during pregnancy is independent of decreases in hematocrit and albumin concentration, ie, the 2 factors conjectured to influence the pregnancy-induced decrease in tHcy concentration. The advantage of this longitudinal study is that we were able to correlate the pregnancy-induced variations in these variables on an individual basis. This type of analysis was not possible in the cross-sectional studies, in which the analysis was based on mean values from groups composed of different women.

Pregnancy involves many complex hormonal changes, from increases or decreases in circulating female hormones that are present in the nonpregnant state to the introduction of pregnancy-specific hormones. There is increasing evidence that female hormones decrease tHcy concentrations. Wouters et al (18) reported a significant correlation between postmethionine plasma homocysteine and 17β-estradiol in premenopausal women. Elderly estrogen users have lower tHcy concentrations than do their elderly postmenopausal counterparts who do not receive estrogen replacement therapy (19). Kim et al (20) showed a significant decrease in plasma tHcy concentration in male rats that were administered estradiol, and Giri et al (21) showed that oral 17β-estradiol reduced tHcy concentrations by 11% in healthy elderly men. Evidence has emerged that the difference between tHcy concentrations in men and women may be due, at least in part, to hormonal differences that become evident as early as puberty. Morris et al (19) reported that at the onset of menarche, tHcy concentrations in females become significantly lower than those in males. Tallova et al (22) reported that in premenopausal women, mean tHcy concentrations in the follicular phase of the menstrual cycle were significantly higher (1.9 μmol/L) than those in the luteal phase. A slight negative correlation was also found between tHcy and estradiol in both phases of the menstrual cycle.

In conclusion, our data refute previous hypotheses for the pregnancy-associated reduction in tHcy concentration. The reduction in tHcy concentration occurs in the absence of folic acid supplementation and is not correlated with reductions in hematocrit or serum albumin concentration. Given the evidence in the literature for the influence of female hormones on tHcy concentration, it is possible that decreases in tHcy concentration during pregnancy are mainly endocrine-based.

REFERENCES

1. Verhoef P, Meleady R, Daly LE, Graham IM, Robinson K, Boers GHJ. Homocysteine, vitamin status and risk of vascular disease. Eur Heart J 1999;20:1234–44.
2. 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.
3. Van der Molen EF, Verbruggen B, Nováková I, Eskes TKAB, Monnens LAH, Blom HJ. Hyperhomocysteinemia and other thrombotic risk factors in women with placental vasculopathy. Br J Obstet Gynaecol 2000;107:785–91.
4. Mills JL, McPartlin JM, Kirke PN, et al. Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet 1995;345:149–51.
5. Goddijn-Wessel TAW, Wouters MGAJ, van den Molen EF, et al.

Hyperhomocysteinemia: a risk factor for placental abruption or infarction. Eur J Obstet Gynecol Reprod Biol 1996;66:23–9.
6. Rajkovic A, Catalano PM, Malinow MR. Elevated homocysteine levels with preeclampsia. Obstet Gynecol 1997;90:168–71.
7. Kang SS, Wong PW, Zhou JM, Cook HY. Total homocyst(e)ine in plasma and amniotic fluid of pregnant women. Metabolism 1986;35: 889–91.
8. Andersson A, Hultberg B, Brattström L, Isaksson A. Decreased serum homocysteine in pregnancy. Eur J Clin Chem Clin Biochem 1992;30:377–9.
9. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. Am J Obstet Gynecol 1999;180:660–4.
10. Cikot RJLM, Steegers-Theunissen RPM, Thomas CMG, de Boo TM, Merkus HMWM, Steegers EAP. Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr 2001;85:49–58.
11. Malinow MR, Rajkovic A, Duell PB, Hess DH, 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.
12. Institute of Statistics, Catalonia. Population census and municipal records of inhabitants, May 1996. Internet: http://www.idescat.es (accessed 19 March 2001) (in Catalan).
13. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. JAMA 1997;277:925–6.
14. Pagán K, Hou J, Goldenberg RL, Cliver SP, Tamura T. Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy. Clin Chim Acta 2001;306:103–9.
15. den Heijer M, Brouwer IA, Bos GMJ, et al. Vitamin supplementation reduces blood homocysteine levels. Arterioscler Thromb Vasc Biol 1998;18:356–61.
16. Steegers-Theunissen RPM, Wathen NC, Eskes TKAB, van Raaij-Selten B, Chard T. Maternal and fetal levels of methionine and homocysteine in early pregnancy. Br J Obstet Gynaecol 1997;104:20–4.
17. Cruikshank DP, Wigton TR, Hays PM. Maternal physiology in pregnancy. In: Gabbe SG, Niebyl JR, Simpson JL, eds. Obstetrics: normal and problem pregnancies. New York: Churchill Livingstone, 1996:91–109.
18. Wouters MG, Moorrees MT, van der Mooren MJ, et al. Plasma homocysteine and menopausal status. Eur J Clin Invest 1995;25:801–5.
19. Morris MS, Jacques PF, Selhub J, Rosenberg IH. Total homocysteine and estrogen status indicators in the Third National Health and Nutrition Examination Survey. Am J Epidemiol 2000;152:140–8.
20. Kim MH, Kim E, Passen EL, Meyer J, Kang SS. Cortisol and estradiol: nongenetic factors for hyperhomocyst(e)inemia. Metabolism 1997;46:247–9.
21. Giri S, Thompson PD, Taxel P, et al. Oral estrogen improves serum lipids, homocysteine and fibrinolysis in elderly men. Atherosclerosis 1998;137:359–66.
22. Tallova J, Tomandi J, Bicikova M, Hill M. Changes of plasma total homocysteine levels during the menstrual cycle. Eur J Clin Invest 1999;29:1041–4.