Homocysteine and Folic Acid Are Inversely Related in Black Women With Preeclampsia

Thelma E. Patrick, Robert W. Powers, Ashi R. Daftary, Roberta B. Ness, James M. Roberts

Abstract—Black women have an increased risk of preeclampsia compared with white women. Plasma homocysteine is increased in preeclampsia. Homocysteine concentrations are affected by nutritional deficiencies, particularly decreased folic acid and B12, leading to increased homocysteine. Previous studies have reported racial differences in nutritional intake including folic acid. Therefore, we investigated whether there were racial differences in plasma homocysteine, folic acid, and vitamin B12 among women with preeclampsia. We tested for an association between homocysteine and folic acid and B12, and we hypothesized an inverse relationship of homocysteine and folic acid in preeclampsia, more so in black women in whom preeclampsia developed. Black women with preeclampsia (n=26) had elevated homocysteine concentrations (8.7±1.4 μmol/L) compared with black women with normal pregnancy (n=52, 7.6±0.5 μmol/L), white women with preeclampsia (n=34, 7.5±0.6 μmol/L), and white women with normal pregnancy (n=48, 5.5±0.3 μmol/L). Folic acid concentrations were lower in black women (14.1±0.8 ng/mL) compared with white women (18.5±0.9 ng/mL, P<0.01). However, plasma homocysteine was inversely related to folic acid only among black women with preeclampsia (r=−0.23, P=0.01). These racial differences may have implications for the higher rates of preeclampsia in this group and may have long-term implications for future cardiovascular risk. Racial differences in diet, adherence to folic acid supplementation, or interactions of nutritional and maternal factors warrant further study by race and pregnancy status. (Hypertension. 2004;43:1279-1282.)

Key Words: preeclampsia ■ race

Preeclampsia, especially in its most severe forms, is more common in black women. Plasma homocysteine concentrations are higher in women with preeclampsia, and this increase is present 10 weeks postpartum in women who have had severe preeclampsia, suggesting elevated homocysteine may increase the risk for preeclampsia. Premenopausal black women have higher plasma total homocysteine than white women. Differences in homocysteine concentrations according to race have not been studied during normal pregnancy and preeclampsia.

Homocysteine concentrations are tightly regulated by 2 main enzymatic pathways. Homocysteine can be remethylated to methionine by a pathway requiring folic acid as a methyl donor. In addition to adequate folic acid, the pathway requires vitamin B12 as an important cofactor. Alternatively, homocysteine can be removed by transsulfuration, a pathway dependent on the cofactor vitamin B6. Enzymatic defects in either of these pathway results in increased homocysteine, as does deficiency of folic acid, vitamin B6, or B12. Interestingly, nonpregnant black women are reported to have lower serum folic acid and B6 levels but higher vitamin B12 levels than white women.

We proposed that the increased frequency of preeclampsia among black women could be secondary to increased serum homocysteine, perhaps because of different dietary intake of vitamins B12 or folic acid. To test this hypothesis, we assessed racial differences in homocysteine, folic acid, and vitamin B12 during normal pregnancy and in pregnancies complicated by preeclampsia.

Methods

Subjects

Patients were recruited at the time of admission to labor and delivery at Magee-Womens Hospital as part of the ongoing investigation of preeclampsia. All subjects participated in the study voluntarily and informed consent was obtained. The study protocol was approved by the Magee-Womens Hospital Institutional Review Board. Women were assigned a diagnosis of normal pregnancy or preeclampsia by a panel of clinical experts. Preeclampsia was defined using the criteria of gestational hypertension, proteinuria, hyperuricemia and the reversal of hypertension and proteinuria after pregnancy. Gestational hypertension was defined as an increase of 30 mm Hg systolic or 15 mm Hg diastolic blood pressure, compared with values obtained before 20 weeks of gestation and an absolute blood pressure >140/90 mm Hg after 20 weeks of gestation if earlier blood pressures were not known. Since these samples were collected, the National High Blood Pressure Education Program (NHBEP) has recommended that the gestational hypertension of preeclampsia be defined by an absolute blood pressure of 140 mm Hg systolic or 90 mm Hg diastolic rather than by incremental blood pressure.
increase. In this cohort, 98% of women also satisfied these criteria for gestational hypertension. Proteinuria was defined as >300 mg/24-hour urine collection or >2+ on a voided or >1+ on a catheterized random urine sample, or a protein-to-creatinine ratio of >0.30. Hyperuricemia was defined as >1 SD above values at gestational age of sampling (at term >5.5 mmol/L). The control population was composed of women with uncomplicated pregnancies. These women were normotensive throughout pregnancy without proteinuria. All patients were nulliparous and no patient was known to have chronic hypertension or renal or metabolic disease. The controls were matched to the preeclampsia group in terms of body mass index.

**Blood Samples**

Maternal venous blood samples were collected in the labor suite before delivery. Plasma was prepared with EDTA, and samples were aliquoted and stored at −80°C until assayed.

**Homocysteine Determination**

Total plasma homocysteine was analyzed according to the procedure of Jacobsen et al. The thiol derivatives were detected fluorometrically with excitation at 390 nm and emission at 470 nm. Calibration curves were generated for every assay and were included at the beginning and end of each analytical set. They consisted of normal human plasma spiked with 0, 2.5, 5, 7.5, 10, 15, 20, and 25 μmol/L L-homocysteine. The coefficient of variation between assays was 8%.

**Folic Acid and B12 Determination**

Serum folic acid and B12 concentrations were determined with a radioimmunoassay from Diagnostics Products Corp. The assay procedure was that described by the manufacturer. The detection limit of the assay for folic acid is 0.3 ng/mL and for B12 is 50 pg/mL. The interassay coefficient of variation for folic acid was 9.4% and 6% for B12.

**Statistical Methods**

Means and standard deviations are reported. Differences in homocysteine, folic acid, and vitamin B12 in the subject groups were analyzed by 2-way ANOVA. Bonferroni/Dunn post-hoc testing was used as appropriate with statistical significance accepted at P<0.01. Correlations were by standard regression analysis with statistical significance accepted at P<0.05.

**Results**

The baseline characteristics and demographics of the 4 subject groups studied are summarized in Table 1. White women with preeclampsia were older than black women with normal pregnancy or preeclampsia and white women with normal pregnancy (P<0.0001). Delivery and gestational age at time of sampling were significantly earlier for women with preeclampsia than for women with normal pregnancy, but were not different by race (P<0.0001). Prepregnancy body mass index was not different by either race or pregnancy outcome (Table 1).

The mean plasma concentration of total homocysteine was significantly higher in black women compared with white women (P<0.01) and in women with preeclampsia compared with normal pregnancy (P<0.03) (Table 2). Plasma folic acid was significantly lower in the black women when compared

<table>
<thead>
<tr>
<th>TABLE 1. Clinical Characteristics of the Patient Groups</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>Maternal age (y)*</td>
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<td>Prepregnant body mass index (kg/m²)</td>
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<td>Weeks gestation at delivery (wk)†</td>
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White women in whom preeclampsia developed were older as compared to all other subject groups. White and black women with preeclampsia delivered at an earlier gestational age at delivery compared to women with normal pregnancy. Regardless of race or diagnosis, subjects were of similar body size. Data are presented as mean±SD. Analysis of variance, with post-hoc analysis, was used to assess differences between groups.

*ANOVA for difference in age (P<0.0001 overall), with white women with preeclampsia older than all other groups (P=0.001).
†ANOVA for difference in gestational age at delivery (P<0.0001 overall), with white and black women with preeclampsia delivering earlier than their respective racial counterparts with normal pregnancy (P<0.001).

<table>
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<th>TABLE 2. Serum Homocysteine, Folic Acid, and B12 Concentrations</th>
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<tr>
<td>Measure</td>
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<tr>
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<tr>
<td>Homocysteine (μM)*</td>
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<tr>
<td>Folic acid (ng/mL)†</td>
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<td>B12 (pg/mL)†</td>
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Homocysteine is higher in black women with preeclampsia compared to black women with normal pregnancy, white women with normal pregnancy, and white women with pregnancies complicated by preeclampsia. There are no differences in folic acid or B12 by diagnosis; however, there are differences noted by race. Folic acid is higher in white women compared to black women, whereas B12 concentration is higher in black women than in white women. Data are presented as mean±SEM. Analysis of variance, with post-hoc analysis, was used to assess differences between groups.

*P<0.01 for difference by race, P<0.02 for difference by diagnosis, nonsignificant interaction.
†P<0.0001 for difference by race.
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the black women. These data suggest a nutritional interven-
significantly lower, and vitamin B12 significantly higher in
women, plasma total homocysteine concentrations did not
differ significantly by race, but plasma folic acid remained
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women, B12 concentrations than white women.4 When these data
compared with white women (P<0.0001) (Table 2); however, no
differences were observed in B12 concentrations when com-
pared by diagnosis.

Discussion
Our findings confirm the previously reported increase of
plasma homocysteine concentration in women with pre-
eclampsia. Furthermore, the higher concentration of homocys-
teine and folic acid in the black women with preeclampsia
(r=0.23, P<0.01) (Figure), but this relationship was not
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Our findings are consistent with a study in nonpregnant
women. Premenopausal black women had higher plasma total
homocysteine, lower plasma folate acid, and higher vitamin
B12 concentrations than white women.4 When these data
were analyzed adjusting for multivitamin use and intake of
ready-to-eat cereal, reported to be more prevalent in white
women, plasma total homocysteine concentrations did not
differ significantly by race, but plasma folate acid remained
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tion may be of value to increase folate acid and lower
homocysteine concentrations in our black population. Al-
though data regarding nutritional intake and compliance with
prenatal vitamins were not obtained, in general, the means
and distribution of plasma folic acid and vitamin B12 were
similar by race regardless of diagnosis.

A growing body of literature indicates the importance of
ethnic and racial factors to considerations of B12 metabolism
and its disorders. Blacks have significantly higher B12 levels
than whites.6–8 Because serum B12 levels are often influ-
enced by factors unrelated to B12 intake, stores, or defi-
cency, it is unclear whether the differences in concentrations
reflect B12 status. The ethnic differences in B12 concentra-
tion, which are present in cord blood, childhood, and preg-
nancy, probably arise from combinations of hereditary and
acquired causes.9 From our data and reported studies,10
continued analysis by race is necessary when addressing
homocysteine, folic acid, and B12 in relation to health and
disease.

Before initiating a nutritional study, other possible sources
of increased homocysteine must be ruled out. Although


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There is an inverse relationship of folic acid and homocysteine.
When analyzed by diagnostic and racial group, this relationship
is significant only for black women with preeclampsia (R²=0.23,
P<0.01).

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weeks, followed by a period of weeks or months when folic acid concentrations are low but there was no other evidence of deficiency;9 however, the mean value of folic acid exceeded this level for all subject groups in this study.

Perspectives
Homocysteine, a risk factor for atherosclerosis, is higher in black pregnant women and higher still in black women with preeclampsia compared with white pregnant women, and these differences are partially related to folic acid. This finding has implications for the higher rates of preeclampsia in blacks and may have long-term implications for future cardiovascular risk. Lastly, racial differences in ath- erosclerotic risk factors merit further exploration for their significance to the higher incidence of preeclampsia in black women.

Acknowledgments
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References
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