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Hypertension. 2004;43:1279-1282; originally published online April 19, 2004;
doi: 10.1161/01.HYP.0000126580.81230.da
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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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.1,2 Plasma homocysteine concentrations are higher in women with preeclampsia, and this increase is present 10 weeks postpartum in women who had severe preeclampsia, suggesting elevated homocysteine may increase the risk for preeclampsia.3 Premenopausal black women have higher plasma total homocysteine than white women.4 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.4

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 1. Clinical Characteristics of the Patient Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>White, Normal Pregnancy (n=51)</th>
<th>White, Preeclampsia (n=34)</th>
<th>Black, Normal Pregnancy (n=52)</th>
<th>Black, Preeclampsia (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)‡*</td>
<td>23.5±4.2</td>
<td>27.3±6.2$§</td>
<td>21.6±4.5</td>
<td>22.2±5.0</td>
</tr>
<tr>
<td>Prepregnant body mass index (kg/m²)</td>
<td>24.1±5.6</td>
<td>24.4±4.4</td>
<td>25.4±6.6</td>
<td>24.8±6.5</td>
</tr>
<tr>
<td>Weeks gestation at delivery (wk)†</td>
<td>37.9±2.5</td>
<td>34.8±3.9‡$</td>
<td>38.2±2.9</td>
<td>35.8±3.5$§</td>
</tr>
</tbody>
</table>

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 2. Serum Homocysteine, Folic Acid, and B12 Concentrations

<table>
<thead>
<tr>
<th>Measures</th>
<th>White, Normal Pregnancy (n=51)</th>
<th>White, Preeclampsia (n=34)</th>
<th>Black, Normal Pregnancy (n=52)</th>
<th>Black, Preeclampsia (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (μM)‡</td>
<td>5.51±0.3</td>
<td>7.48±0.6</td>
<td>7.59±0.5</td>
<td>8.73±1.4</td>
</tr>
<tr>
<td>Folic acid (ng/mL)†</td>
<td>18.54±0.9</td>
<td>18.39±1.2</td>
<td>14.10±0.7</td>
<td>14.45±0.9</td>
</tr>
<tr>
<td>B12 (pg/mL)†</td>
<td>205.03±9.9</td>
<td>281.40±28.0</td>
<td>359.52±27.4</td>
<td>353.29±29.7</td>
</tr>
</tbody>
</table>

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.
though data regarding nutritional intake and compliance with homocysteine concentrations in our black population. Al-

tation may be of value to increase folic acid and lower the black women. These data suggest a nutritional interven-
significantly lower, and vitamin B12 significantly higher in already-to-eat cereal, reported to be more prevalent in white women, plasma total homocysteine concentrations did not differ significantly by race, but plasma folic acid remained significantly lower, and vitamin B12 significantly higher in the black women. These data suggest a nutritional intervention may be of value to increase folic acid and lower homocysteine concentrations in our black population. Although 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. Because serum B12 levels are often influenced by factors unrelated to B12 intake, stores, or deficiency, it is unclear whether the differences in concentrations reflect B12 status. The ethnic differences in B12 concentration, which are present in cord blood, childhood, and pregnancy, probably arise from combinations of hereditary and acquired causes. From our data and reported studies, 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 genetic mutations were not explored in this study, one of the most common genetic polymorphisms associated with mild hyperhomocysteinemia is a point mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, a C -to-T substitution at nucleotide 677 (C677T). This mutation has a very low incidence among black populations. Several studies have investigated the incidence of this polymorphism among women with preeclampsia. The majority of these studies report no significant increase in the prevalence of this polymorphism among women with preeclampsia compared with women with a normal pregnancy outcome.

We did not assess the vitamin B6, the cofactor in the transsulfuration pathway. The association of low levels of vitamin B6 and cardiovascular disease has been reported to be independent of homocysteine when studied in general populations and in the assessment of racial differences between Asian Americans and whites. Further, the addition of B vitamins to folic acid supplementation achieves little additive effect in lowering homocysteine.

In general, homocysteine concentration increases with age and decreases as pregnancy advances. Walker et al report a concentration of 5.5 µmol/L (95% CI: 3.3 to 7.5) at 36 to 42 weeks of gestation, compared with 7.9 µmol/L (95% CI: 6.2 to 9.6) in nonpregnant women. The homocysteine concentrations in this study were higher in the younger black group and, despite sampling at an earlier gestational age, were higher in the presence of a diagnosis of preeclampsia.

Finally, it may be beneficial in future studies to assess erythrocyte folic acid concentration to differentiate between short-term and long-term nutritional deficits of folic acid. Inadequate folic acid intake first leads to a decrease in serum folic acid concentration, then to a decrease in erythrocyte folic acid concentration, a rise in homocysteine concentration, and eventually to megaloblastic changes in the bone marrow and other tissues with rapidly dividing cells. Serum folic acid concentration of <3 ng/mL indicates a negative folic acid balance at the time that a blood sample is drawn. Erythrocyte folic acid concentration does not reflect recent or transient changes in dietary folic acid intake. In all experimental studies subjecting volunteers to folic acid deprivation, a decrease in folate concentration occurred within 1 to 3

Discussion

Our findings confirm the previously reported increase of plasma homocysteine concentration in women with pre-
eclampsia. Furthermore, the higher concentration of homocysteine previously reported in premenopausal black women compared with white women was confirmed during pregnancy. Folic acid was lower in black women and was correlated with increased homocysteine concentration only in black women with preeclampsia. As previously reported, vitamin B12 was not reduced and in fact was increased in black women, and thus did not contribute to the increased homocysteine in these women. Decreased folic acid and increased vitamin B12 concentrations were specific to racial comparisons and were not different by pregnancy outcome.

Our findings are consistent with a study in nonpregnant women. Premenopausal black women had higher plasma total homocysteine, lower plasma folic acid, and higher vitamin B12 concentrations than white women. 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 folic acid remained significantly lower, and vitamin B12 significantly higher in the black women. These data suggest a nutritional intervention may be of value to increase folic acid and lower homocysteine concentrations in our black population. Although data regarding nutritional intake and compliance with

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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; 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 atherothrombotic risk factors merit further exploration for their significance to the higher incidence of preeclampsia in black women.

**Acknowledgments**

Supported by funding from National Institutes of Health 2PO1 HD30367, 5MO1 RR00056, and R55 NR04988-01.

**References**