Plasma Homocysteine Concentrations in a Healthy Population Living in Burkina Faso

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ABSTRACT

Background: The low circulating levels of homocysteine observed in a South African black population have been associated with a low occurrence of coronary heart disease.

Objective: The aim of this study was to determine whether the observation of reduced homocysteine levels in South African black subjects is valid in a black population living in West Africa.

Methods: Plasma homocysteine and cysteine levels were determined by high-performance liquid chromatography in 171 healthy black adults of different tribal groups and 28 healthy black Mossi children, all living in Ouagadougou, Burkina Faso. The same determinations were made in 67 healthy white adults and 25 healthy white children living in Ouagadougou. The black subjects habitually consumed a diet of millet flour with vegetable sauce, whereas the white subjects consumed a Mediterranean-type diet consisting of pasta and tomato sauce, providing adequate amounts of calories and vitamins.

Results: Plasma homocysteine levels were lower in the black adults and children, particularly the females, than in the white subjects. However, no difference between males and females was observed in either white adults or children.

Conclusions: These results showed that the previously observed difference in homocysteine metabolism in black South Africans also exists in the black population of Burkina Faso, particularly in females of all ages. This difference between black and white people living in the same area could be due to genetic and environmental factors, even if the role of diet cannot be excluded. The lower plasma homocysteine levels in black people may play a protective role against coronary artery disease by reducing endothelial damage.

Key words: homocysteine, black people, Burkina Faso. (Curr Ther Res Clin Exp. 2000;61:659–668)
INTRODUCTION

Plasma levels of homocysteine are the result of an interplay between congenital and environmental factors. Homocysteine is the demethylated derivative of methionine, an essential amino acid derived from dietary and recycled endogenous proteins. Fifty percent of intracellular homocysteine is remethylated to methionine, and the remainder may be transsulfurated to cystathionine through a reaction catalyzed by the vitamin B₆-dependent cystathionine β-synthase. Cystathionine then forms cysteine, which is required for the synthesis of many compounds, including glutathione, the most important thiol.

Several studies carried out in the past 2 decades have demonstrated that mild to moderate hyperhomocystinemia may be associated with a higher risk of coronary and other vascular diseases. The effect on thrombotic risk of reducing plasma levels of homocysteine through supplementation of vitamins B₆, B₁₂, folic acid, and others has not been clearly demonstrated. A study of plasma homocysteine levels in a South African black population has demonstrated that the lower circulating levels of homocysteine observed in this population were associated with a low occurrence of coronary heart disease.

Burkina Faso, formerly known as Upper Volta, is a country in West Africa near the Niger River with a population of 11 million living in an area of 274,200 km². The inhabitants of Burkina Faso are distributed among 8000 villages, although 27% of the population currently lives in urban centers, the result of a strong movement from the country over recent years. The landlocked nature of this region and its difficult environmental conditions have served to keep the genetic composition of the population largely unchanged. The southern savanna in which Ouagadougou is located is inhabited by the Mossi people, whereas the arid Sahel contains a variety of ethnic groups, including the Songhrais, Mellebe, Peuhl, and Tuaregs. The economic system is based on agriculture, which involves >95% of the working population. The rural living conditions of this population differ from those of the largely urban South African population. The subjects in the present study consumed a traditional diet and lived in a depressed area with a high incidence of malaria and intestinal parasites.

The reduced levels of plasma homocysteine seen in black people living in South Africa suggest the existence of an imbalance in homocysteine metabolism in black compared with white people that is confirmed by the low increase in plasma homocysteine observed after oral methionine loading. If racial differences can explain these lower levels of homocysteine, then the higher levels of this amino acid reported in black people living in the United States, with their Western lifestyle and greater incidence of obesity, support a role for environmental factors.
The aim of the present study was to measure circulating plasma homocysteine levels in healthy black and white subjects living in Burkina Faso, a depressed area of Africa with a high incidence of malaria, traditional nutritional habits, and a low incidence of coronary heart disease.

SUBJECTS AND METHODS

Selection and Screening of Subjects

Subjects were recruited at random from a population of 1200 patients visiting the Centre Medical St Camille in Ouagadougou for a basic health screening. Exclusions included refusal to undergo more laboratory tests; weight >97th percentile for the subject's age; systolic blood pressure >2 SDs above normal for the subject's age; clinical evidence of atherothrombotic cardiovascular disease, diabetes mellitus, renal or hepatic disease, thyroid disease, or cardiomyopathy; use of anticonvulsant medication; chronic alcohol abuse; and the presence of other pathologies associated with increased plasma homocysteine levels (eg, low levels of folate or vitamin B12).

Written informed consent was obtained from all study participants. The study protocol was reviewed and approved by the ethics committee of the Centre Medical St Camille. Measures of subjects' health status and organ function were recorded at the beginning of the study, including body weight, body mass index (BMI) (weight in kg/height in m²), and an electrocardiogram. Routine laboratory tests were also performed.

Using a standard protocol, blood pressure was measured in triplicate in both arms with the subject in a seated position. Other measures included heart rate (measured at the radial artery) and ventilation rate. Clinical chemistry tests were performed in the medical center laboratory using standard methods. These included hemoglobin electrophoresis, osmotic fragility of erythrocytes, and serum folate and vitamin B₁₂ determinations, as required.

Blood Sampling

Blood samples were collected at the end of the screening interview. Ten milliliters of peripheral blood was collected, 5 mL in plain tubes and 5 mL in EDTA. The tubes containing blood in EDTA were centrifuged at 1500g for 10 minutes at 4°C, whereas the tubes containing blood without additive were left to stand at room temperature for 30 minutes. Plasma and serum were then separated and stored at -80°C (in 250-μL aliquots).

Measurement of Plasma Homocysteine

High-performance liquid chromatography was used to determine levels of circulating plasma homocysteine and cysteine.¹³
**Statistical Analysis**

Results are expressed as medians and ranges, or means ± SD. The SDs of the data from all groups were analyzed using the Student t test. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

The study enrolled 291 subjects with normal results on the health screening. Subjects were separated into 2 groups by race. The first group included 171 healthy black adults (41 males, 130 females) aged from 17 to 60 years (median, 26 years) from different tribal groups (151 Mossi, 7 Gaurussi, 5 Bisse, 4 Dagara, 4 Peuhl) and 28 healthy black Mossi children (14 males, 14 females) aged from 4 to 24 months (median, 11 months). The second group was composed of 67 healthy white adults (30 males, 37 females) aged from 10 to 60 years (median, 29 years) and 25 healthy white children (12 males, 13 females) aged from 6 to 24 months (median, 12 months).

All subjects were from Ouagadougou and had conventional dietary habits. The black subjects habitually ate cereals, millet flour with vegetable sauce, seasonal fruits, and, once a week, chicken, pork, mutton, or beef. The white subjects ate a Mediterranean-type diet that included pasta and tomato sauce, beef or pork every day, cooked vegetables, and seasonal fruits, with adequate intake of calories and vitamins.

Results of the clinical and laboratory investigations showed all subjects to be in good health, with a BMI between 23 and 28 mg/dL and blood pressure, heart rate, and ventilation rate all in the normal range for their age (<95th percentile). Serum total cholesterol was 178.2 ± 66.6 mg/dL in black subjects and 196.5 ± 68.2 mg/dL in white subjects, a nonsignificant difference. Triglyceride levels were also higher in white subjects (103.7 ± 38.9 mg/dL) compared with black subjects (97.9 ± 31.6 mg/dL). The hemoglobin (Hb) level was significantly lower in black women compared with white women, a difference not observed between black and white males. Hemoglobin electrophoresis demonstrated that 70 of 199 subjects were carriers of hemoglobinopathy (Hb S or Hb C), but none were homozygous or heterozygous for both. Levels of folic acid and vitamin B₁₂ were within the normal range (folic acid, 15.2 ± 6.7 ng/mL in black subjects and 13.6 ± 5.8 ng/mL in white subjects; vitamin B₁₂, 425.2 ± 46.4 pg/mL in black subjects and 418.9 ± 44.1 pg/mL in white subjects).

Mean homocysteine values were lower in black adults than in white adults (Table I). Black males had higher levels of homocysteine compared with black females. No such difference between the sexes was observed in the white subjects. Plasma homocysteine levels were also lower in black children than in white children (Table II). Furthermore, a statistically significant difference in homocysteine levels was observed between male
Table I. Homocysteine and cysteine levels in black and white adults living in Burkina Faso, by sex. Values were measured as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Homocysteine (µmol/L)</th>
<th>Cysteine (µmol/L)</th>
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<tbody>
<tr>
<td>Black adults (n = 171)</td>
<td></td>
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</tr>
<tr>
<td>Male (n = 41)</td>
<td>4.12 ± 2.30*</td>
<td>85.24 ± 26.28*</td>
</tr>
<tr>
<td>Female (n = 130)</td>
<td>3.59 ± 1.73*</td>
<td>83.21 ± 22.52*</td>
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<tr>
<td>White adults (n = 67)</td>
<td></td>
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<tr>
<td>Male (n = 30)</td>
<td>8.61 ± 3.20</td>
<td>113.54 ± 20.00</td>
</tr>
<tr>
<td>Female (n = 37)</td>
<td>8.98 ± 3.61</td>
<td>118.23 ± 17.14</td>
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* P < 0.001, black versus white adults (Student t test).
† P < 0.001, black males versus black females (Student t test).
‡ P < 0.001, black females versus white females (Student t test).

and female black children (P = 0.02). Levels of cysteine were significantly lower in black adults than in white adults (Table I), with no significant difference between males and females in either group. As with homocysteine levels, cysteine levels were lower in black children than in white children, with no significant difference between males and females. As expected, a positive correlation was found between homocysteine and cysteine levels in both black adults (Figure 1) and black children (Figure 2). Homocysteine levels in black adults and children are compared with those in white adults and children in Figures 3 and 4.

Considering the black subjects of Mossi origin, who were more numerous, no significant difference in mean homocysteine (4.27 ± 2.50 µmol/L) or cysteine levels (86.13 ± 26.32 µmol/L) was observed between them and black subjects of ethnic groups that were less well represented.

No significant correlation was found between subjects' age and plasma homocysteine or cysteine levels.

DISCUSSION

In the past 10 years, elevation in plasma homocysteine levels has been widely studied as an independent risk factor for atherosclerosis. Homocys-

Table II. Homocysteine and cysteine levels in black and white children living in Burkina Faso, by sex. Values were measured as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Homocysteine (µmol/L)</th>
<th>Cysteine (µmol/L)</th>
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<tbody>
<tr>
<td>Black children (n = 28)</td>
<td></td>
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</tr>
<tr>
<td>Male (n = 14)</td>
<td>4.11 ± 2.06*</td>
<td>55.72 ± 31.96*</td>
</tr>
<tr>
<td>Female (n = 14)</td>
<td>3.69 ± 1.23*</td>
<td>57.72 ± 23.81*</td>
</tr>
<tr>
<td>White children (n = 25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 12)</td>
<td>8.55 ± 3.26</td>
<td>93.62 ± 19.05</td>
</tr>
<tr>
<td>Female (n = 13)</td>
<td>8.77 ± 3.54</td>
<td>100.45 ± 18.53</td>
</tr>
</tbody>
</table>

* P < 0.001, black versus white children (Student t test).
† P = 0.02, black males versus black females (Student t test).
Tinemia, an inborn error of metabolism, was first described in 1962.\textsuperscript{14,15} This rare disorder, for which a homozygous defect in the enzyme cystathionine $\beta$-synthase is the most frequent etiology, produces multiple abnormalities, including virulent atherosclerotic plaque formation and widespread arterial and venous thrombosis, usually resulting in death as early as the first decade.\textsuperscript{16} Similar vascular pathology observed in patients with elevated plasma homocysteine levels secondary to disparate enzymatic
Figure 3. Homocysteine and cysteine concentrations in 171 black adults, by sex, as a proportion of those in white adults. Homocys = homocysteine; T = total; M = male; F = female.

Figure 4. Homocysteine and cysteine concentrations in 28 black children, by sex, as a proportion of those in white children. Homocys = homocysteine; T = total; M = male; F = female.
defects suggests that homocysteine is the causative agent. Moreover, the findings in fraternal and identical twins suggest that homocysteine levels may be genetically controlled.\textsuperscript{17}

A study in a large population of American adolescents and adults has demonstrated that homocysteine concentrations are lower in females than in males in various racial and ethnic groups; and significantly lower in Mexican American females than in non-Hispanic white and Hispanic black females.\textsuperscript{18} A significant interaction between homocysteine level, age, and sex was seen in this study, with homocysteine levels in females diverging from those in males at a younger age and converging with those in males at an older age. These observations confirm previous observations in black traditional people from South Africa\textsuperscript{9} and support our findings of a difference in homocysteine concentrations between males and females and between black and white subjects.

In another study, 6 weeks of supplementation with 1.0 mg of folic acid, 400 \( \mu \)g of vitamin \( B_{12} \), and 10 \( \mu \)g of vitamin \( B_{6} \) reduced mean fasting homocysteine concentrations in white subjects, whereas this effect was less evident in black subjects, suggesting a genetic factor.\textsuperscript{10} Few studies to date have examined the risk associated with elevated plasma homocysteine levels in women.\textsuperscript{19,20} However, like premenopausal white women with the same metabolic status, premenopausal black women living in the United States and having a high BMI, hypertension, greater consumption of saturated fat and cholesterol, and high levels of lipoprotein(a) have been shown to have high plasma homocysteine levels and an increased risk of coronary heart disease.\textsuperscript{21} This suggests that environmental conditions may have a major or minor impact on the genetic factors that regulate plasma homocysteine levels.

The plasma homocysteine concentration in white subjects is positively skewed with age. Furthermore, children with a positive parental history of coronary artery disease have significantly greater age-adjusted mean homocysteine levels than do those without such parental history.\textsuperscript{22} Individuals with high plasma homocysteine concentrations probably acquire this characteristic during or after young adulthood, because the plasma homocysteine frequency distribution is normal in white children.

Diet may have been an important factor in our study, since there were several differences in the composition of the meals consumed by black and white subjects. The black diet consisted primarily of millet flour and vegetables (beans and potatoes), whereas the white diet consisted of pasta, rice, and meats. Both consumed sufficient amounts of local seasonal fruits to ensure similar intake of calories and vitamins. Moreover, when we consider that black people living in the United States, where the Western lifestyle is conducive to obesity, have elevated levels of plasma homocysteine,\textsuperscript{12} it is probable that diet, as well as genetic and racial factors, may influence circulating homocysteine concentrations.
CONCLUSIONS

The results of this study confirm previous observations that black people are biochemically less responsive to atherogenic factors than are white people and that these differences are present since infancy.²³ The lower incidence of coronary artery disease observed in black people living in Africa⁹ may be a consequence of the protective role of modified homocysteine metabolism on endothelial damage.

Acknowledgments

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References:


