Hormone Levels During Dietary Changes in Premenopausal African-American Women

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Background: In the United States, 5-year survival rates of 69% and 84%, respectively, have recently been reported for African-American and Caucasian women diagnosed with breast cancer. Differences in the levels of endogenous sex hormones in these populations could explain some of the variation in survival rates, since estrogen is recognized as a risk factor for this type of cancer. Purpose: Dietary factors are known to affect endogenous hormone levels; therefore, our study was designed to determine the serum hormone levels of African-American women consuming a typical North American diet, to determine the effect of a low-fat and high-fiber diet on their serum hormone levels, and to compare the base-line serum hormone levels in the African-American women with hormone data from our study of Caucasian women (n = 68) consuming the same control diet. Methods: Twenty-one healthy, premenopausal, African-American women who agreed to eat only food prepared in a clinical study unit were recruited into the study. The control diet was similar to their usual diet, being high in fat (40% of calories from fat) and low in fiber (12 g/day); it was consumed on average for 3 weeks. The concentrations of estrone (E1), estrone sulfate (E1SO4), estradiol (E2), free E2, androstenedione, and sex hormone-binding globulin (SHBG) in serum samples obtained from the participants during the last week of the control diet and during the follicular phase of their menstrual cycle were determined. The women were then switched to a diet low in fat (20% of calories as fat) and high in fiber (40 g/day); they consumed this diet for two menstrual cycles before blood samples were collected for determination of serum hormone levels. Repeated-measures regression modeling was used to investigate the relationship between diet and hormone levels in African-American and Caucasian women. All P values resulted from two-sided statistical tests. Results: Analysis of serum hormone levels in the African-American women indicated that the change in diet caused a significant decrease in E2 (-8.5%; 95% confidence interval [CI] = -16.1% to -0.3%; \( P \leq .03 \)) and E1SO4 (-16.2%; 95% CI = -22.1% to -9.8%; \( P < .0001 \)) and a significant increase in androstenedione levels (+18.3%; 95% CI = +10.3% to +26.8%; \( P < .0001 \)). SHBG levels of the African-American women were 5.6% (95% CI = -14.0% to +3.7%) lower for those on the experimental diet compared with those on the control diet, but the difference was not statistically significant. Comparison of control serum hormone values in the African-American women in this study with those in Caucasian women previously studied indicated that the Caucasian women had statistically significant lower levels of E1 (-37%; 95% CI = -61.2% to -16.4%; \( P \leq .0002 \)), E2 (-54.5%; 95% CI = -90.9% to -25.1%; \( P \leq .0001 \)), free E2 (-30.2%; 95% CI = -65.7% to -2.3%; \( P < .03 \)), and androstenedione (-48.3%; 95% CI = -83.7% to -19.7%; \( P \leq .0004 \)). Conclusion: African-American women appear to have higher levels of serum hormones than Caucasian women, and dietary modification can result in a lowering of serum estrogens. [J Natl Cancer Inst 1996;88:1369-74]

In the United States, mortality rates for breast cancer are higher in African-Americans (1981-1992), and the 5-year survival rate (1986-1991) for African-American women with breast cancer is 69% compared with 84% in Caucasian women (1). Differences in mortality rates were not related to system delay (2) or patient delay (3) in obtaining medical care. Controlling for stage of disease still did not eliminate all of the survival difference (4-6). Eley et al. (7) reported that 75% of the additional mortality in African-American women could be explained by stage of disease, treatment, comorbid illness, and pathologic and sociodemographic variables, leaving 25% unaccounted for. It has been speculated that nutritional factors may play a role in both the incidence of and mortality from breast cancer. African-American women have higher body weight, decreased intake of vitamins A and C, and lower serum levels of vitamin C (8,9).

Differences in sex hormone metabolism as well as interactions between diet and sex hormones in African-American women could also explain, in part, the disparity in mortality. Estrogen has been cited as a risk factor for breast cancer, based on its role in increasing mitotic activity, a factor known to result in increased neoplasms (10,11). A recent nested cohort study...
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Subjects and Methods

Participants

African-American female participants were recruited for the study (August 1993 through October 1994) by advertisements in newspapers in Boston, MA, and by posters in the New England Medical Center complex (Boston, MA). Subjects were initially screened by use of a medical history questionnaire. They were excluded from participation if they had a history of chronic disease such as cancer, heart disease, arthritis, hypertension, diabetes mellitus, renal disease, or liver disease. All subjects were healthy, premenopausal women who were not taking prescribed drugs and who had normal values for their blood chemistry analyses, which included complete blood cell count, liver-function tests, and measurements of serum calcium, phosphorous, creatinine, cholesterol, triglycerides. They reported regular menses (cycle length of 26-32 days) and had not used oral contraceptives within the previous 6 months. Exclusion criteria included use of alcohol on a regular basis (greater than three drinks per week), use of intrauterine devices, use of megavitamin dosing, and higher levels of physical activity (aerobic exercise lasting more than 30 minutes occurring more than three times per week). The distributions of age, height, weight, and body mass index (BMI) in the study populations are presented in Table 1.

Before entering the study, all women filled out a screening form and a food-frequency questionnaire and were interviewed by a nutritionist to ensure that they consumed a typical American omnivore diet regarding macronutrients and that they did not engage in frequent reducing diets or binge eating. Women were excluded from the study if their 4-day food record indicated an intake of total fat of less than 30% of calories, a polyunsaturated-saturated (P:S) ratio of greater than 0.65, or a total fiber intake of greater than 20 g/day. These African-American women consumed a diet that was similar to that of Caucasian women enrolled in our previous studies (19,21,24). Twenty-four women were sought for the study. Our sample size calculation indicated that 20 subjects were required to observe a 25% difference in $E_1SO_4$ with a power of 0.83. We have reported observing a 30%-35% difference in $E_1SO_4$ under similar conditions (24). Twenty-one women were recruited for the study in four cohorts over an 18-month period and are included in the data analysis. Eighteen women completed the protocol, and their data are included in analysis of the data on the experimental diet. Three women did not complete the protocol because of pregnancy, illness requiring antibiotic treatment, or lack of compliance. A 4-day base-line food record was obtained from all recruited and/or eligible study participants during the screening period (Table 2). The study protocol was reviewed and approved by the Institutional Review Board of Tufts University School of Medicine. Prior to starting the study, all subjects gave written informed consent.

Diet

Food for the study was prepared by the New England Medical Center inpatient dining service. A trained dietary aide weighed the food to the nearest 0.5 g, and the study dietitian checked the food tray. The food was served in the Clinical Study Unit. The study eating plan repeated the same menu every 3 days. Participants committed themselves to eating only foods prepared for purposes of this study. Twelve subjects ate three meals a day, and six ate two meals a day at the Clinical Study Unit during the week. Other meals were packed up for consumption at home or at work. The control diet had 40% of calories from fat (saturated-monounsaturated-polyunsaturated fat [S:M:P] ratio of 2:2:1), 16% protein, 44% carbohydrate, 409 mg of cholesterol, and 12 g of dietary fiber (Table 2). This combination of dietary fat, fiber, and cholesterol is typical of diets consumed in the United States and other Western countries where the incidence of breast cancer is high (16,28). The dietary fiber of the control diet was a mixture of fiber sources, with approximately equal contribution from grains, legumes, fruits, and vegetables. The study dietitian calculated caloric intakes on the basis of the participant’s weight, age, sex, and usual level of physical activity and adjusted these intakes, if needed, in order to maintain body weight (± 3 lb) throughout the study. The participants consumed the control diet for a mean of 21 days (range, 4-28 days) before blood was collected during the follicular phase of the menstrual cycle.

Table 1. Demographic variables for African-American women and Caucasian women

<table>
<thead>
<tr>
<th>Variable</th>
<th>African-American women (n = 21)</th>
<th>Caucasian women (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Standard deviation)</td>
<td>Range</td>
</tr>
<tr>
<td>Age, y</td>
<td>26.7 (5.1)</td>
<td>18.5-35.7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.6 (6.1)</td>
<td>16.7-38.4</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.63 (0.07)</td>
<td>1.5-1.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.1 (16.8)</td>
<td>43.2-104.1</td>
</tr>
</tbody>
</table>

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of the menstrual cycle. (Two participants were entered into the study with only 4 and 6 days on the control diet; the range for the remaining 19 women was 14-28 days.)

The participants were switched directly from the control diet to the experimental diet, which was isocaloric with the control diet but which was low in fat (20% of calories from fat) and high in dietary fiber (40 g/day) and had a S:M:P ratio of 1:1:1: dietary cholesterol of 150 mg/day, 16% protein, and 68% carbohydrate (Table 2). The dietary fiber was composed of mixed sources, with 43%, 25%, 18%, and 14% from grains, fruits, vegetables, and legumes, respectively. Two special high-fiber baked products (bread and muffins) were prepared specifically for the study in order to meet the fiber requirements. The experimental diet represents a diet consisting of levels of macronutrients more typical of a population with low risk of breast cancer, which are low-fat, high-fiber, low in dietary cholesterol and with a more equal distribution of saturated, monounsaturated, and polyunsaturated fatty acids (16,29). The foods were typical of a North American diet. The African-American women remained on the experimental diet for 7-10 weeks before blood samples were collected during the follicular phase of their second menstrual cycle. Participants were weighed two to five times per week to ensure maintenance of body weight (±3 lb) at entry levels. Caloric intake was adjusted if changes in weight were observed.

The study protocol and control diet used in this study of African-American women were the same as those used in our study of Caucasian women, described elsewhere (24). Hormone values of 48 Caucasian women on the control diet and the effect of dietary changes in fat and fiber on hormone values have been reported (24). Twenty additional Caucasian subjects have been recruited into the study since our last publication, and hormone data for 68 Caucasian women on the experimental diet are used for comparison with data for the African-American women on the same control diet.

Blood Collection and Determinations of Hormone Levels

Blood collection protocols and determinations of hormone levels were the same for the African-American and Caucasian women. On three consecutive mornings during the women’s follicular phase of the menstrual cycle (days 4-7 after the first sign of bleeding), we collected 20-ml blood samples from them between 7 and 10 AM after they had fasted overnight for 8-12 hours. The blood samples were centrifuged at 1400g for 22 minutes at 4 °C, and the sera were collected and stored at -70 °C for an average of 3 months but no more than 6 months before hormone concentrations were analyzed. Hormones were analyzed in cohort batches so that all the serum samples from an individual woman were included in the same batch. The concentrations of serum E2, E1, SHBG, and androstenedione were measured by radioimmunoassays involving solvent extraction and Celite chromatography as previously described (29,30). The DAE-cellusel filter technique was used to measure the level of sex-hormone-binding globulin (SHBG) (31,32). All samples were coded by use of a random-number system, and a set of blinded duplicates and a standard pooled serum sample were included for quality-control purposes.

Table 2. Dietary intake of African-American women at base line and on study diets (n = 21)

<table>
<thead>
<tr>
<th>Macronutrients per day</th>
<th>Base line*</th>
<th>Control diet†</th>
<th>Experimental diet‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilocalories per day</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>1914</td>
<td>618</td>
<td></td>
<td>2114</td>
</tr>
<tr>
<td>Fat, g</td>
<td>74</td>
<td>24</td>
<td>94</td>
</tr>
<tr>
<td>Fat, %</td>
<td>35</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Polyunsaturated fat, g</td>
<td>14</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>27</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Monounsaturated fat, g</td>
<td>28</td>
<td>9</td>
<td>35</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>274</td>
<td>116</td>
<td>409</td>
</tr>
<tr>
<td>Protein, %</td>
<td>15</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>50</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>10</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

*4-day food record.
†Menu was repeated every 3 days.
‡Menu was repeated every 3 days.

Statistical Analyses

Because the distributions of all hormones measured in the current study were skewed to the left, natural logarithm transformation was used to normalize the distributions. As a result of the logarithmic transformation, data are reported as geometric means and 95% confidence intervals (CIs) for the geometric mean.

\[ y_i = \log(\text{geometric mean}) \]

where \( y_i \) is the natural logarithm of the mean on the natural logarithm scale and \( s_e \) is the standard error of the mean on the natural logarithm scale. Repeated-measures regression analyses were carried out to compare the hormone values of the African-American women on the control diet with those of the African-American women on the experimental diet (33), as well as to compare hormonal responses of the two groups to a similar dietary change. To adjust for BMI and age, we directly entered them as continuous covariates into the repeated-measures regression model. The adjustment can be considered an analysis of covariance.

For investigating the effect of diet within strata of race, the repeated-measures regression model used was

\[ Y_{ij} = \beta_0 + \beta_1 \times \text{DIET}_{ij} + \beta_2 \times \text{BMI}_i + \beta_3 \times \text{AGE}_i + h_i + \epsilon_{ij} \]

where \( Y_{ij} \) is participant \( i \)'s hormone measurement on cycle day \( j \); \( \text{DIET}_{ij} \) is 1 if participant \( i \) is on the experimental diet on cycle day \( j \) and 0 if participant \( i \) is on the standard North American diet on cycle day \( j \); \( \text{BMI}_i \) and \( \text{AGE}_i \) are participant \( i \)'s base-line BMI and age, respectively; \( h_i \) is the random between-person variation assumed to have mean 0 and variance \( \sigma^2_h \); and \( \epsilon_{ij} \) is the random within-person variation assumed to have mean 0 and variance \( \sigma^2_{\epsilon_{ij}} \). The effect of diet is represented by \( \beta_1 \) and has the interpretation of the amount of change in hormone \( Y \) as a result of changing the diet. When \( Y \) is the natural logarithm of the hormone measurement, \( e^{(\beta_1 - 1) \times 100} \) gives the percent change in the hormone observed for the change in diet, and the 95% CI for percent change uses the standard error of the regression coefficient in the usual manner (Table 3).

To compare hormone levels of Caucasian women with those of African-American women within strata of diet, the same model as above was used, replacing the term for diet \( (\text{DIET}_{ij}) \) with an indicator variable for race, \( \text{RACE}_{ij} \), where \( \text{RACE}_{ij} \) is less than 1 if participant \( i \) was African-American and 0 if otherwise (Table 4).

To investigate modification of the effect of diet by race, the model variables included were base-line age \( (\text{AGE}_i) \) and BMI \( (\text{BMI}_i) \); the main effects for diet were represented by \( \text{DIET}_{ij} \); variable, the main effects for race were represented by the \( \text{RACE}_{ij} \) variable, and an interaction term was obtained by multiplying the indicator variable for \( \text{RACE}_{ij} \) (1 = African-American; 0 = Caucasian) by the indicator variable for \( \text{DIET}_{ij} \). The Wald test for significance of this interaction term was used to obtain the \( P \) value for this test of effect modification. All \( P \) values reported in this article are two-sided.

Blinded duplicates were sent for hormone analysis from eight subjects (three measurements each for a total of 24 measurements) to determine laboratory reproducibility. The reliability coefficients (the ratio of the between-person variance divided by the total variance) ranged from 54% to 84% for all hormones.
Abstracts of the American Cancer Society's Third National Cancer Conference on Nutrition and the Prevention of Cancer

The American Cancer Society's Third National Cancer Conference on Nutrition and the Prevention of Cancer was held in Chicago in 1995. The conference focused on the role of nutrition in the prevention of cancer and the implications for public health policy. The presentations and discussions covered a wide range of topics, including the role of diet in the causes and prevention of cancer, the impact of nutrition on cancer treatment, and the implications of nutrition science for cancer control and public health policy.

The conference featured a variety of speakers, including experts in nutrition, cancer prevention, and public health. The presentations included research on the relationship between diet and cancer risk, as well as discussions on the implications of nutrition science for public health policy and practice.

The conference also included panel discussions and workshops on specific topics, such as the role of diet in the prevention of breast cancer, the role of diet in the prevention of colorectal cancer, and the role of nutrition in cancer treatment.

Overall, the conference provided a comprehensive overview of the current state of knowledge in nutrition and cancer prevention, as well as a forum for discussing the implications of this knowledge for public health policy and practice.
diet in the African-American women resulted in statistically significant decreases in the levels of \( E_1 \cdot SO_4 \) and \( E_2 \) and a statistically significant increase in the level of androstenedione. Previous investigations from our laboratory (23,24) and from other laboratories (20-22,25) have consistently reported decreases in the level of estrogens in response to this type of dietary change. Only our laboratory (24) reported on androstenedione or testosterone levels, both of which were decreased in premenopausal Caucasian women on the low-fat-high-fiber experimental diets compared with those on a high-fat-low-fiber diet. The mechanism that has been proposed to explain the ability of the low-fat-high-fiber diet to decrease serum estrogen levels involves increased fecal excretion and a decreased enterohepatic circulation of estrogens (36). Dietary fat and fiber both alter the activity of the bacterial enzyme glucuronidase, which in turn affects the degree of reabsorption of estrogen from the intestines (36). The decrease in estrogen levels with an increase in androstenedione level observed in these African-American women is not consistent with increased fecal excretion of steroids and suggests that a different dietary mechanism may be in effect, such as the down-regulation (i.e., reduced activity) of the enzyme aromatase that converts the androstenedione to \( E_1 \). Our participants consumed the low-fat-high-fiber diet for only 7-10 weeks; therefore, it was not possible to determine whether the androstenedione level would decrease over time as a result of a negative feedback of decreased conversion to estrogen.

The observation that the African-American women had higher control serum levels of \( E_1 \) (by 37.0%), \( E_2 \) (by 54.5%), and free \( E_2 \) (by 30.2%) than the Caucasian women is striking. The elevated estrogen levels on the typical American diet may offer an explanation for the increased mortality from breast cancer in African-American women, since these women have increased exposure to the promotional effects of estrogen. Measurements of the effect of body fat distribution and endogenous hormone levels may be pertinent to our observation. Waist–hip ratio has been reported to be a better predictor of breast cancer than obesity or BMI (37-40). Kirschner et al. (41) reported an association between increased testosterone and free \( E_2 \) levels and decreased SHBG levels in women with greater upper body obesity. Higher androstenedione levels have also been associated with increased waist–hip ratio (42,43). Waist–hip ratio was not measured in our studies, so we could not evaluate it directly or control for it in the analyses. Preliminary data from our current study of body fat distribution and hormone levels in Caucasian and African-American women, however, indicate a higher waist–hip ratio in our sample of African-American compared with Caucasian women as well as increases in \( E_2 \cdot SO_4 \) and androstenedione levels associated with greater waist–hip ratio (i.e., greater upper body adiposity). All analyses in this study were adjusted for base-line BMI, but this adjustment may be insufficient to address any possible effects of differences in waist–hip ratio between the two populations.

Among the study populations, the African-American women had somewhat greater parity than the Caucasian women. When adjustment was made for parity in the analysis, there was little difference in the results (data not shown). The difference in parity should have favored lower hormone values in the African-American women.

to determine if our results were affected by the different time periods during which the hormone values were collected and analyzed in the Caucasian versus African-American women (8 years versus 18 months, respectively), we compared the hormone values in a subset of the Caucasian women (n = 10) who were in the follicular phase of their menstrual cycle and were eating the control diet; this subset of women was studied concurrently with the African-American women (n = 21). We found that this subset had lower mean hormone levels than those of the entire group of Caucasian women (n = 68) (data not shown). This result provided support that our observation of elevated levels of hormones in the African-American women on the control diet compared with those in the total Caucasian women studied was not being unduly affected by differences in study time periods. Since this analysis resulted in larger CIs as a result of the small number of observations, we have reported on the total population.

Although the estrogen levels on the control diet were higher in the African-American women than in the Caucasian women, both groups responded to the change to a low-fat–high-fiber diet with a lowering of their estrogen levels. This result suggests that a substantial reduction in breast cancer risk can be achieved in African-American women as well as in Caucasian women if they would adopt a low-fat–high-fiber diet, which would result in a decrease in the levels of circulating estrogen. The relevance of increased androstenedione levels in the African-American women while on the low-fat–high-fiber diet, which was not observed in the Caucasian population, is unclear and deserves further investigation.

References


Notes

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