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DIETARY FIBER AND SERUM 16 α -HYDROXYESTRONE, AN ESTROGEN METABOLITE ASSOCIATED WITH LOWER SYSTOLIC BLOOD PRESSURE

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Abstract

Objective—We recently identified an inverse relationship between systolic blood pressure (SBP) and serum 16 α -hydroxyestrone, a metabolite of 17 β -estradiol, in postmenopausal women. Formation of 16 α -hydroxyestrone is catalyzed primarily by CYP1A2, a cytochrome P450 enzyme. The objective of this study was to evaluate the relationships between known modifiers of CYP1A2 activity and serum 16 α -hydroxyestrone in postmenopausal women. We hypothesized that fruits, vegetables, and grains, which contain more soluble fiber (a known inducer of CYP1A2) as a proportion of total fiber, would be more positively associated with serum 16 α -hydroxyestrone than legumes, which contain less soluble fiber as a proportion of total fiber.

Materials and Methods—Serum from a population-based sample of 42 postmenopausal women aged 55–69 living in Cook County, Illinois, was assayed for 16 α -hydroxyestrone using mass spectrometry. Ordinal logistic regression was used to evaluate the cross-sectional relationship between dietary fiber and serum 16 α -hydroxyestrone after adjusting for multiple covariates.

Results—Relative to dietary fiber from legumes, dietary fiber from fruits and vegetables was associated with a greater log odds ($B = 0.201$, $p = 0.036$) of having higher serum concentrations of 16 α -hydroxyestrone. The log odds of having higher serum concentrations of 16 α -hydroxyestrone was also lower among African-American women ($B = -2.300$, $p = .030$) compared to white women.

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Conclusion—These results are consistent with previous studies demonstrating a negative relationship between SBP and dietary fruits and vegetables and a positive relationship between African-American race and SBP. Further research is needed regarding dietary factors that may influence the serum concentration of 16 α -hydroxyestrone.

Keywords

nutrition; fiber; fruits; vegetables; estrogen; metabolism; blood pressure

Introduction

Systolic blood pressure (SBP) increases with age in both men and women [1]. While SBP is lower, on average, among women compared to men prior to age 60, this pattern reverses after age 60 [2]. Because production of 17 β -estradiol (E2) declines with menopause, it has been suggested that lower serum E2 explains the higher prevalence of hypertension among women compared to men after age 60 [3]. Although exogenous estrogen did not reduce SBP among postmenopausal women in the Women's Health Initiative (WHI) [4], recent studies show that the estrogen utilized in that study, conjugated equine estrogen, is not bioequivalent to E2 [5]. Therefore, it is too early to discount the potential blood-pressure lowering effects of exogenous sex hormones. Our group recently found an inverse relationship between the serum concentration of 16 α -hydroxyestrone (a metabolite of E2) and SBP among postmenopausal women after adjusting for age, BMI, race/ethnicity, and use of antihypertensive medications [6]. Previous studies have shown that 16 α -hydroxyestrone is a potent antioxidant [7] which can increase endothelial cell production of prostacyclin (a vasodilator) at twice the rate of E2 [8]. It may also increase endothelial nitric oxide synthase (eNOS) gene expression, nitric oxide (also a vasodilator) production, and vascular endothelial cell proliferation [9].

E2 exists in a steady state with estrone (E1) and the conversion of these hormones to 16 α -hydroxyestrone is catalyzed by at least two enzymes: CYP1A2 [10] and CYP3A7 [11]. Because CYP1A2 plays such an important role in 16 α -hydroxyestrone production, the goal of this study was to determine the relationship between known modifiers of this enzyme and serum levels of 16 α -hydroxyestrone in postmenopausal women. A meta-analysis of 24 trials found evidence that diets rich in soluble, but not insoluble, fiber are associated with lower systolic and diastolic blood pressure [12]. Dietary fiber is a collective term for plant substances that are resistant to digestion. Analysis of several foods revealed that soluble fiber, as a proportion of total fiber, is higher in fruits (38%) compared to vegetables (32%), grains (32%), and legumes (25%) [13]. Among rats, a diet rich in cocoa seeds (which contain 32% soluble fiber [14]) doubled hepatic CYP1A2 enzyme concentration while a diet rich in oat hulls (which contain 4% soluble fiber [14]) reduced hepatic CYP1A2 concentration [15]. We hypothesized that compared to dietary fiber from legumes, dietary fiber from fruits, vegetables, and grains would be more positively associated with serum 16 α -hydroxyestrone after adjusting for multiple covariates.

Materials and Methods

Participants

The data for this study were gathered from the fifth year of the Chicago Health, Aging, and Social Relations Study (CHASRS), which is a population-based, longitudinal study designed to examine the biological and psychosocial aspects of social isolation and health. The participants of CHASRS were all born between 1935 and 1952, reside in Cook County, Illinois, and can be described as non-Hispanic white, African-American, or non-Black Latino-American. A multistage probability design, described elsewhere, was utilized for

selection of the sample [16]. With attrition over five years, the initial sample size declined from 229 to 163. In year 5, we had serum estrogen metabolite data from 51 women, all of whom were postmenopausal. Four participants were excluded because they were taking hormone replacement therapy. Of the 47 women whose sera were analyzed for estrogen metabolites, the current analysis included 42 women who had no missing dietary or demographic data. Participants were compensated \$90 for about eight hours of testing in the laboratory beginning at approximately 9 a.m. Their day included informed consent, self-report questionnaires, interviews, lunch, blood pressure measurement, and phlebotomy. All procedures were approved by the University of Chicago Institutional Review Board.

Assessment of dietary intake and exercise

Daily fiber intake from fruits, vegetables, grains, and legumes was assessed using the Brief Block 2000 Nutritional Questionnaire, which evaluates the participant's dietary habits by asking two types of questions about different foods. First, subjects were asked to classify how often they ate a particular food during the past year using the following categories: never, a few times per year, once per month, 2–3 times per month, once per week, twice per week, 3–4 times per week, 5–6 times per week, and every day. Second, subjects were asked to classify how much of the food they typically ate at each sitting by referring to pictures that depict amounts of ¼ cup, ½ cup, 1 cup, or 2 cups of food. The answers to these questions were used to calculate values (in grams) for daily fiber from fruits and vegetables (combined), grains, and legumes. Participants were also asked to estimate the number of minutes they exercise on a typical day and whether they had engaged in any exercise in the previous week.

Estrogen Metabolite Assay

Frozen serum samples were shipped on dry ice to the Laboratory of Proteomics and Analytical Technologies at SAIC in Frederick, MD and were assayed for endogenous estrogens according to the established protocol [17]. Briefly, stock and working standard solutions of estrogen metabolites (EM's) and stable isotope-labeled estrogen metabolites (SI-EM) were prepared. Calibration standards and quality control (QC) samples were then prepared using charcoal-stripped human serum with no detectable levels of any EM. Next, the unknown serum samples, as well as the calibration standards and QC samples, were hydrolyzed, extracted, and derivatized. All samples were then analyzed using capillary liquid chromatography-electrospray ionization-tandem mass spectrometry analysis (LC-ESI-MS). The precision, or percent recovery of a low concentration (8 pg/mL) of EM's assayed, ranged from 91 to 113%. Serum EM's from study participants were quantified using Xcalibur Quan Browser (Thermo Electron, Waltham, MA) [17].

Statistical Methods

Serum 16 α -hydroxyestrone was undetectable in 11 of 42 (26%) participants, and the distribution of the remaining values was positively skewed: 10 (23.8%) had values between 0 and 13 pg/mL and 21 (50%) had values $\geq$ 13 pg/mL (i.e., 13 to 90 pg/mL). A one-way ANOVA showed that women in these three concentration categories did not differ in age, racial/ethnic composition, or BMI (all p 's $>$.4). The three categories of serum 16 α -hydroxyestrone concentration served as the dependent variable in ordinal logistic regression models that examined the contribution of dietary fiber to metabolite concentration. Total serum estrogen (natural log to correct for positive skew), age, BMI, and race/ethnicity were included as covariates to account for their influence on metabolite concentrations. Significance was set at $p <$.05, two-tailed. Parallel models that used alternative categorizations of serum 16 α -hydroxyestrone concentrations (e.g., quartiles) produced substantively equivalent results.

Results

Sample characteristics regarding age, body mass index (BMI), race/ethnicity, serum 16 α -hydroxyestrone, total serum estrogens, and dietary fiber from fruits and vegetables, grains, and legumes, are presented in Table 1. Place Table 1 about here.

In an initial model, total daily dietary fiber was noted to be a significant predictor of 16 α -hydroxyestrone concentration ($B = 0.164$, $SE = 0.056$, $p = .004$). In the subsequent model, dietary fiber was categorized based upon its source (e.g., fruits and vegetables, grains, or legumes). Table 2 demonstrates that dietary fiber from fruits and vegetables was associated with an increased log odds ($B = 0.201$, $SE = 0.096$, $p = 0.036$) of falling in the next higher category of serum 16 α -hydroxyestrone concentration when the other covariates are held constant. A test of the assumption of “parallel lines” was not rejected ($p = .428$), indicating that the association between fiber intake and serum 16 α -hydroxyestrone concentration was constant across the range of metabolite concentration categories. The log odds for fiber from dietary fruits and vegetables translates into a 1.22 increase in the odds of having a higher serum 16 α -hydroxyestrone level for each additional gram per day of fiber from dietary fruits and vegetables.

In the same model, being African-American was associated with a decreased log odds ($B = -2.300$, $SE = 1.062$, $p = 0.030$) of having a higher serum 16 α -hydroxyestrone concentration when the other covariates are held constant. This translates into a 9.97 lower odds of having a higher serum 16 α -hydroxyestrone concentration in postmenopausal African-American women compared to postmenopausal white women. None of the other covariates was associated with serum 16 α -hydroxyestrone concentrations, although a trend existed for an association between dietary fiber from grains and increased log odds ($B = 0.198$, $SE = 0.111$, $p = 0.074$) of falling in the next higher category of serum 16 α -hydroxyestrone concentration. Neither minutes per day of exercise nor any exercise in the past week was correlated with 16 α -hydroxyestrone or any of the independent variables. Including exercise in the logistic regression model did not substantively change the results shown in Table 2. Place Table 2 about here.

Discussion

Consistent with our hypotheses, we found that the serum concentration of 16 α -hydroxyestrone among postmenopausal women was more strongly associated with dietary fiber from fruits, vegetables, and grains than with legumes. The association between fruits and vegetable fiber and serum 16 α -hydroxyestrone was significant while the association between grain fiber and serum 16 α -hydroxyestrone did not quite reach significance, most likely because among grains, soluble fiber as a proportion of total fiber is quite variable (7.0% to 51.7%) [13]. The associations we found may be due to activation of hepatic CYP1A2 by soluble fiber [15] and increased conversion of E1 to 16 α -hydroxyestrone [10], a potent anti-oxidant. Unlike fiber from fruits, vegetables, and grains, fiber from legumes is less water soluble [13] and is less likely to stimulate hepatic CYP1A2 [15]. Our results are consistent with a recent meta-analysis, which found that compared to insoluble fiber, soluble fiber has more significant negative associations with systolic and diastolic blood pressure [12]. In addition, Japanese studies have found negative associations between systolic blood pressure and diets high in fruits and vegetables compared to diets rich in meat products and fats [18,19]. Conversely, diets low in fruits and vegetables are associated with increased blood pressure [20].

Our results are also consistent with a recent study which demonstrated increased risk of cataract formation among individuals who consumed diets low in fruits and vegetables [21].

Because the anti-oxidative defense mechanism is considered important to protection of the eye lens from UV-B radiation, this result could be due to the anti-oxidant effects of 16 α -hydroxyestrone or to anti-oxidants within fruits and vegetables.

Of note, 16 α -hydroxyestrone was not detected in 26% of our sample. Prior research has shown that postmenopausal women have lower levels of this metabolite compared to premenopausal women [17]. Because the assay we used is precise at low concentrations (8 pg/mL), we can assume the serum concentration of 16 α -hydroxyestrone among these women was below this cut-off. However, undetectable levels of 16 α -hydroxyestrone do not detract from our findings because results from these women were included in our analysis.

A previous study demonstrated an inverse relationship between dietary fiber from fruits and vegetables and the urine concentration of 16 α -hydroxyestrone among women aged 42–52 [22]. While this is not consistent with our results, the correlations between ln urine and ln serum estrogens in our cohort were low. For example, the correlation between urine and serum ln 17 β -estradiol was .226 while the correlation between urine and serum ln 16 α -hydroxyestrone was .185. These results suggest further investigation is needed regarding the relationship between urine and serum concentrations of endogenous estrogens.

The negative association between African-American race and serum 16 α -hydroxyestrone is consistent with the higher rate of hypertension among postmenopausal African-American women compared to similarly-aged white women [2] and with our recent finding of an inverse relationship between serum 16 α -hydroxyestrone and SBP [6]. The etiology of this negative association is unclear. It does not appear to be due to racial differences in dietary fiber from fruits and vegetables as ancillary analysis indicated that African-American women reported greater daily intake of fruits and vegetables (13.2 g/d) than white women (7.8 g/d), $p < .05$. The negative association may be related to racial differences in the activity of CYP1A2 or CYP3A7, the enzymes which convert E1 to 16 α -hydroxyestrone. Further investigation, including an evaluation of high and low activity single nucleotide polymorphisms (SNP's) of these enzymes is needed to better understand racial differences in serum 16 α -hydroxyestrone concentration and its impact on SBP.

This study has a number of limitations, including its cross-sectional design, which prevents causal inference regarding the relationships between the independent and dependent variables. Secondly, it is possible that dietary fiber from fruits and vegetables does not lead to higher levels of serum 16 α -hydroxyestrone but that a third factor accounts for this association. For example, individuals who eat more fruits and vegetables may be more likely to exercise, and exercise has been shown to influence the metabolism of CYP1A2 [23]. However, adjusting for exercise in our analysis did not change the results. Alternatively, fruits and vegetables contain more vitamin C than grains and legumes [19] and some authors have postulated that vitamin C contributes to the inverse relationship between fruits and vegetables and systolic blood pressure [24]. However, despite lower levels of vitamin C in grains, grain fiber and fruit and vegetable fiber had similar effect sizes in our analysis. This suggests that soluble fiber is more likely than vitamin C to account for the associations between serum 16 α -hydroxyestrone and dietary fiber from fruits, vegetables, and grains. An additional limitation is the small sample size of this study. A larger study cohort may have revealed that the positive association between fiber from grains and 16 α -hydroxyestrone is significant. Given the relatively high proportion of soluble fiber to total fiber in certain grains, such a finding would not be surprising [13].

This is the first study to evaluate predictors of serum 16 α -hydroxyestrone, an endogenous estrogen associated with lower blood pressure among postmenopausal women [6]. If replicated, results from this study may provide insight into the mechanisms by which dietary

fruits and vegetables are associated with lower SBP. In addition, our results suggest that postmenopausal African-American women have lower levels of serum 16 α -hydroxyestrone. This finding may help explain the higher prevalence of hypertension among older African-American women compared to similarly-aged women in other racial/ethnic groups.

Conclusion

Dietary fiber from fruits, vegetables, and grains was more strongly associated with serum 16 α -hydroxyestrone concentration compared to fiber from legumes. Given the potential importance of 16 α -hydroxyestrone to systolic blood pressure, our results highlight the need for additional research regarding the effects of dietary fiber on estrogen metabolism.

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Table 1

Characteristics of postmenopausal women not taking estrogen replacement therapy (N = 42)

	Mean/N	Standard Deviation	Minimum	Maximum
Age (years)	60.93	3.73	55	69
Body mass index (kg/m ²)	32.39	7.37	19.81	50.64
Serum 16 α -hydroxyestrone (pg/mL)	19.37	23.24	.00	89.96
Total serum estrogens (pg/mL)	1266.46	600.34	423.74	3551.66
Dietary fiber from fruits and vegetables (g/day)	9.05	6.03	2.20	31.65
Dietary fiber from grains (g/day)	6.47	3.58	1.75	15.95
Dietary fiber from legumes (g/day)	2.70	2.56	.13	9.48
White (N)	19			
African-American (N)	11			
Hispanic (N)	12			

Table 2

Ordinal logistic regression predicting the log odds of a higher serum 16 α -hydroxyestrone concentration (N = 42)

	Estimate (B)	Standard Error	P value
Age	.099	.114	.388
Body Mass Index (kg/m ²)	.041	.054	.440
ln Total serum estrogens (pg/mL)	1.639	.910	.072
Dietary fiber from fruits and vegetables (g/day)	.201	.096	.036
Dietary fiber from grains (g/day)	.198	.111	.074
Dietary fiber from legumes (g/day)	.037	.196	.849
African-American*	-2.300	1.062	.030
Hispanic*	.133	.890	.881

* Reference group is white.