

Estrogen Metabolites and Systolic Blood Pressure in a Population-Based Sample of Postmenopausal Women

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Context: Lower systolic blood pressure (SBP) and lower rates of coronary heart disease among premenopausal women compared with similarly aged men and postmenopausal women suggest that female sex hormones may confer cardiovascular protection. 2-Hydroxyestradiol, a product of 17 β -estradiol oxidative metabolism, inhibits the proliferation of vascular smooth muscle cells *in vitro*. The other major product of 17 β -estradiol oxidative metabolism, 16 α -hydroxyestradiol, does not demonstrate similar inhibitory effects. Concentrations of 2-hydroxyestrone (2-OHE) and 16 α -hydroxyestrone (16-OHE) in urine reflect the relative activity of the 2- and 16 α -hydroxylation pathways of 17 β -estradiol.

Objective: The objective of this study was to determine the relationship between SBP and the ratio of 2-OHE to 16-OHE in urine.

Design and Participants: This was a cross-sectional study of 80 postmenopausal women living in Cook County, Illinois.

Setting: This study was performed in an academic clinical laboratory.

Main Outcome Measure: The main outcome measure was SBP.

Results: Women taking hormone replacement therapy had higher levels of urinary 2-OHE and 16-OHE, but their mean 2:16-OHE ratio and SBP did not differ from that of women not taking hormone replacement therapy. In a multivariate regression model that controlled for age, body mass index, race/ethnicity, and antihypertensive medication use, a SD increase in the 2:16-OHE ratio was associated with a 6.7-mm Hg decrease ($P < 0.05$) in SBP.

Conclusions: The ratio of urinary 2-OHE to 16-OHE is a significant predictor of SBP among postmenopausal women and may reflect the effects of 2-hydroxyestradiol, a potent inhibitor of vascular smooth muscle cell proliferation. (*J Clin Endocrinol Metab* 91: 1015–1020, 2006)

HYPERTENSION AFFECTS APPROXIMATELY 50 million people in the United States and 1 billion people worldwide (1). Risk factors for essential hypertension include salt sensitivity, cigarette smoking, glucose intolerance, and obesity (2). Gender is also important to blood pressure, and population-based studies have shown that mean systolic blood pressure (SBP) is higher among men compared to women until about age 60 yr. After age 60 yr, mean SBP is higher among women compared to men (3). Hypertension is a significant risk factor for coronary heart disease (CHD), and although the risk of death from CHD in women lags 10 yr behind that in men, the gap in incidence narrows with advancing age (4). These phenomena have led to speculation that female sex hormones confer a cardiovascular benefit that is attenuated with the onset of menopause. The Women's Health Initiative (WHI) examined the effect of oral estrogen supplementation on CHD risk among postmenopausal women and found no evidence of reduced CHD incidence among those receiving conjugated equine estrogen (CEE) alone (5). Among those receiving CEE plus medroxyprogesterone acetate (MPA), CHD incidence was significantly increased compared to controls (6).

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The WHI results were somewhat surprising given the vascular benefits of 17 β -estradiol, which include vasodilation via ion channel- (7) and nitric oxide (8)-mediated relaxation of vascular smooth muscle cells and enhanced gene expression for vasodilatory enzymes, such as prostacyclin synthase and nitric oxide synthase (9, 10). In addition, several studies have demonstrated an association between transdermal 17 β -estradiol usage and SBP reduction (11). However, CEE differs chemically from 17 β -estradiol, and the constituents of CEE vary in their estrogenic effects. For example, estrone sulfate is the largest constituent by weight of CEE, and estrone exhibits 1/10th the estrogenic potency of 17 β -estradiol in yeast cell screening assays (12).

Differences in activity of the metabolites of 17 β -estradiol and CEE may also contribute to differences in the apparent effects of 17 β -estradiol and CEE on blood pressure. For example, metabolites of 17 β -estradiol, particularly, 2-hydroxyestradiol and 2-methoxyestradiol, inhibit the proliferation of vascular smooth muscle cells (VSMC) (13–15), cardiac fibroblasts (16), and glomerular mesangial cells (17). In addition, 2-hydroxyestradiol improves endothelial function in rats prone to obesity (18), whereas 2-methoxyestradiol attenuates angiotensin II-induced hypertension in male Sprague Dawley rats (19). By contrast, metabolites of estrone, including 2-hydroxyestrone (2-OHE), 2-methoxyestrone, and 4-OHE, inhibit the proliferation of VSMC, cardiac fibroblasts, and glomerular mesangial cells only at supraphysi-

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Abbreviations: BMI, Body mass index; CEE, conjugated equine estrogen; CHD, coronary heart disease; HRT, hormone replacement therapy; MPA, medroxyprogesterone acetate; 2-OHE, 2-hydroxyestrone; 16-OHE, 16 α -hydroxyestrone; SBP, systolic blood pressure; VSMC, vascular smooth muscle cell.

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ological concentrations (13, 16, 17). A review of the effect of hormone replacement therapy on blood pressure found that oral estrogens, which include estrone sulfate as a primary constituent, were associated with SBP reduction in only a minority of studies (11).

In humans, 17 β -estradiol exists in a steady state with its oxidized and less metabolically active form, estrone (20). Metabolism of both 17 β -estradiol and estrone occurs via hydroxylation, glucuronidation, sulfonation, and/or *O*-methylation (21). In the primary hydroxylation pathways, 2-hydroxyestrogens (2-hydroxyestradiol and 2-OHE) and 16 α -hydroxyestrogens [16 α -hydroxyestradiol and 16 α -hydroxyestrone (16-OHE)] are formed (22). These pathways are competitive, and an increase in 2-hydroxylation is tantamount to a decrease in 16 α -hydroxylation (23). Once formed, 2-hydroxyestrogens are rapidly methylated to yield 2-methoxyestradiol and 2-methoxyestrone (24). Previous studies have identified an inverse relationship between the ratio of urinary 2-OHE to 16-OHE and the risk of breast and cervical cancer (25, 26). Because 2-OHE does not exhibit anticarcinogenic properties, this association may reflect the activity of the 2-hydroxylation pathway of 17 β -estradiol and production of 2-hydroxyestradiol and 2-methoxyestradiol, which both inhibit breast and cervical cancer cell proliferation (27).

We examined the relationship between the urinary 2:16-OHE ratio (a marker of the ratio of 2-hydroxyestradiol to 16 α -hydroxyestradiol production) and SBP in a population-based sample of postmenopausal women. Given the inhibitory effects of 2-hydroxyestradiol and 2-methoxyestradiol on VSMC and glomerular mesangial cells *in vitro*, we hypothesized that an inverse relationship would exist between the urinary 2:16-OHE ratio and resting SBP.

Subjects and Methods

Data for this study were collected in 2002, the first year of the Chicago Health, Aging, and Social Relations Study (CHASRS), a longitudinal, population-based study of individuals born between 1935 and 1952. The Chicago Health, Aging, and Social Relations Study was designed to examine the social, psychological, and biological aspects of social isolation and health. The target population included Caucasian, African-American, and Latino-American subjects between the ages of 50 and 67 yr living in Cook County, Illinois, who were English speaking and sufficiently ambulatory to come to the University of Chicago for a daylong visit to the laboratory. The sample was selected using a multistage probability design in which African-Americans and Latino-Americans were oversampled. A sample of households was selected, then screened by telephone for the presence of an age-eligible person. Age-eligible subjects were asked to participate in the study. If a household contained more than one age-eligible person, the person with the most recent birthday was selected. A quota-sampling strategy was used to achieve an approximately equal distribution of respondents across racial/ethnic groups. The response rate among eligible subjects was 45%, which is comparable to that of other well-conducted telephone surveys. The final sample size was 229 (120 females and 109 males). Additional details were published previously (28).

For the purposes of this study, we focused on 85 postmenopausal women for whom we had complete estrogen metabolite data. Of these, 33 were Caucasian, 29 were African-American, and 23 were Latino-American. Fifty-eight reported taking no hormone replacement therapy (HRT), and 27 reported taking HRT. Forms of supplemental estrogen included oral CEE or estrone ($n = 11$), oral CEE plus MPA ($n = 13$), oral esterified estrogens plus methyltestosterone ($n = 2$), and estrogen injections ($n = 1$). There were no racial/ethnic differences in the percentage of women taking HRT ($\chi^2 = 0.4$; $P > 0.8$).

Procedures

Before each participant's scheduled day in our laboratory, the participant was mailed a urine collection bottle containing a preservative (50% acetic acid). Enclosed in the package were urine collection instructions that asked participants to thoroughly void, but not into the container, before going to bed the night before the laboratory tests. The container was to be used for any nighttime voiding and for the first morning void the next day. A follow-up telephone call before the laboratory day verified that participants understood the sampling instructions.

Participants arrived at the laboratory between 0800 and 0900 h, whereupon the urine sample volume was measured, and aliquots of urine were frozen at -80 C and batched for later testing. Participants provided informed consent and then began a day of assessments that included standard psychological and medication surveys, interviews, lunch, and a cardiovascular protocol. The study design and protocol were approved by the University of Chicago Social Science Division institutional review board.

Cardiovascular measures

Participants were seated in a comfortable padded chair and provided with a footrest if their legs were too short to rest on the floor. A Colin Vital Statistics Monitor (model BP-508, Vital Signs, Minster, OH) was used to obtain systolic, diastolic, and mean arterial blood pressure readings from the nondominant arm, which was supported at the heart level by a cushion resting on the arm of the participant's chair. The Colin Monitor records a pulse wave tonometrically by partial occlusion of the radial artery against the radius at the wrist, allowing for beat to beat measurement of blood pressure. The tonometer was calibrated against an initial blood pressure reading obtained using an oscillometric cuff and was periodically recalibrated either automatically or on experimenter initiation. Electrocardiogram and impedance cardiogram data were also obtained, but are not reported here.

During a 15-min adaptation period, participants completed questionnaires while experimenters established good signal quality. Blood pressure was recorded during an orthostatic stress protocol that consisted of a 2-min sitting epoch, followed by a 4-min standing epoch and ending with another 2-min sitting epoch. A 2-min adaptation period followed each postural change before recording commenced. Our analysis used the average of the approximately 280 beat to beat blood pressure values obtained during 4 min in the seated posture. These repeated measures provide a reliable index of individual differences in blood pressure. For example, the yr 1–2 correlation of SBP using this method was 0.58 ($P < 0.001$).

Hypertension was defined as 1) a physician having told the participant she had hypertension or high blood pressure, 2) SBP greater than 140 mm Hg in the laboratory, or 3) use of prescribed antihypertensive medications. Antihypertensive agents were categorized as either vasoactive (*i.e.* α_2 -agonists, α -blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, and β -blockers) or volume active (*i.e.* diuretics) for inclusion in multiple linear regression models. Twenty-one percent of participants were taking vasoactive medications, 7% were taking volume active medications, and 13% were taking both types of medication.

Urinary measures

Urine samples were sent in a single batch on dry ice to Genova Diagnostics (Asheville, NC) for enzyme immunoassay of 2-OHE and 16-OHE levels. The specificity of the 2-OHE assay was 100% for 2-OHE and 2-hydroxyestradiol, 68% for estetrol, and less than 1% for other relevant estradiol metabolites (29). The 2-OHE assay therefore reflects a composite end point of the 2-hydroxylation pathway. The specificity for the 16-OHE assay was 100% for 16-OHE and less than 1% for other relevant estradiol metabolites (29).

Individual 2-OHE and 16-OHE concentrations were divided by urinary creatinine concentration to correct for hydration and urinary volume differences that could bias estimates of hormone concentration. Creatinine standardization is typically employed in estimates of urinary biomarker concentrations. However, creatinine excretion varies with muscle mass (30), and group differences in muscle mass require a stan-

standardization technique that takes these differences into consideration (31). We conducted preliminary analysis and found that muscle mass did not differ as a function of ethnicity or HRT usage. We therefore employed the usual creatinine standardization and divided the 2-OHE and 16-OHE concentrations (nanograms per milliliter) by creatinine concentrations (milligrams per milliliter) to correct for hydration and urine volume differences. Estrogen metabolite concentrations are therefore expressed as nanograms per milliliter of creatinine.

Statistical analysis

A series of *t* tests was conducted to compare age, body mass index (BMI), SBP, 2-OHE, 16-OHE, and the 2:16-OHE ratio in postmenopausal women who were either taking or not taking HRT. We focused on SBP based on the recommendations of Chobanian *et al.* (1), who reported that SBP is superior to diastolic blood pressure in predicting cardiovascular disease, especially in adults over the age of 50 yr. Correlational analyses were conducted to examine bivariate associations between estrogen metabolite variables and SBP. Ordinary linear regression analyses were used to test the magnitude of the effects of estrogen metabolites on SBP independent of demographic characteristics (age, BMI, and race/ethnicity), use of vasoactive blood pressure medications (0 = no; 1 = yes), and use of volume-active blood pressure medications (0 = no; 1 = yes). Before regression analysis, estrogen metabolite values were standardized, and the ratio values were calculated from the standardized values. Regression coefficients are therefore interpretable as the magnitude of change in SBP associated with a 1 sd difference in the predictor variable. All analyses employed only those cases with no missing data for estrogen metabolites and covariates used in the regression analyses (*n* = 80). Statistical significance was set at *P* < 0.05. All analyses were conducted using SPSS 13.0 (SPSS, Inc., Chicago, IL).

Results

In the study group, 61% of women had hypertension, and this proportion did not differ by HRT status (*P* > 0.9). Women receiving HRT did not differ from women not receiving HRT in age, BMI, or SBP (*P* > 0.2; Table 1). As would be expected, women receiving HRT had significantly higher urinary levels of both 2-OHE and 16-OHE than women not receiving HRT. However, the 2:16-OHE ratio did not differ based upon HRT status. A supplementary regression analysis was conducted to examine whether the relationship between the 2:16-OHE ratio and SBP differed as a function of HRT status. A nonsignificant interaction between the 2:16-OHE ratio and HRT status (*P* > 0.8) indicated that the regression slopes did not differ as a function of HRT status (Fig. 1). Therefore, subsequent analyses collapsed across HRT groups.

Correlational analysis revealed a significant inverse association between SBP and the 2:16-OHE ratio (*r* = -0.30; *P* < 0.01). Age and BMI showed modest positive associations with SBP (*r* = 0.21 and 0.20; *P* = 0.06 and 0.08, respectively). Neither age nor BMI was significantly associated with the 2:16-OHE ratio (*P* > 0.4).

A multivariate linear regression analysis showed that the

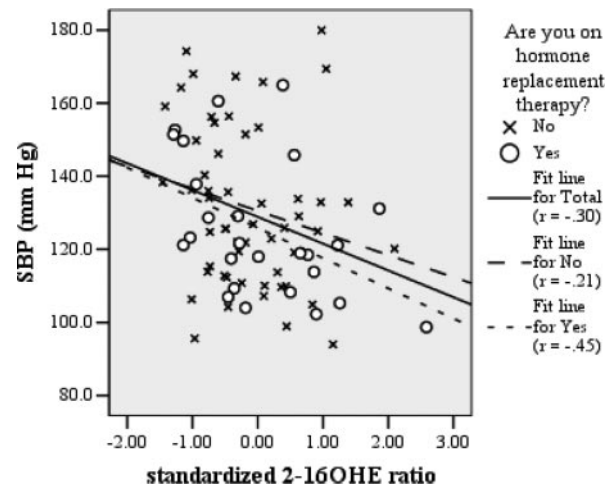


FIG. 1. Scatterplot showing the bivariate relationships, overall and by HRT group, between the urinary 2:16-OHE ratio and SBP. Regression lines are fit using the least squares method.

2:16-OHE ratio retained a significant association with SBP when age, BMI, race/ethnicity, and vasoactive and volume active hypertension medications were kept constant. As Table 2 shows, a sd increase in the 2:16-OHE ratio was associated with a 6.7-mm Hg decrease in SBP. When the 2:16-OHE ratio was replaced by either 2-OHE or 16-OHE in the multivariate regression model, neither 2-OHE nor 16-OHE was significantly related to SBP.

Discussion

The present research establishes for the first time an association between the urinary 2:16-OHE ratio and SBP in humans. In a population-based sample of postmenopausal women, this ratio was a significant predictor of SBP after controlling for age, BMI, race/ethnicity, and use of antihypertensive medications. Although women taking HRT had higher levels of urinary 2-OHE and 16-OHE compared with women not taking HRT, the 2:16-OHE ratios and the mean SBP did not differ between these two groups. Although we measured urinary 2- and 16-OHE and not the metabolites known to affect VSMC proliferation, our findings are consistent with the hypothesis that an elevated 2:16-OHE ratio reflects greater relative activity of the 2-hydroxylation (*vs.* the 16 α -hydroxylation) pathway of 17 β -estradiol metabolism and increased relative production of 2-hydroxyestradiol and 2-methoxyestradiol.

Previous research indicates that the inhibitory effects of 2-hydroxyestradiol and 2-methoxyestradiol on cellular proliferation are not mediated by estrogen receptors. Rather,

TABLE 1. Comparison of baseline characteristics of 80 postmenopausal women according to HRT status

Measure	Not on HRT (n = 54) (mean)	On HRT (n = 26) (mean)	t-statistic (df = 78)	<i>P</i>
Age (yr)	57.2	57.7	0.0483	0.63
BMI (kg/m ²)	32.9	30.8	1.23	0.22
SBP (mm Hg)	131.4	125.4	1.204	0.23
Urinary 2-OHE (ng/mg creatinine)	9.9 (4.1) ^a	23.3 (9.8)	-5.183	<0.001
Urinary 16-OHE (ng/mg creatinine)	6.9 (2.9)	15.4 (6.4)	-6.198	<0.001
Urinary 2:16-OHE ratio	1.5	1.7	-0.973	0.33

^a Numbers in parentheses are in SI units (picomoles/micromoles creatinine).

TABLE 2. Regression coefficients predicting SBP in 80 postmenopausal women

	Coefficient (SE)	Lower and upper bounds of 95% confidence interval
Urinary estrogen predictor		
2:16-OHE ratio	−6.7 (2.6) ^a	−11.96, −1.41
Covariates		
Age	0.9 (0.6)	−0.28, 2.14
BMI	0.6 (0.3)	−0.11, 1.27
Ethnicity ^b		
African-American	−1.9 (3.5)	−8.92, 5.20
Latino-American	0.3 (3.5)	−6.69, 7.33
Vasactive BP medications	8.5 (5.3)	−2.02, 19.10
Volume active BP medications	1.4 (6.4)	−11.35, 14.10
Intercept	53.5 (37.9)	−21.98, 129.02

BP, Blood pressure.

^a $P < 0.05$.^b The reference group is Caucasian Americans.

these metabolites operate via other mechanisms, including microtubule depolymerization and disruption of mitosis via colchicine-binding sites, increased production of cellular reactive oxygen species, and induction of apoptosis by up-regulation of death receptor 5 (32). In contrast to 2-hydroxyestradiol and 2-methoxyestradiol, 16 α -hydroxyestradiol inhibits VSMC and glomerular mesangial cell proliferation only at supraphysiological concentrations (17). We found that 2-OHE and 16-OHE alone were not significant predictors of SBP in linear regression models that controlled for age, BMI, race/ethnicity, and use of antihypertensive medications. This suggests that the relative concentrations of these metabolites are more strongly associated with SBP than the concentration of either metabolite alone and may reflect competitive inhibition of 2-hydroxyestradiol and 2-methoxyestradiol by 16 α -hydroxyestradiol at the colchicine-binding sites. Although women taking HRT had higher urinary levels of 2-OHE, their ratio of 2:16-OHE did not differ from those not taking HRT, and we found no difference in SBP between these groups, suggesting that the ratio of 2-hydroxylation metabolites to 16 α -hydroxylation metabolites is more important to SBP than the concentration of either alone.

In the WHI, CHD incidence was increased among women who received CEE plus MPA (6), but not among those taking CEE alone (5). Interestingly, progesterone inhibits 2-hydroxylation of estradiol *in vitro* (33). If MPA has a similar effect in humans, it may reduce the production of 2-hydroxyestradiol and 2-methoxyestradiol and permit VSMC proliferation, a risk factor for hypertension (34). In our study population, the mean urinary 2:16-OHE ratio among those taking CEE plus MPA did not differ from that of those taking CEE alone or from all other women in the study ($P > 0.2$).

Although we have hypothesized that the inverse relationship between 2:16-OHE and SBP reflects preferential activation of the 2-hydroxylation (*vs.* the 16 α -hydroxylation) pathway of 17 β -estradiol metabolism and increased production of 2-hydroxyestradiol and 2-methoxyestradiol, other explanations should be considered. For example, lower SBP may somehow lead to preferential activation of the 2-hydroxylation pathway, or an unmeasured third factor may affect both SBP and the balance between the 2- and 16 α -hydroxylation pathways. Of these possibilities, however, most evidence

supports the idea that 2-hydroxylation metabolites of 17 β -estradiol lead to lower blood pressure via established vascular effects.

A potential limitation of this study is our use of urinary 2-OHE, instead of urinary 2-hydroxyestradiol or 2-methoxyestradiol as a study marker. The 2-OHE enzyme immunoassay we used cross-reacts with 2-hydroxyestradiol (29), so our measure of 2-OHE is actually a composite of 2-OHE and 2-hydroxyestradiol. In addition, previous studies have shown that urinary excretion of estrone is positively correlated with urinary excretion of 17 β -estradiol (35), whereas the urinary 2-OHE concentration is positively correlated with the urinary 2-methoxyestradiol concentration (36). 2-Hydroxyestradiol is rapidly converted to 2-methoxyestradiol *in vivo*, and the concentrations of these metabolites are positively correlated in rat plasma (37). We therefore believe urinary 2-OHE is a suitable marker for the production of both 2-hydroxyestradiol and 2-methoxyestradiol, and the 2:16-OHE ratio reflects the relative activity of the 2- and 16 α -hydroxylation pathways of 17 β -estradiol.

The urinary concentrations of 2-OHE and 16-OHE in this study are consistent with the results of a previous study of postmenopausal women not taking HRT (38). As expected, the urinary 2-OHE and 16-OHE levels among women not taking HRT in our study were lower than the urinary 2-OHE and 16-OHE levels in premenopausal women in previous studies (39, 40). The mean urinary 2:16-OHE ratios among premenopausal women in those studies were 2.0 and 1.8, respectively, slightly higher than the mean ratio (1.5) among postmenopausal women not taking HRT in our study. Although this may suggest a higher 2:16-OHE ratio among premenopausal compared with postmenopausal women, a study that compared the mean 2:16-OHE ratio of premenopausal and postmenopausal women found no significant difference between the groups (38).

Our results suggest that shifting the balance of estrogen metabolism from 16 α -hydroxylation to 2-hydroxylation is associated with lower blood pressure. Given the competitive nature of the 2- and 16 α -hydroxylation pathways (23), such a shift could occur by increasing the activity of CYP1A1, which catalyzes 2-hydroxylation of 17 β -estradiol (41). A potent inducer of 2-hydroxylation is indole-3-carbinol, which is present in cruciferous vegetables such as broccoli, Brussels sprouts, and cabbage (23). Administration of indole-3-carbinol to healthy volunteers significantly increases the ratio of urinary 2-OHE to 16-OHE (42).

An alternative way to increase the 2:16-OHE ratio is to inhibit CYP3A4, which catalyzes 16 α -hydroxylation of 17 β -estradiol. CYP3A4 is inhibited by grapefruit juice as well as certain medications, including calcium channel blockers (diltiazem), macrolide antibiotics (erythromycin and clarithromycin), and selective somatostatin release inhibitors (fluoxetine) (43). The 2-methoxyestradiol concentration may also be increased directly through oral supplementation. Phase I and phase II clinical trials of 2-methoxyestradiol, an oral anticancer agent, have shown it to be well tolerated (32), although the cardiovascular effects of this therapy have not been described.

Future research into estrogen metabolism and hypertension should address several questions. First, does the asso-

ciation between the 2:16-OHE ratio and SBP exist in other populations, including men and premenopausal women? The similarity of our findings among women who were and were not taking HRT suggests that the association between the 2:16-OHE ratio and SBP may hold across a wide range of basal estrogen levels. Second, could therapies that increase the 2:16-OHE ratio reduce age-associated increases in blood pressure and provide protection against hypertension or CHD incidence? Although oral 2-methoxyestradiol is currently being tested as an anticancer agent, larger studies are needed to determine whether oral supplementation alone is sufficient to shift the 2:16-OHE balance and to identify any risks associated with such therapies. Finally, can the relationship between estrogen metabolites and SBP shed light on the WHI results? It is possible that the WHI intervention failed to reduce CHD risk not because it did not increase circulating estrone, but because oral CEE does not affect the 2:16 α -hydroxylation ratio and because estrone metabolites are not effective inhibitors of VSMC proliferation?

In summary, we found the urinary 2:16-OHE ratio to be a significant predictor of SBP in a population-based sample of postmenopausal women. This finding is consistent with previous studies documenting inhibition of VSMC and glomerular mesangial cell proliferation by 2-hydroxyestradiol and 2-methoxyestradiol *in vitro* as well as inhibition of angiotensin II-induced hypertension by 2-methoxyestradiol in rats (13, 17, 19). Future studies should focus on the relationship between estrogen metabolism and SBP in men and younger women as well as the role of therapies that shift the balance of 17 β -estradiol oxidative metabolism toward 2-hydroxylation.

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