

Published in final edited form as:

Am J Hypertens. 2009 November ; 22(11): 1148–1153. doi:10.1038/ajh.2009.155.

Serum Estrogen Metabolites and Systolic Blood Pressure Among Middle-Aged and Older Women and Men

Christopher M. Masi¹, Louise C. Hawkey², Xia Xu³, Timothy D. Veenstra³, and John T. Cacioppo²

¹Section of General Internal Medicine and the Department of Medicine, University of Chicago, Chicago, Illinois, USA

²Center for Cognitive and Social Neuroscience and the Department of Psychology, University of Chicago, Chicago, Illinois, USA

³Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., Advanced Technology Program, National Cancer Institute at Frederick, Frederick, Maryland, USA

Abstract

Background—Hypertension is more common among men at younger ages and among women after age 60, suggesting a possible link between endogenous estrogens and systolic blood pressure (SBP). We tested whether serum 17 β -estradiol (E₂) or any of its metabolites were associated with SBP among middle-aged and older adults.

Methods—Using a cross-sectional study design, we examined data from a population-based sample of 98 adults living in Cook County, Illinois. Age ranged between 55 and 69 years and body mass index (BMI) ranged between 19.8 and 50.6 kg/m². Serum was analyzed for 17 β -E₂ and 14 estrogen metabolites (EMs) using mass spectrometry. SBP was measured using a tonometric device that records a pulse wave at the radial artery. Demographic and health history information were obtained via questionnaires.

Results—Univariate analysis revealed an inverse relationship between SBP and both natural log (ln) 16 α -hydroxyestrone (OHE₁) ($r = -0.360$, $P < 0.05$) and ln 16-ketoestradiol (ketoE₂) ($r = -0.360$, $P < 0.05$) among women but not men. No significant correlations were found between SBP and 17 β -E₂ in either sex. In multivariate analysis which adjusted for age, race, ethnicity, BMI, and use of cardiovascular medications, ln 16 α -hydroxyestrone (16 α -OHE₁) ($B = -5.3$, s.e. = 2.1, $P < 0.05$) and ln 16-ketoE₂ ($B = -4.7$, s.e. = 1.9, $P < 0.05$) continued to be negatively associated with SBP among postmenopausal women.

Conclusions—These data suggest that serum 16 α -OHE₁ or 16-ketoE₂ may be important for vascular health among postmenopausal women but not among similarly aged men.

Systolic blood pressure (SBP) increases with age among both men and women. The slope of this increase is steeper among men before age 60 but this pattern reverses after age 60 (ref. 1). As a result, hypertension is more prevalent among men at younger ages² and among women at older ages.³ This pattern is consistent with age- and sex-related differences in 17 β -estradiol (E₂) production, a hormone with known vasodilatory effects.⁴

The mechanisms by which E₂ influences vascular tone include genomic and nongenomic effects on endothelial and vascular smooth muscle cells (VSMCs).⁵ Genomic effects on

Correspondence: Christopher M. Masi (cmasi@medicine.bsd.uchicago.edu).

Disclosure: The authors declared no conflict of interest.

vascular endothelium include increased mitogen-activated protein kinase–induced endothelial nitric oxide (NO) synthase gene expression and NO production, as well as increased endothelial cell production.⁵ Nongenomic effects of E₂ on endothelial cells include increased endothelial NO synthase activity, increased NO generation, enhanced prostacyclin production, and reduced free radical (superoxide) formation.⁵ NO and prostacyclin from the endothelium have vasodilatory effects on VSMCs. In VSMC, E₂ inhibits growth factor-mediated activation of mitogen-activated protein kinase, impairs gene transcription, and reduces VSMC growth and proliferation.⁵ Nongenomically, E₂ reduces Ca²⁺ influx into VSMCs, thereby inhibiting Ca²⁺-dependent myosin light chain phosphorylation and VSMC contraction.⁵

Several estrogen metabolites (EMs), including D-ring metabolites such as 16 α -hydroxyestrone (OHE₁), bind to both α and β estrogen receptors.⁶ This metabolite induces endothelial cell prostacyclin production to a greater extent than E₂⁷ and inhibits oxidation of low-density lipoproteins,⁸ suggesting it may have vasculoprotective effects. The purpose of the current study is to examine the relationship between endogenous serum estrogen concentrations and SBP in a population-based sample of middle-aged and older adults. Given the established vascular effects of 17 β -E₂ and the potential vasculoprotective effects of 16 α -hydroxyestrone (16 α -OHE₁), we hypothesized significant inverse relationships between these estrogens and SBP in our study population.

Methods

Study population

Data for this study were collected in 2005, the fifth year of the Chicago Health, Aging, and Social Relations Study, 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 years 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 previously described multistage probability design.⁹ With attrition, the original sample size of 229 adults (120 females and 109 males) in year 1 declined to 163 adults (91 females and 72 males) in year 5. All procedures were approved by the University of Chicago Institutional Review Board and all participants provided informed consent.

After obtaining height and weight measurements, 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 of the wrist, allowing for beat-to-beat measurement of blood pressure. The tonometer was calibrated against an initial blood pressure reading using an oscillometric cuff and was periodically recalibrated either automatically or on experimenter initiation. As described in a previous report, our analysis used an average of 280 beat-to-beat blood pressure values obtained during 4 min in the seated posture.⁹ Participants listed their medications, and 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.

Following the blood pressure measurement, we obtained permission to collect venous blood samples from 52 women and 51 men. All women reported being postmenopausal at the time of collection. Serum aliquots were stored at -80°C and batched for analysis. Samples were then shipped on dry ice to the Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Maryland for analysis of EM concentrations.

EM assay

Reagents and materials—Fifteen EMs including estrone (E_1), E_2 , estriol (E_3), 16-epiestriol (16-epi E_3), 17-epi E_3 , 16-ketoestradiol (16-keto E_2), 16 α -OHE $_1$, 2-methoxyestrone (2-MeOE $_1$), 4-MeOE $_1$, 2-OHE $_1$ -3-methyl ether (3-MeOE $_1$), 2-methoxyestradiol (2-MeOE $_2$), 4-MeOE $_2$, 2-OHE $_1$, 4-OHE $_1$, and 2-hydroxyestradiol (2-OHE $_2$) were obtained from Steraloids, (Newport, RI). Stable isotope-labeled estrogens (SI-EMs), including E_2 -13,14,15,16,17,18- $^{13}\text{C}_6$ ($^{13}\text{C}_6$ - E_2) and E_1 -13,14,15,16,17,18- $^{13}\text{C}_6$ ($^{13}\text{C}_6$ - E_1) were purchased from Cambridge Isotope Laboratories, (Andover, MA); E_3 -2,4,17- d_3 (d_3 - E_3), 2-OHE $_2$ -1,4,16,16,17- d_5 (d_5 -2-OHE $_2$), and 2-MeOE $_2$ -1,4,16,16,17- d_5 (d_5 -2-MeOE $_2$), were obtained from C/D/N Isotopes, (Pointe-Claire, Quebec, Canada). 16-epi E_3 -2,4,16- d_3 (d_3 -16-epi E_3) was purchased from Medical Isotopes (Pelham, NH). All EM and SI-EM analytical standards have reported chemical and isotopic purity $\geq 98\%$, and were used without further purification. Dichloromethane, methanol, and formic acid were obtained from EM Science (Gibbstown, NJ). Glacial acetic acid, sodium bicarbonate, and L-ascorbic acid were purchased from J.T. Baker (Phillipsburg, NJ) whereas sodium hydroxide and sodium acetate were purchased from Fisher Scientific (Fair Lawn, NJ). β -Glucuronidase/sulfatase (*Helix pomatia*, Type HP-2) was obtained from Sigma Chemical (St Louis, MO). Dansyl chloride and acetone were purchased from Aldrich Chemical (Milwaukee, WI). All chemicals and solvents used in this study were high performance liquid chromatography or reagent grade unless otherwise noted.

Preparation of stock and working standard solutions—Stock solutions of EM and SI-EM were each prepared at 80 $\mu\text{g/ml}$ by dissolving 2 mg of the estrogen powders in methanol with 0.1% L-ascorbic acid to a final volume of 25 ml in a volumetric flask. The stock solutions are stable for at least 2 months while stored at -20°C . Stock solutions were analyzed at the beginning of each analysis to verify no time-dependent degradation of the EM and SI-EM standards had occurred. Working standards of EM and SI-EM at 8 ng/ml were prepared by dilutions of the stock solutions using methanol with 0.1% L-ascorbic acid.

Calibration standards and quality control samples—Charcoal-stripped human serum (Golden West Biologicals, Temecula, CA) with no detectable levels of any EM and containing 0.1% (wt/vol) L-ascorbic acid, was employed for preparation of calibration standards and quality control samples. Calibration standards were prepared in charcoal-stripped human serum by adding 2 μl of the SI-EM working internal standard solution (16 pg of each SI-EM) to various volumes of the EM working standard solution. These calibration standards typically contain 0.2–200 pg of each EM in 0.4 ml of charcoal-stripped serum and were assayed in duplicate. The calibration standards cover three orders of magnitude. The quality control samples were prepared at three levels: 0.5, 20, and 250 pg/ml of each EM.

Sample preparation—To 400 μl of each serum sample, 500 μl of freshly prepared enzymatic hydrolysis buffer containing 2 mg of L-ascorbic acid, 5 μl of β -glucuronidase/sulfatase (*Helix pomatia*, Type HP-2), and 0.5 ml of 0.15 mol/l sodium acetate buffer (pH 4.6) was added. The samples were incubated for 20 h at 37°C . Aliquots then underwent slow inverse extraction at 8 r.p.m. (RKVSD; ATR, Laurel, MD) with 6 ml dichloromethane for 30 min. After extraction, the aqueous layer was discarded and the organic solvent portion

was transferred into a clean glass tube and evaporated to dryness at 60 °C under nitrogen gas (Reacti-Vap III; Pierce, Rockford, IL).

To each dried sample, 32 µl of 0.1 mol/l sodium acetate buffer (pH at 9.0) and 32 µl of dansyl chloride solution (1 mg/ml in acetone) were added. After vortexing, the sample was heated at 60 °C (Reacti-Therm III Heating Module; Pierce) for 5 min to form the EM and SI-EM dansyl derivatives (EM-Dansyl and SI-EM-Dansyl, respectively). Calibration standards and quality control samples were hydrolyzed, extracted, and derivatized following the same procedure as that used for unknown serum samples.

Capillary liquid chromatography-electrospray ionization-tandem mass spectrometry analysis—After derivatization, all samples were analyzed using capillary liquid chromatography-electrospray ionization-tandem mass spectrometry analysis.¹⁰ Capillary liquid chromatography-electrospray ionization-tandem mass spectrometry analysis was performed using an Agilent 1200 series nanoflow LC system (Agilent Technologies, Palo Alto, CA) coupled to a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Electron, San Jose, CA). The LC separation was carried out on a 150 mm long × 300 µm internal diameter column packed with 4 µm Synergi Hydro-RP particles (Phenomenex, Torrance, CA) and maintained at 40 °C. A total of 8 µl of each sample was injected onto the column. The mobile phase, operating at a flow rate of 4 µl/min, consists of methanol as solvent A and 0.1% (vol/vol) formic acid in water as solvent B. A linear gradient from 72 to 85% solvent B in 75 min. was employed for separation of EM and SI-EM. The mass spectrometry conditions were: source: ESI; ion polarity: positive; spray voltage: 3,500 V; sheath and auxiliary gas: nitrogen; sheath gas pressure: seven arbitrary units; ion transfer capillary temperature, 270 °C; scan type: selected reaction monitoring; collision gas: argon; collision gas pressure: 1.5 mTorr; scan width: 0.7 u; scan time: 0.50 s; Q1 peak width: 0.70 u full-width half-maximum; Q3 peak width: 0.70 u full-width half-maximum. The optimized selected reaction monitoring conditions for the protonated molecules [MH]⁺ of EM-Dansyl and SI-EM-Dansyl were similar to those previously described.¹⁰

Quantitation of serum EMs—Quantitation of serum EM was carried out using Xcalibur Quan Browser (Thermo Electron, Waltham, MA). Briefly, calibration curves for the each EM were constructed by plotting EM-Dansyl/SI-EM-Dansyl peak area ratios obtained from calibration standards vs. amounts of EM injected on column and fitting these data using linear regression with 1/X weighting. The amounts of EM in sera were then interpolated using this linear function. Based on their similarity of structures and retention times, ¹³C₆-E₂ was used as the internal standard for E₂; ¹³C₆-E₁ for E₁; d₃-E₃ for E₃, 16-ketoE₂, and 16α-OHE₁; d₃-16-epiE₃ for 16-epiE₃ and 17-epiE₃; d₅-2-MeOE₂ for 2-MeOE₂, 4-MeOE₂, 2-MeOE₁, 4-MeOE₁, and 3-MeOE₁; d₅-2-OHE₂ for 2-OHE₂, 2-OHE₁, and 4-OHE₁, respectively.

Statistical analysis—Statistical analysis focused on SBP because it is superior to diastolic blood pressure in predicting cardiovascular disease, especially in adults over the age of 50 years.¹¹ For the purposes of this study, we utilized data from the 47 postmenopausal females who were not on hormone replacement therapy and from 51 men. Correlational analyses were conducted to examine the bivariate associations between serum EM concentrations and SBP in both women and men. Ordinary linear regression analyses were used to test the magnitude of effects of EMs on SBP independent of demographic characteristics (age, body mass index (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). EM values exhibited a positively skewed distribution and were therefore subjected to a natural log (ln) transformation. Regression coefficients are therefore

interpretable as the magnitude of change in SBP associated with one ln unit increase of the predictor metabolite. Regression analysis among women employed only those cases ($N = 32-33$) with no missing data on any of the variables used in the regression model. Statistical significance was set at $P < 0.05$. All analyses were conducted using SPSS 16.0 (SPSS, Chicago, IL).

Results

Among women in the study, 23 were white, 11 were African American, and 13 were Latino American. Among men, 22 were white, 15 were African American, and 14 were Latino American. Age, BMI, and SBP data, as well as EM concentrations for cases with detectable levels of metabolites are provided in Table 1. Concentrations of E_1 , E_2 , E_3 , and $2-OHE_1$ were at measurable levels in all participants; the remaining metabolites exhibited varying prevalence of undetectable levels. Consistent with previous research, the concentrations of several serum estrogens were higher among men compared to women in this age group.¹²

Correlational analysis revealed significant inverse relationships between SBP and both $16\alpha-OHE_1$ ($r = -0.360$; $P = 0.031$) and $16\text{-keto}E_2$ ($r = -0.360$; $P = 0.029$) among women. Figures 1 and 2 demonstrate the scatterplots of the associations between SBP and $16\alpha-OHE_1$ and between SBP and $16\text{-keto}E_2$, respectively. None of the other EMs was significantly correlated with SBP in women. Among men, there were no significant correlations between SBP and E_2 or any other serum estrogen. Although serum concentrations of certain EMs varied by sex, for cases with detectable levels, serum metabolite concentrations did not differ as a function of race or ethnicity, $P_s > 0.05$. Fifty percent of participants were taking vasoactive medications, 17% were taking volume-active medications, and the remainder were taking both vasoactive and volume-active medications.

Multivariate regression analysis of data from 32 to 33 women for whom we had both EM and covariate information showed that both $16\alpha-OHE_1$ and $16\text{-keto}E_2$ retained significant associations with SBP when age, BMI, race/ethnicity, and vasoactive and volume active hypertension medications were kept constant. Table 2 shows that a ln unit increase in $16\alpha-OHE_1$ was associated with a 5.3 mm Hg decrease in SBP whereas a ln unit increase in $16\text{-keto}E_2$ was associated with a 4.7 mm Hg decrease in SBP. A ln unit increase in the sum of $16\alpha-OHE_1$ and $16\text{-keto}E_2$ was associated with a 4.6 mm Hg decrease in SBP after adjusting for multiple covariates. As combining these metabolites provided no increase in effect magnitude, it is unlikely that $16\alpha-OHE_1$ and $16\text{-keto}E_2$ have additive vasodilatory effects. When both metabolites were simultaneously entered in the regression model, neither had a significant effect on SBP, $P_s > 0.4$, suggesting a high correlation between these variables. Ancillary analysis revealed the correlation coefficient between $16-OHE_1$ and $16\text{-keto}E_2$ was 0.91. Therefore it is likely that $16\text{-keto}E_2$ is not independently associated with SBP but serves as a marker for $16\alpha-OHE_1$ (or vice versa).

Discussion

The present study is the first to examine the relationship between *serum* EMs and blood pressure in a population-based sample of middle-aged and older adults. Consistent with prior studies, postmenopausal women had lower levels of serum E_1 and $17\beta-E_2$ compared to similarly aged men.¹² In addition, the abundance of E_3 in the serum is consistent with studies showing it is the most abundant urinary estrogen.¹² Among postmenopausal women, we found an inverse relationship between serum $16\alpha-OHE_1$ and SBP after adjusting for multiple covariates. $16\text{-keto}E_2$, an EM similar in structure to $16\alpha-OHE_1$, also demonstrated an inverse relationship with SBP in this population. However, contrary to our hypothesis, we did not find an inverse relationship between $17\beta-E_2$ and SBP in either women or men.

Previous research has shown that endothelial production of the vasodilator prostacyclin can be induced by 16α -OHE₁.⁷ 16α -OHE₁ also has potent antioxidant effects.⁸ These properties may contribute to the association between 16α -OHE₁ and lower blood pressure among postmenopausal women. In addition, at least one study suggests that 16α -OHE₁ can form covalent bonds with estrogen receptors, possibly leading to longer-lasting estrogenic effects.¹³ Within the arterial vasculature, these effects may include increased vasodilator production, enhanced endothelial cell production, inhibition of reactive oxygen species, and inhibition of VSMC proliferation.⁵ A prolonged effect of 16α -OHE₁ may explain its greater association with SBP than 17β -E₂, which may exert shorter-term effects.

Previous research suggests lower serum concentrations of 16α -OHE₁ and 16 -ketoE₂ among postmenopausal compared to premenopausal women. In a recent article describing an assay for EMs, the mean serum concentrations of 16α -OHE₁ and 16 -ketoE₂ among premenopausal women ($N = 4$) were 21.4 pg/ml (s.d. = 16.1) and 25.4 pg/ml (s.d. = 16.6), respectively.¹⁰ Among postmenopausal women ($N = 2$), the mean serum concentrations of 16α -OHE₁ and 16 -ketoE₂ were 8.8 pg/ml (s.d. = 0.6) and 11.0 pg/ml (s.d. = 0.9), respectively.¹⁰ Due to the small sample sizes in that article, neither of the metabolite concentration differences associated with menopausal status was significant. Our study population included only postmenopausal women and the mean serum concentrations for 16α -OHE₁ and 16 -ketoE₂ were comparable to the mean values among postmenopausal women in the previous report.¹⁰ The trend toward decreased serum 16α -OHE₁ and 16 -ketoE₂ among postmenopausal women is consistent with our hypothesis that a decline in one of these metabolites contributes to increased blood pressure among postmenopausal women.

Among men in our study population, SBP was not associated with 17β -E₂, 16α -OHE₁, or 16 -ketoE₂. This is not surprising given results of animal and human studies which show sex differences in vascular function. For example, endothelium-dependent vascular relaxation is greater among female than male spontaneously hypertensive rats,¹⁴ total endothelial NO release is greater in premenopausal women than in men,¹⁵ superoxide production is greater in the blood vessels of male compared to female rats,⁵ and less endothelin-1 is released from female spontaneously hypertensive rat endothelial cells than from those of male spontaneously hypertensive rats.¹⁶ Therefore, our findings do not contradict research demonstrating important sex differences in vascular physiology.

A limitation of this study is its relatively small sample size. Because we did not find correlations between endogenous estrogens and SBP among men, our analysis focused on the 32–33 postmenopausal women for whom all EM and covariate data were available. Therefore, our results should be viewed as preliminary until replicated in a larger cohort of postmenopausal women. Another potential limitation of this study was our use of total (conjugated plus unconjugated) serum EMs. As 16α -OHE₁ circulates primarily unbound to sex hormone binding globulin,¹⁷ the total concentration of this metabolite may approximate its unbound (and therefore metabolically active) concentration. In contrast, most other EMs are significantly bound to sex hormone binding globulin and the lack of association between total serum concentration of those metabolites and SBP may be due more to their unavailability to estrogen receptors than to their inability to induce vasodilatory changes. Future studies should evaluate the relationship between SBP and both conjugated and unconjugated EMs.¹⁰ Finally, our study was cross-sectional in design. Therefore, we cannot infer that higher serum concentrations of 16α -OHE₁ resulted in lower SBP among postmenopausal women. The reverse may be true or a third factor may account for this inverse relationship. Causal inferences will require prospective analysis of the relationship between serum EMs and SBP.

The mechanism by which 16 α -OHE₁ and 16-ketoE₂ are associated with lower blood pressure in postmenopausal women is unknown but we hypothesize that 16 α -OHE₁ is the active metabolite whereas 16-ketoE₂ simply serves as a marker for 16 α -OHE₁. This hypothesis is based upon studies which show that 16 α -OHE₁ can induce endothelial prostacyclin production,⁷ has antioxidant effects,⁸ and can bind covalently to estrogen receptors.¹³ If our findings are replicated, future studies should investigate dietary and genetic predictors of 16 α -OHE₁ production. Such information may lead to screening tests which identify women at risk for hypertension. It may also lead to therapies which mimic the potentially vasculoprotective effects of 16 α -OHE₁.

Acknowledgments

This work was supported by a National Institute on Aging Career Development Award 5K08AG027200-02 (principal investigator: C.M. Masi) and a National Institute on Aging R01 AG034052-01 (principal investigator: J.T. Cacioppo). This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract N01-CO-12400. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

References

- Franklin SS, Gustin W, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation*. 1997; 96:308–315. [PubMed: 9236450]
- Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988-2000. *JAMA*. 2003; 290:199–206. [PubMed: 12851274]
- Ostchega Y, Dillon CF, Hughes JP, Carroll M, Yoon S. Trends in hypertension prevalence, awareness, treatment, and control in older U.S. adults: data from the National Health and Nutrition Examination Survey 1988 to 2004. *J Am Geriatr Soc*. 2007; 55:1056–1065. [PubMed: 17608879]
- Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol*. 2004; 286:R233–R249. [PubMed: 14707008]
- Qiao X, McConnell KR, Khalil RA. Sex steroids and vascular responses in hypertension and aging. *Gend Med*. 2008; 5 A:S46–S64. [PubMed: 18395683]
- Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis*. 1998; 19:1–27. [PubMed: 9472688]
- Seeger H, Mueck AO, Lippert TH. Effect of estradiol metabolites on prostacyclin synthesis in human endothelial cell cultures. *Life Sci*. 1999; 65:PL167–170. [PubMed: 10503964]
- Seeger H, Mueck AO, Lippert TH. Effect of estradiol metabolites on the susceptibility of low density lipoprotein to oxidation. *Life Sci*. 1997; 61:865–868. [PubMed: 9284079]
- Hawkley LC, Masi CM, Berry JD, Cacioppo JT. Loneliness is a unique predictor of age-related differences in systolic blood pressure. *Psychol Aging*. 2006; 21:152–164. [PubMed: 16594800]
- Xu X, Roman JM, Issaq HJ, Keefer LK, Veenstra TD, Ziegler RG. Quantitative measurement of endogenous estrogens and estrogen metabolites in human serum by liquid chromatography-tandem mass spectrometry. *Anal Chem*. 2007; 79:7813–7821. [PubMed: 17848096]
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA*. 2003; 289:2560–2572. [PubMed: 12748199]
- Bulun, SE.; Adashi, EY. The Physiology and pathology of the female reproductive axis. In: Larson, PR.; Kronenberg, HM.; Melmed, S.; Polonsky, KS., editors. *Williams Textbook of Endocrinology*. 10th. Saunders; Philadelphia: 2003. p. 587-664.
- Swanek GE, Fishman J. Covalent binding of the endogenous estrogen 16 alpha-hydroxyestrone to estradiol receptor in human breast cancer cells: characterization and intranuclear localization. *Proc Natl Acad Sci USA*. 1988; 85:7831–7835. [PubMed: 3186693]

14. Pare G, Krust A, Karas RH, Dupont S, Aronovitz M, Chambon P, Mendelsohn ME. Estrogen receptor-alpha mediates the protective effects of estrogen against vascular injury. *Circ Res.* 2002; 90:1087–1092. [PubMed: 12039798]
15. Forte P, Kneale BJ, Milne E, Chowienczyk PJ, Johnston A, Benjamin N, Ritter JM. Evidence for a difference in nitric oxide biosynthesis between healthy women and men. *Hypertension.* 1998; 32:730–734. [PubMed: 9774371]
16. Fetalvero KM, Martin KA, Hwa J. Cardioprotective prostacyclin signaling in vascular smooth muscle. *Prostaglandins Other Lipid Mediat.* 2007; 82:109–118. [PubMed: 17164138]
17. Fishman J, Martucci C. Biological properties of 16 alpha-hydroxyestrone: implications in estrogen physiology and pathophysiology. *J Clin Endocrinol Metab.* 1980; 51:611–615. [PubMed: 7190977]

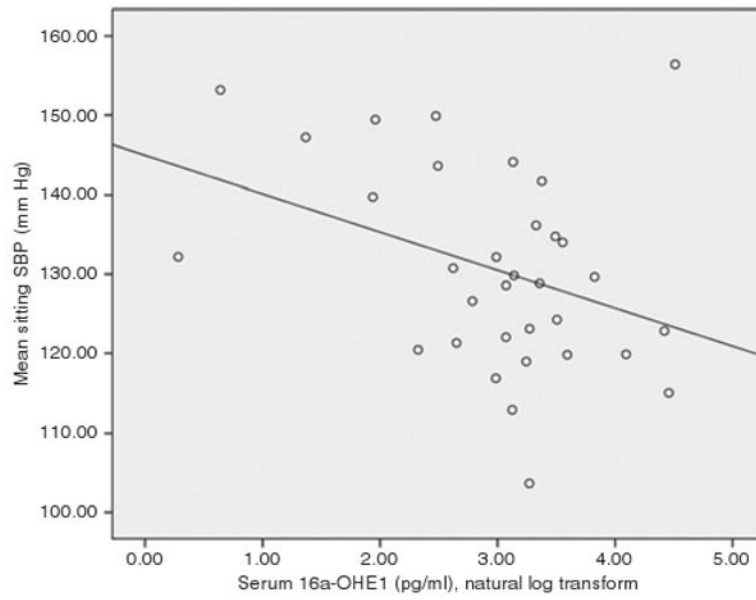


Figure 1. Scatterplot showing the bivariate relationships between natural log serum 16 α -hydroxyestrone and systolic blood pressure among postmenopausal women not taking hormone replacement therapy. The regression line was fit using the least squares method.

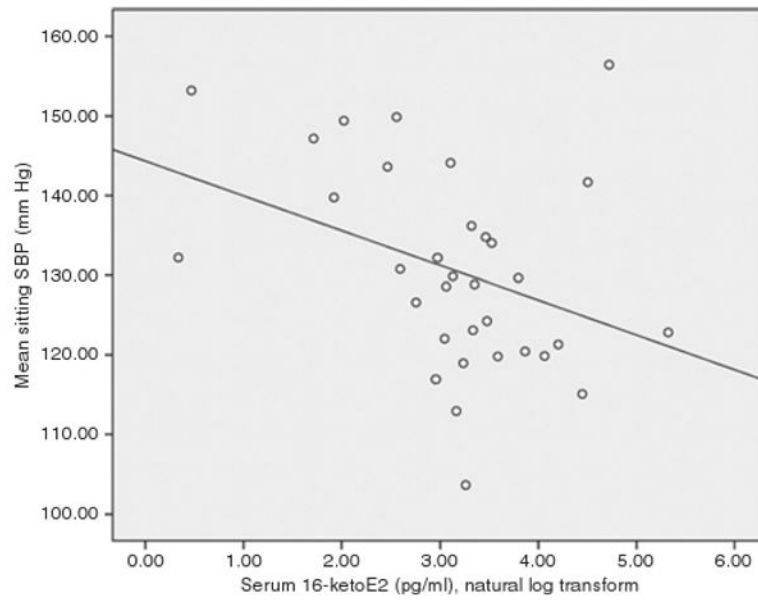


Figure 2. Scatterplot showing the bivariate relationships between natural log serum 16-ketoestradiol and systolic blood pressure among postmenopausal women not taking hormone replacement therapy. The regression line was fit using the least squares method.

Table 1
Participant demographics and serum estrogen metabolite concentrations

Variable	N	Female Mean (s.d.) ^a	N	Male Mean (s.d.) ^a	ANOVA P
Age	47	61.1 (3.9)	51	61.3 (4.3)	0.823
BMI (kg/m ²)	46	32.0 (7.3)	50	31.0 (5.5)	0.472
SBP (mm Hg)	46	131.5 (12.6)	50	133.8 (16.6)	0.432
Serum estrogen metabolites (pg/ml)					
Estrone	47	196.7 (226.0)	51	409.2 (229.1)	0.000
17 β -estradiol	47	31.6 (30.5)	51	62.3 (33.3)	0.000
Estriol	47	741.6 (437.2)	51	878.4 (430.8)	0.122
2-Hydroxyestrone	47	136.4 (77.8)	51	146.0 (111.6)	0.624
2-Methoxyestrone	45 ^b	32.8 (26.5)	48 ^b	25.5 (18.2)	0.199
2-Hydroxyestradiol	46 ^b	15.9 (26.1)	51	18.1 (22.0)	0.598
2-Methoxyestradiol	42 ^b	19.0 (17.6)	46 ^b	16.1 (18.1)	0.810
2-Hydroxyestrone-3-methyl ether	36 ^b	8.9 (10.4)	41 ^b	6.7 (6.7)	0.958
4-Hydroxyestrone	0 ^b	ND	0 ^b	ND	NA
4-Methoxyestrone	38 ^b	16.6 (29.4)	41 ^b	8.7 (11.4)	0.267
4-Methoxyestradiol	29 ^b	9.2 (9.9)	27 ^b	4.1 (6.5)	0.339
16 α -Hydroxyestrone	34 ^b	26.4 (22.5)	43 ^b	45.3 (53.6)	0.002
16-Ketoestradiol	35 ^b	34.7 (38.8)	43 ^b	55.4 (56.6)	0.003
16-Epiestriol	37 ^b	4.7 (6.3)	47 ^b	7.0 (7.6)	0.002
17-Epiestriol	39 ^b	3.4 (4.6)	38 ^b	3.1 (5.2)	0.803
Total estrogen concentration	47	1,247.4 (603)	51	1,686.1 (607.7)	0.001

ND, analyte not detected.

^aDescriptive statistics for cases with detectable metabolite levels.

^bNumber of cases for which metabolite levels were detectable.

Table 2
Regression coefficients predicting SBP in postmenopausal women not taking hormone replacement therapy

	<i>B</i> (s.e.) (<i>N</i> = 32)	<i>B</i> (s.e.) (<i>N</i> = 33)	<i>B</i> (s.e.) (<i>N</i> = 32)
Serum estrogen predictor			
ln 16 α -Hydroxyestrone	-5.3 (2.1)*		
ln 16-Ketoestradiol		-4.7 (1.9)*	
ln (16 α -Hydroxyestrone + 16-ketoestradiol)			-4.6 (1.9)*
Covariates			
Age	0.7 (0.6)	0.6 (0.6)	0.7 (0.6)
BMI	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)
Race/ethnicity			
African American	-2.0 (4.8)	-2.7 (4.9)	-2.4 (4.9)
Latino American	-6.9 (4.7)	-7.6 (4.7)	-7.3 (4.7)
Vasoactive medications	-0.7 (4.9)	-1.8 (5.0)	-1.6 (4.9)
Volume active medications	3.1 (5.7)	4.7 (5.5)	4.1 (5.6)
Intercept	97.6 (37.6)	102.2 (38.8)	103.0 (38.7)

SBP, systolic blood pressure.

* $P < 0.05$.