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# Endogenous sex hormones, blood pressure change, and risk of hypertension in postmenopausal women: The Multi-Ethnic Study of Atherosclerosis

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ABSTRACT

*Objective:* Sex steroid hormones have been postulated to involve in blood pressure (BP) regulation. We examine the association of endogenous sex hormone levels with longitudinal change of BP and risk of developing hypertension in initially normotensive postmenopausal women.
*Methods:* We conducted prospective analysis among 619 postmenopausal women free of hypertension at baseline in the Multi-Ethnic Study of Atherosclerosis (MESA). Change of BP and development of incident hypertension were assessed during a mean of 4.8 years follow-up.
*Results:* After adjusting for age, race/ethnicity, and lifestyle factors, baseline serum estradiol ($E_2$), total and bioavailable testosterone (T), dehydroepiandrosterone (DHEA) were each positively associated and sex-hormone binding globulin (SHBG) was inversely associated with risk of hypertension. Additional adjustment for body mass index eliminated the associations for $E_2$ and T but only attenuated the associations for DHEA and SHBG. The corresponding multivariable hazard ratios (95% CIs) in the highest quartile were 1.28 (0.83–1.97) for $E_2$, 1.38 (0.89–2.14) for total T, 1.42 (0.90–2.23) for bioavailable T, 1.54 (1.02–2.31) for DHEA, and 0.48 (0.30–0.76) for SHBG. Adjustment for fasting glucose, insulin, and C-reactive protein further attenuated the association for DHEA but not for SHBG. Associations of sex hormones with longitudinal BP change were similar.
*Conclusion:* In postmenopausal women, higher endogenous $E_2$, T, and DHEA and lower SHBG were associated with higher incidence of hypertension and greater longitudinal rise in BP. The associations for $E_2$, T, and DHEA were mostly explained by adiposity, while the association for SHBG was independent of measures of adiposity, insulin resistance, and systemic inflammation.


## 1. Introduction

High blood pressure (BP) is one of the largest contributors to death in the U.S. and world-wide [1]. BP differs by sex [2], which may be explained partly by endogenous sex steroid hormones. Evidence from laboratory studies shows that both estrogens and therefore androgens can affect BP. Estrogens can promote vasodilation and lower BP [3]; whereas estrogens may also cause impairment in insulin signaling pathway [4], affect normal immune function [5], induce cytokine activities [6], and subsequently raise BP. Androgens also have been shown to activate both vasoconstricting [7] and vasorelaxing [8] mechanisms.

Epidemiologic studies that examined the relations between circulating sex hormone levels and BP or hypertension status are largely confined to cross-sectional studies and retrospective case–control studies, with inconsistent results. In women, higher estradiol ($E_2$) levels were found among those with elevated BP or hypertension than those with normal BP in some [9], but not all [10,11] studies. Similarly, total or free testosterone (T) concentrations were positively correlated with BP among postmenopausal women in some studies [11,12] but not in others [13]. Sex-hormone binding globulin (SHBG), a serum protein that affects free circulating sex hormone levels [14], has been shown either not associated [9,10] or inversely associated [12] with BP in women.

To further evaluate the possible role of sex steroid hormones in BP regulation and development of hypertension, we examined the

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prospective associations of baseline serum concentrations of endogenous sex hormones, including $E_2$, T, dehydroepiandrosterone (DHEA, a pre-androgen), and SHBG, with the subsequent change of BP and the incidence of hypertension among postmenopausal women free of baseline hypertension.

## 2. Materials and methods

### 2.1. Study subjects

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multicenter, prospective cohort study of subclinical cardiovascular disease (CVD). Study design has been described in detail elsewhere [15]. In brief, 6814 men and women from 4 ethnic origins (Caucasian, African American, Hispanic, and Asian American), aged 45–84 years and free of clinically diagnosed CVD, were enrolled from 6 US field centers. The baseline, second, third, and fourth clinic examination took place from 2000 through 2002, 2002 through 2004, 2004 through 2005, and 2005 through 2007, respectively. The study procedures were approved by Institutional Review Boards at all participating sites. All participants provided written consent. For the current study, we considered 3199 postmenopausal female participants in the MESA, excluding those with baseline hypertension ($n = 1556$) and those who had ever used hormone replacement therapy ($n = 1526$), had no sex hormone measurements ($n = 303$), or had insufficient information to ascertain incident hypertension status during follow-up ($n = 222$). As a result, 619 postmenopausal women remained for current analyses, of whom 595, 570, and 558 attended the second, third, and fourth examination, respectively.

### 2.2. Measurement of blood pressure and ascertainment of hypertension

At baseline and each follow-up examination, physician diagnosis of hypertension was obtained from self-report, use of BP-lowering medications was assessed by reviewing participants' medications brought to clinic, and resting seated BP was measured three times in the right arm using a Dinamap model Pro 100 automated sphygmomanometer (Critikon, Tampa, FL), with average of the last two readings used for analysis. Hypertension was defined as measured systolic BP (SBP) ≥140 mmHg, or diastolic BP (DBP) ≥90 mmHg, or using medications for physician diagnosed hypertension. Incident hypertension was defined as newly developed hypertension during follow-up among those free of hypertension at baseline. Time of event was assigned to the mid-point between the last visit without hypertension and the first visit with hypertension.

### 2.3. Measurement of sex hormones

Fasting blood samples were obtained from participants between 7:30 and 10:30 AM at baseline examination. Serum samples were extracted by centrifugation at 2000× *g* for 15 min or 3000× *g* for 10 min, immediately frozen at −70 °C, and then shipped to the University of Vermont for long-term storage and future analyses. Serum sex hormone concentrations were measured in the Sex Hormone Laboratory at the University of Massachusetts Medical Center in Worcester, MA. Total $E_2$ was measured using an ultrasensitive RIA kit from Diagnostic Systems Laboratory (Webster, TX). Total T and DHEA were measured directly using RIA kits. SHBG was measured by chemiluminescent enzyme immunometric assay using Immulite kits from Diagnostic Products Corp. (Los Angeles, CA). Concentrations of free T, SHBG-bound T, and albumin-bound T were calculated according to the method of Södergard et al. [16]. Bioavailable T was determined as total T minus SHBG-bound T. Calculated bioavailable T has been shown to be comparable to free T concentration obtained by equilibrium dialysis [17]. Assay quality was blindly monitored by assessing 5% randomly selected duplicate participant samples and quality control sera included with the kits. The overall coefficients of variation for total $E_2$, total T, DHEA, and SHBG were 10.5, 12.3, 11.2 and 9.0%, respectively.

### 2.4. Assessment of other baseline covariates

Participants came to clinic examinations after an 8- to 12-h overnight fast. Information on demographics, lifestyle factors, reproductive history, and medical history were collected from standardized questionnaires. Physical activity was assessed using the MESA Typical Week Physical Activity Survey, which was adapted from the Cross-Culture Activity Participation Study [18] and designed to assess the time spent in and frequency of various physical activities during a typical week in the past month [19]. Smoking and alcohol consumption were self-reported. Anthropometrics including weight, height, and waist circumference were measured using standard protocols as described previously [15]. Body mass index (BMI) was calculated. Serum concentrations of

**Table 1**
Baseline characteristics of postmenopausal women who developed incident hypertension compared to those who remained free of hypertension, MESA 2000–2007.

| Characteristics | Incident HTN ($N = 194$) | No HTN ($N = 425$) | $P$[a] |
|---|---|---|---|
| Systolic blood pressure, mmHg | 125.2 ± 0.77[b] | 111.3 ± 0.61 | <0.0001 |
| Diastolic blood pressure, mmHg | 68.6 ± 0.57 | 63.7 ± 0.39 | <0.0001 |
| Age, years | 66.0 ± 0.62 | 62.3 ± 0.44 | <0.0001 |
| Age at menopause, years | 47.8 ± 0.47 | 48.1 ± 0.29 | 0.59 |
| **Years after menopause, %** | | | <0.0001 |
| <5 | 10.8 | 24.7 | |
| 5–<10 | 15.0 | 16.7 | |
| 10–<20 | 27.8 | 30.1 | |
| ≥20 | 46.4 | 28.5 | |
| **Race/ethnicity, %** | | | 0.35 |
| Caucasian | 32 | 32 | |
| African American | 25 | 20 | |
| Hispanic | 28 | 28 | |
| Asian American | 15 | 20 | |
| **Cigarette smoking, %** | | | 0.97 |
| Current | 12.4 | 12.5 | |
| Former | 24.7 | 23.8 | |
| Never | 62.9 | 63.7 | |
| **Alcohol use, %** | | | 0.60 |
| Current | 43.8 | 45.3 | |
| Former | 22.2 | 18.7 | |
| Never | 34.0 | 36.0 | |
| Physical activity, MET-min/week | 1208 ± 116.2 | 1354 ± 100.1 | 0.34 |
| **Adiposity** | | | |
| Body mass index, kg/m$^2$ | 29.2 ± 0.43 | 27.4 ± 0.27 | 0.0002 |
| Waist circumference, cm | 98.3 ± 1.07 | 93.9 ± 0.69 | 0.0005 |
| **Metabolic and inflammatory markers**[c] | | | |
| Fasting glucose, mg/dL[d] | 97.3 (85, 101) | 90.7 (81, 94) | 0.001 |
| Fasting insulin, mU/L | 5.90 (3.90, 8.60) | 4.95 (3.40, 7.40) | 0.0006 |
| C-reactive protein, mg/L[e] | 2.39 (1.09, 5.58) | 1.76 (0.72, 3.97) | 0.003 |
| **Sex hormone variables**[c] | | | |
| $E_2$, nmol/L | 0.053 (0.040, 0.081) | 0.054 (0.037, 0.081) | 0.75 |
| Total T, nmol/L | 0.98 (0.73, 1.35) | 0.88 (0.59, 1.28) | 0.03 |
| Bioavailable T, nmol/L | 0.28 (0.17, 0.42) | 0.23 (0.14, 0.38) | 0.002 |
| DHEA, nmol/L | 11.2 (7.84, 16.2) | 10.9 (7.95, 15.5) | 0.57 |
| SHBG, nmol/L | 46.8 (35.1, 62.3) | 52.5 (36.7, 72.4) | 0.005 |

[a] $P$ values are derived from $t$-test for continuous variables and $\chi^2$ test for categorical variables.
[b] Mean ± standard error and all such values.
[c] Geometric means (interquartile range) are shown for all biomarkers because of the skewed distribution.
[d] To convert glucose value from mg/dl to mmol/l, multiply by 0.0555.
[e] To convert C-reactive protein value from mg/L to nmol/l, multiply by 9.524.