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Prospective Study of Endogenous Circulating Estradiol and Risk of Stroke in Older Women

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Abstract

Objective—To test the hypothesis that circulating endogenous estradiol is associated with stroke risk in older postmenopausal women. Stroke incidence increases after menopause, when endogenous estrogen levels fall, yet exogenous estrogen increases strokes in older postmenopausal women. The relation between endogenous estrogen and stroke is unclear.

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Author Contributions: Dr Cummings and Ms Lui had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Lee, Yaffe, Browner, and Cummings. *Acquisition of data:* Cauley, Browner, and Cummings. *Analysis and interpretation of data:* Lee, Yaffe, Lui, Taylor, Browner, and Cummings. *Drafting of the manuscript:* Lee. *Critical revision of the manuscript for important intellectual content:* Lee, Yaffe, Lui, Cauley, Taylor, Browner, and Cummings. *Statistical analysis:* Yaffe, Lui, and Browner. *Obtained funding:* Lee, Yaffe, Cauley, Browner, and Cummings. *Administrative, technical, and material support:* Lee, Cauley, and Taylor. *Study supervision:* Cummings.

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Design—Prospective case-control study.

Setting—Study of Osteoporotic Fractures.

Patients or Other Participants—Women at least age 65 years (99% follow-up) who were not taking estrogen at baseline.

Main Outcome Measures—Free estradiol index (FEI) was calculated by dividing total estradiol by sex hormone–binding globulin concentrations measured in banked baseline serum. Using logistic regression, odds ratios were estimated for a first-ever atherothrombotic stroke associated with endogenous FEI in 196 women who had a subsequent validated stroke (median follow-up, 8 years) compared with 219 randomly selected women who did not. Potential mediators were assessed in multivariable models.

Results—The age-adjusted odds of atherothrombotic stroke increased with increasing FEI quartiles (P_{trend}=.007). Women in the highest FEI quartile had an age-adjusted 2.31-fold (odds ratio, 2.31; 95% confidence interval, 1.28–4.17) higher odds than women in the lowest quartile. Women with greater central adiposity had a suggestively stronger association (P =.08). Atherogenic dyslipidemia, type 2 diabetes mellitus, and C-reactive protein level were potential mediators of this relation.

Conclusions—Endogenous estradiol level is an indicator of stroke risk in older postmenopausal women, especially in those with greater central adiposity. Potential mediators, including atherogenic dyslipidemia, insulin resistance, and inflammation, might underlie this association. Whether estradiol, independent of atherogenic adiposity, influences such mediators and stroke risk needs to be determined. Estrogen-altering agents might be harmful or beneficial depending on endogenous estradiol levels, especially in women with greater central adiposity.

Stroke is the third leading cause of death in women in the United States and a major source of serious long-term disability and dementia.[1] Most strokes are ischemic (88%), with the remaining hemorrhagic.[1,2] Endogenous, or naturally occurring, estrogen production declines during menopause. Postmenopausal women experience approximately a doubling every 10 years in stroke incidence, with stroke events accounting for about 1 in 6 deaths.[1] Estradiol, the most potent estrogen, affects many mechanisms that impact the occurrence of atherothrombotic ischemic stroke, including lipid metabolism, inflammation, oxidative stress, fibrinolysis, and thrombosis.[3–5] Circulating free estradiol, which is not bound to sex hormone–binding globulin (SHBG), interacts with target tissues throughout the body and is inversely proportional to circulating SHBG concentration.[6]

Use of exogenous estrogen, which increases circulating estrogen levels, increased stroke events in large clinical trials of older postmenopausal women.[7–9] Endogenous estrogen's effects on stroke occurrence in postmenopausal women are unclear. Some studies have focused on the risk of cardiovascular disease[10–13] or coronary artery disease[14–17] associated with endogenous circulating levels of estrogen and SHBG. These studies have provided inconsistent results; they use different laboratory assays and measures of circulating estrogen and do not assess specifically stroke risk.

A better understanding of the relation between endogenous estradiol and atherothrombotic stroke may provide insights into how estrogen influences stroke pathogenesis and risk in

older women. The current study tested the hypothesis that circulating endogenous estradiol is associated with stroke risk in older postmenopausal women. In a prospective case-control study with 8 years of follow-up, baseline serum levels of endogenous estradiol were compared in postmenopausal women who had a subsequent first-ever atherothrombotic stroke and those who did not.

METHODS

From 1986 to 1988, 9704 white women 65 years or older were recruited into the Study of Osteoporotic Fractures[18,19] from Baltimore, Maryland; Minneapolis, Minnesota; Pittsburgh, Pennsylvania; and Portland, Oregon. Women with a prior bilateral hip replacement or who were unable to walk without help were excluded. Written informed consent was obtained from all participants after the pertinent institutional review boards had approved the study protocol.

QUESTIONNAIRE AND EXAMINATION DATA COLLECTION

At baseline, participants completed an interview-based questionnaire during a 3-hour examination. The questionnaire elicited use of hormone medications, social habits, and medical history. At baseline examination, height, weight, waist circumference, and blood pressure were measured. The diuretics were the antihypertensive drugs collected by the Study of Osteoporotic Fractures. Hypertension was defined as taking a diuretic or having a measured blood pressure higher than 140/90mmHg. Height was measured using wall-mounted stadiometers and weight, using balance-beam scales. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

BLOOD HORMONE MEASUREMENTS

Blood was collected between 9 AM and 2 PM after a fat-free diet overnight, and sera were immediately frozen at −20°C. The samples were shipped on dry ice and then stored in liquid nitrogen at −190°C at Biomedical Research Institute (Rockville, Maryland). At Endocrine Sciences, Inc (Calabasas Hills, California), all blood measurements were measured blinded to any clinical information. Serum total estradiol concentration was measured by a sensitive indirect radioimmunoassay that included an initial extraction by column chromatography. It is important to measure accurately the very low estradiol levels typically seen in postmenopausal women; this assay's interassay and intraassay coefficients of variation, indicators of dispersion in measurements, are less than 15% at such low estradiol levels. The interassay coefficient of variation was 12% at 2.6 pg/mL (to convert to picomoles per liter, multiply by 3.671). The intraassay coefficient of variation was 13.1% at 6.5 pg/mL. The limit of detection was 2 pg/mL. Sex hormone–binding globulin was measured using an immunoradiometric assay. At a level of 0.14 μg/mL (to convert to nanomoles per liter, multiply by 8.896), the intraassay coefficient of variation was 2.4% and the interassay coefficient of variation was 8%.

Total estradiol includes both protein-bound and unbound estradiol. Free estradiol index (FEI) was calculated by dividing total estradiol by SHBG in molar concentrations. Fasting total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels

were measured using an automated chemistry analyzer; low-density lipoprotein cholesterol levels were derived from these measurements using the standard Friedewald equation.[20] Total homocysteine level was measured using high-performance liquid chromatography. C-reactive protein (CRP) levels were measured with rate nephelometry. A participant was considered to have diabetes mellitus if she had physician-diagnosed diabetes or if baseline serum fructosamine level was greater than 51 mg/L (to convert to millimoles per liter, multiply by 5.581) (upper limit of the reference range). Fructosamine was measured using a standard colorimetric assay based on the ability of ketoamines to reduce nitro blue tetrazolium to formazan.

IDENTIFICATION AND SELECTION OF INCIDENT STROKE CASES AND CONTROLS

Every 4 months, participants or their proxy returned a postcard, supplemented by telephone calls for outstanding postcards and an annual questionnaire that asked about incident strokes. Death certificates and hospital discharge summaries were reviewed for those who died and causes of death were coded by a blinded investigator (cardiovascular disease included *International Classification of Diseases, Ninth Revision, Clinical Modification* codes 394–440). Medical records were obtained from participants who reported strokes or transient ischemic attacks. Validation of an atherothrombotic stroke event required (1) a clinical presentation that was relatively sudden or stuttering onset of a new neurological deficit with residua that lasted at least 24 hours, (2) no information suggesting a noncardiovascular explanation, (3) if available, computed tomographic or magnetic resonance imaging evidence consistent with the diagnosis of atherothrombotic stroke, as interpreted by the responsible radiologist, and (4) determination that cardiac or transcardiac embolism, global hypoperfusion, or other unusual causes, such as systemic disease or isolated retinal infarction, were not probable. Women with a previous atherothrombotic stroke were excluded. Using a prospective case-control design that was designed primarily to ascertain predictors of stroke, cases of first-ever incident atherothrombotic stroke (n=247) were validated during the follow-up to the end study date of February 18, 1998. Two investigators independently reviewed each potential incident case; disagreements were resolved by consensus. Controls (n=243) were randomly selected from the entire cohort who did not report a prior stroke at baseline and did not experience a stroke during the follow-up period. Women undergoing estrogen therapy at baseline (51 cases, 24 controls) were excluded such that 196 women with incident (first-ever) atherothrombotic stroke and 219 controls composed the case-control study population nested in the Study of Osteoporotic Fractures.

STATISTICAL ANALYSIS

The associations between levels of endogenous estradiol, both total estradiol and calculated FEI, and SHBG and the risk of incident atherothrombotic stroke were assessed. Distribution normality was assessed for each hormone as a continuous variable. Results did not change using log-transformed estradiol variables, so results for non–log transformed variables are presented. Mean levels of continuous variables were compared using the *t* test or analysis of variance. Cut points for estradiol and SHBG quartiles were based on the distribution of hormone level in the controls. Categorical variables were compared using the χ^2 test. Pearson correlation coefficients (*r*) with *P* values were calculated between hormone measures and continuous cardiovascular factors.

Using logistic regression modeling, odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for incident stroke by levels of endogenous estradiol. Associations were assessed in age-adjusted and multivariable models that adjusted for potential confounders and putative cardiovascular risk factors. Baseline smoking status also was available during the follow-up period; potential confounding due to change in smoking status during follow-up was assessed. Analyses were repeated after excluding women undergoing estrogen therapy at any time during the follow-up period (53 cases, 11 controls).

To identify potential biological mediators of the relation between endogenous estrogen and stroke, representative covariates were included 1 by 1 in separate logistic regression models. If the OR estimate for FEI changed by more than 10% with the inclusion of a covariate that could biologically lie in the causal pathway between estradiol and stroke, the covariate was considered a biological mediator. A priori, age 71 years or younger or older than 71 years, anthropometric measures of adiposity (BMI 25 or less or more than 25 and waist circumference 88 cm or less or more than 88 cm), current smoking status, diabetes status, and markers of atherogenic dyslipidemia (triglyceride: HDL-C ratio and TC:HDL-C ratio) were considered potential effect modifiers of the relation between endogenous estrogen and the risk of stroke. Tests for interaction between each potential modifier and endogenous FEI and age-adjusted OR estimates on stratification by each potential modifier were determined using logistic regression.

All statistical analyses were conducted using SAS software (SAS Institute Inc, Cary, North Carolina). A 2-sided *P* value of less than .05 was designated to be statistically significant. The FEI was calculated by dividing serum total estradiol level (in picomoles per liter) by SHBG (in nanomoles per liter).

RESULTS

BASELINE CHARACTERISTICS

The 196 postmenopausal women who experienced a first-ever stroke in 8 years of follow-up were older and more likely to smoke and drink less alcohol at baseline than the 219 women who did not experience a stroke. Incident stroke cases also were more likely to have hypertension, diabetes, higher CRP levels, and worse atherogenic dyslipidemia (higher triglyceride, lower HDL-C, and higher non–HDL-C levels, higher TC:HDL-C ratio) (Table 1). Mean waist circumference was greater in women who experienced a stroke, whereas mean weight and BMI did not differ between the groups. Last estrogen use was on average more than 10 years prior to baseline in both groups. Physical activity, aspirin use, age at menopause, plasma homocysteine level, and parental history of stroke did not differ between the 2 groups.

Across increasing quartiles of FEI, circulating total estradiol level increased on average by 6.5 pg/mL and SHBG level decreased on average by 4 μg/mL (Table 2). Baseline FEI positively correlated with total estradiol level (r=0.78; P <.001) and inversely correlated with SHBG level (r=−0.30; P <.001). The FEI also positively correlated with BMI (r=0.43), waist circumference (r=0.42), CRP level (r=0.28), triglyceride level (r=0.26), and TC: HDL-C ratio (r=0.25) and inversely correlated with HDL-C level (r=−0.20) in women who

did not experience a stroke (P <.001 for each correlation coefficient). Waist circumference, a marker of central adiposity, correlated with BMI (r=0.83; P <.001).

ENDOGENOUS ESTRADIOL AND INCIDENT STROKE

Serum FEI was more strongly associated with incident stroke than total estradiol level (Table 3). Women with increasing FEI by quartiles had an increasing age-adjusted odds of having a stroke over 8 years (P for linear trend=.007). Women in the highest FEI quartile had the highest odds, an age-adjusted OR of 2.31 (95% CI, 1.28–4.17) compared with women in the lowest quartile. Women in mid-quartiles had 1.5-fold higher odds but the corresponding CIs included unity. Excluding women who did not undergo estrogen therapy during follow-up (53 women with incident stroke and 11 controls) did not materially alter the OR estimates; women in the highest FEI quartile had an age-adjusted OR of 2.38 (95% CI, 1.29–4.42).

A separate multivariable model that included age, hypertension, alcohol use, current smoking, CRP level, diabetes, triglyceride level, and TC:HDL-C ratio attenuated the OR estimates for the FEI quartiles (P for trend=.51; OR for highest FEI quartile, 1.26; 95% CI, 0.63–2.52) (Table 3). Additional adjustment for waist circumference did not materially alter the OR estimates. To identify potential biological mediators, covariates were included 1 by 1 into separate logistic regression models (Table 4). The OR for the highest FEI quartile was attenuated by more than 10% after individual adjustment for hypertension, CRP level, diabetes status, waist circumference, and the following atherogenic lipid markers: triglyceride level, TC:HDL-C ratio, HDL-C level, and low-density lipoprotein cholesterol level. The OR for the highest FEI quartile was no longer associated with stroke after adjustment for triglyceride level (OR,1.79;95% CI, 0.95–3.39) or TC:HDL-Cratio (OR, 1.81;95%CI, 0.96–3.40) and remained borderline associated after adjustment for diabetes (OR, 1.91; 95%CI, 1.05–3.50) or CRP level (OR, 1.94; 95% CI, 1.05–3.59). Adjustment for waist circumference also attenuated the OR for the highest FEI quartile (OR, 1.90; 95% CI, 0.99–3.66) by more than 10%, whereas adjustment for BMI or weight did not. Controlling for smoking during follow-up did not alter the risk estimates.

Adipose tissue is a source of endogenous estrogen production, especially in postmenopausal women. Waist circumference might be an effect modifier for the association between FEI and stroke, with a suggestively significant interaction (P value=.08). Among women with waist circumference more than 88cm (78 cases, 73 noncases), those in the highest FEI quartile had an OR of 6.27 (95% CI, 1.10–35.6; P =.04) compared with those in the lowest FEI quartile, whereas among women with waist circumference 88 cm or less (117 cases, 146 noncases), those in the highest FEI quartile had an OR of 1.40 (95% CI, 0.64–3.06; P =.41). Age, overweight BMI, current smoking, diabetes, and markers of atherogenic dyslipidemia were not effect modifiers (P for interaction >.10 for each).

COMMENT

This study observed that older postmenopausal women with high endogenous free estradiol levels had a 2.3-fold greater odds of stroke, independent of age. Atherogenic dyslipidemia, insulin resistance/diabetes, and inflammation were biological mediators of the relation

between endogenous estradiol and stroke risk. The association between endogenous estradiol and stroke risk was stronger among older postmenopausal women who had greater central adiposity. Previous studies of anthropometric measures and stroke risk also support the mechanism that adiposity and the metabolic syndrome contribute to stroke pathogenesis.[21] Central adiposity increases endogenous estradiol production and circulating estradiol levels. Higher endogenous estradiol levels and atherogenic adiposity, separately and/or combined, could promote inflammation, atherogenic dyslipidemia, and diabetes to increase stroke risk in older postmenopausal women. This study does not rule out the possibility that higher endogenous estradiol concentration is just a by-product of adiposity and, by itself, does not promote these mediators and stroke risk. However, estradiol is known to affect inflammation, lipid metabolism, insulin resistance, and other processes in atherothrombosis.[3–5] Further study is needed to address these possibilities.

Recent randomized clinical trials indicate that exogenous estrogen use in older postmenopausal women increases stroke occurrence by 1.4-fold.[7,9,22,23] Thus, increasing free estradiol levels in older postmenopausal women might increase the risk of subsequent stroke; the current study supports this concern. Few studies have assessed circulating levels of endogenous free (or bio-available) estrogen and the risk of cardiovascular disease,[10–13] coronary artery disease alone,[14–17] or carotid atherosclerosis.[24–26] None have focused on stroke occurrence alone, to the our knowledge. Postmenopausal women with high estrone levels had an age-adjusted 86% increased odds of carotid atherosclerosis than those with levels in the lowest quartile in the Atherosclerosis Risk in Communities study.[24] Similar to the current study, this association was no longer observed in multivariable models, but estradiol was not measured.

The Women's Health Study did not observe an association between endogenous FEI and cardiovascular disease,[11] and the Rancho Bernardo Study reported that estradiol did not predict mortality due to cardiovascular or ischemic heart disease.[10] However, these studies did not assess stroke separately. The role of endogenous estradiol may differ among the specific cardiovascular disease entities of stroke and ischemic heart disease in postmenopausal women. In the 2-year study Estrogen in the Prevention of Atherosclerosis Trial, recently menopausal women taking micronized estradiol with higher endogenous free estradiol levels had less progression of carotid intima-medial thickness than those taking micronized estradiol with lower endogenous estradiol levels or women taking placebo.[25,26] However, the Estrogen in the Prevention of Atherosclerosis Trial did not study older postmenopausal women or stroke, unlike this study, and the effect of endogenous estradiol on stroke occurrence might differ depending on age and/or duration of menopause. Thus, inconsistent findings across studies could be due to the merging of heterogeneous vascular disease outcomes, differences in age and other characteristics of the study populations, and the varying forms of estrogen studied.

This study does not rule out the possibility that lower SHBG levels might affect stroke risk beyond its effect of increasing circulating free estradiol levels, since it used SHBG level to calculate FEI. In prior studies, low SHBG levels were not associated with either subsequent overall cardiovascular disease or mortality from cardiovascular disease or ischemic heart disease[11,12] but were associated with worse carotid intima-medial thickness[24] and

cardiovascular risk profile.[11,13,16,17,24,27–36] The current study results may not apply to older postmenopausal women of different race/ethnicities or ages. Data were unavailable on some stroke risk factors at baseline and during follow-up. However, the presence of a heart murmur did not differ between cases and controls (Table 1), and adjustment for smoking during the follow-up period did not alter the risk estimates. Statins were newly introduced around the study's baseline (only 13 cases and 15 controls took a statin at study years 4–5), so their use is unlikely to confound the current study. Endogenous androgens, which may influence the risk of stroke independently of or in addition to endogenous estradiol,[11] were not evaluated.

This study has important strengths. It is a large, prospective, and community-based study of well-characterized postmenopausal women. Incident stroke events during a long follow-up period were validated. It uses a well established and sensitive estradiol radioimmunoassay method with good functional sensitivity at low postmenopausal concentrations.[37]

In summary, endogenous circulating estradiol levels are an indicator of an increased risk of stroke in older postmenopausal women, independent of age. Potential mediating mechanisms, including atherogenic dyslipidemia, insulin resistance, and inflammation, might underlie this association and warrant further study. Greater waist circumference might potentiate the association between high endogenous estradiol levels and stroke risk, but further study is needed to determine if endogenous estradiol, independent of atherogenic adiposity, affects inflammation, atherogenic dyslipidemia, and insulin resistance in atherothrombotic stroke. The current study implies that estrogen-altering agents might be harmful or beneficial depending on endogenous circulating free estradiol levels in older postmenopausal women, especially among those with greater central adiposity. However, studies are needed to determine whether (1) therapies that raise the levels or enhance the effects of estradiol have more adverse effects in older postmenopausal women with low endogenous serum estradiol levels and (2) agents that decrease estradiol levels or mitigate their effect may be more beneficial for women with high endogenous estradiol levels.

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Table 1

Baseline Characteristics of Postmenopausal Women With Incident Stroke and Controls in a Prospective Case-Control Study of Incident Stroke From the Study of Osteoporotic Fractures

Baseline Characteristic	Mean (SD)		
	Women With Incident Stroke (n=196)	Controls (n=219)	*P* Value[a]
Age, y	73.5 (5.6)	70.8 (5.0)	<.001
Age at menopause, y	47.9 (6.0)	48.6 (5.2)	.24
Weight, kg	68.2 (12.5)	68.0 (12.6)	.89
BMI	27.1 (4.8)	26.8 (4.8)	.45
Waist circumference, cm	86.4 (12.1)	83.6 (11.5)	.02
Smoking, %			
Never	70.1	61.2	.03
Past	19.6	31.1	
Current	10.3	7.8	
No. of alcoholic drinks/wk	1.2 (3.6)	2.1 (4.5)	.03
Walk for exercise, %	45.4	49.3	.43
Weekly total calories burned	1499 (1761)	1625 (1625)	.45
Diabetes mellitus, %	23.2	8.3	<.001
Hypertension (>140/90 mm Hg or diuretic use), %	79.1	55.7	<.001
At least 1 parent with stroke, %	40.5	42.4	.71
Aspirin use, %	39.7	38.4	.79
Presence of a heart murmur, %	11.9	12.5	.87
Prior myocardial infarction, %	15.4	4.5	.001
Angina, %	20.8	12.9	.05
Past estrogen use, %	27.9	35.9	.08
Years since last estrogen use	17.6 (9.8)	15.3 (7.8)	.14
TC level, mg/dL	242.3 (39.4)	234.6 (38.1)	.05
LDL-C level, mg/dL	154.4 (34.2)	149.9 (31.5)	.17
HDL-C level, mg/dL	50.0 (13.0)	53.6 (14.4)	.008
Non–HDL-C level, mg/dL	192.2 (39.9)	181.0 (38.6)	.004
TC:HDL-C ratio	5.13 (1.32)	4.65 (1.23)	<.001
Triglyceride level, mg/dL	192.0 (118.4)	158.1 (93.6)	.001
C-reactive protein level, mg/dL	0.51 (0.82)	0.37 (0.59)	.04
Total homocysteine level, μmol/L	11.9 (3.7)	11.5 (3.7)	.21

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein level; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

SI conversion factors: To convert TC, LDL-C, and HDL-C to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113, C-reactive protein to nanomoles per liter, multiply by 9.524; homocysteine to milligrams per liter, divide by 7.397.

[a] By *t* test for continuous variables and χ^2 test for categorical variables.

Table 2

Baseline Serum Levels of E_2 and SHBG According to Quartiles of FEI in Postmenopausal Women in a Prospective Case-Control Study of Incident Stroke From the Study of Osteoporotic Fractures

	Mean (SD)	
FEI Quartile[a]	E_2 Concentration, pg/mL	SHBG, µg/mL
Quartile 1	4.4 (1.3)	6.9 (1.7)
Quartile 2	5.6 (1.4)	4.9 (1.3)
Quartile 3	8.0 (2.5)	4.3 (1.4)
Quartile 4 (highest)	10.9 (4.8)	2.7 (1.1)

Abbreviations: E_2, total estradiol; FEI, free estradiol index; SHBG, sex hormone–binding globulin.

SI conversion factors: To convert E_2 to picomoles per liter, multiply by 3.671; SHBG to nanomoles per liter, multiply by 8.896.

[a]The FEI was calculated by dividing E_2 concentration (in picomoles per liter) by SHBG concentration (in nanomoles per liter): quartile 1=0.096 to 0.373, quartile 2 ≥ 0.373 to 0.612, quartile 3 ≥ 0.612 to 0.952, and quartile 4 > 0.952.

Table 3

OR Estimates for Stroke by Baseline Serum Quartiles of E_2, FEI, and SHBG in Age-Adjusted and MV-Adjusted Models in Postmenopausal Women in a Prospective Case-Control Study of Incident Stroke From the Study of Osteoporotic Fractures

	OR Estimate (95% CI)		
Endogenous Estradiol	**Age-Adjusted**	**MV Model[a]**	**MV Model[a] Plus Waist Circumference**
E_2 level[b]			
Quartile 1	1 [Reference]	1 [Reference]	1 [Reference]
Quartile 2	1.26 (0.62–2.57)	1.24 (0.58–2.64)	1.24 (0.58–2.63)
Quartile 3	1.55 (0.87–2.74)	1.37 (0.73–2.54)	1.35 (0.72–2.52)
Quartile 4 (highest)	1.71 (0.94–3.13)	1.13 (0.58–2.22)	1.09 (0.55–2.19)
P_{trend}	.07	.69	.76
FEI[c]			
Quartile 1	1 [Reference]	1 [Reference]	1 [Reference]
Quartile 2	1.46 (0.80–2.68)	1.10 (0.57–2.10)	1.09 (0.57–2.09)
Quartile 3	1.52 (0.83–2.78)	1.15 (0.59–2.24)	1.14 (0.58–2.24)
Quartile 4 (highest)	2.31 (1.28–4.17)	1.26 (0.63–2.52)	1.23 (0.59–2.57)
P_{trend}	.007	.51	.58
SHBG level[d]			
Quartile 1	1 [Reference]	1 [Reference]	1 [Reference]
Quartile 2	0.60 (0.34–1.05)	0.66 (0.35–1.25)	0.67 (0.36–1.25)
Quartile 3	0.33 (0.18–0.58)	0.42 (0.22–0.81)	0.42 (0.22–0.82)
Quartile 4 (highest)	0.58 (0.34–1.01)	0.84 (0.44–1.62)	0.85 (0.43–1.67)
P_{trend}	.01	.37	.40

Abbreviations: CI, confidence interval; CRP, C-reactive protein; E_2, total estradiol; FEI, free estradiol index; HDL-C, high-density lipoprotein cholesterol; MV, multivariable; OR, odds ratio; SHBG, sex hormone–binding globulin; TC, total cholesterol.

SI conversion factors: See Table 2.

[a] The MV model includes age, hypertension, smoking, alcohol intake, CRP level, diabetes mellitus, triglyceride level, and TC:HDL-C ratio.

[b] Total estradiol level quartile 1=2 to 4 pg/mL, quartile 2=5 to 6 pg/mL, quartile 3=7 to 8 pg/mL, and quartile 4 ≥ 9 pg/mL.

[c] The FEI was calculated by dividing level E_2 (in picomoles per liter) by SHBG level (in nanomoles per liter).

[d] Sex hormone–binding globulin level quartile 1=0.6 to 3.2 μg/mL, quartile 2=3.5 to 4.4 μg/mL, quartile 3=4.5 to 5.7 μg/mL, and quartile 4 ≥ 5.8 μg/mL.

Table 4

OR Estimates for Stroke in Bivariate Logistic Regression Models According to Quartiles of FEI in Postmenopausal Women in a Prospective Case-Control Study of Incident Stroke From the Study of Osteoporotic Fractures[a]

	OR Estimate (95% CI)			
Covariate	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4 (Highest)**
Age only	1 [Reference]	1.46 (0.80–2.68)	1.52 (0.83–2.78)	2.31 (1.28–4.17)
Age + hypertension	1 [Reference]	1.36 (0.73–2.53)	1.51 (0.81–2.79)	2.04 (1.12–3.73)
Age + current smoking	1 [Reference]	1.43 (0.78–2.63)	1.47 (0.80–2.69)	2.29 (1.27–4.13)
Age + alcohol intake	1 [Reference]	1.44 (0.79–2.64)	1.51 (0.83–2.77)	2.28 (1.26–4.11)
Age + weight	1 [Reference]	1.48 (0.81–2.72)	1.56 (0.84–2.90)	2.41 (1.26–4.59)
Age + BMI	1 [Reference]	1.47 (0.80–2.70)	1.53 (0.82–2.86)	2.34 (1.22–4.49)
Age + waist circumference	1 [Reference]	1.39 (0.76–2.57)	1.36 (0.73–2.55)	1.90 (0.99–3.66)
Age + TG level	1 [Reference]	1.46 (0.79–2.70)	1.41 (0.76–2.63)	1.79 (0.95–3.39)
Age + TC:HDL-C ratio	1 [Reference]	1.39 (0.75–2.58)	1.33 (0.71–2.49)	1.81 (0.96–3.40)
Age + HDL-C level	1 [Reference]	1.46 (0.79–2.69)	1.43 (0.76–2.67)	2.06 (1.10–3.85)
Age + LDL-C level	1 [Reference]	1.48 (0.80–2.74)	1.53 (0.83–2.82)	2.06 (1.13–3.77)
Age + TC level	1 [Reference]	1.49 (0.81–2.75)	1.52 (0.82–2.80)	2.24 (1.23–4.08)
Age + diabetes mellitus	1 [Reference]	1.34 (0.72–2.47)	1.40 (0.76–2.59)	1.91 (1.05–3.50)
Age + CRP level	1 [Reference]	1.34 (0.73–2.48)	1.37 (0.75–2.53)	1.94 (1.05–3.59)

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; FEI, free estradiol index; HDL-C, high-density lipoprotein level; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglyceride.

[a]The FEI was calculated by dividing total estradiol level (in picomoles per liter) by SHBG level (in nanomoles per liter) (to convert estradiol to picomoles per liter, multiply by 3.671; SHBG to nanomoles per liter, multiply by 8.896).