Low Dehydroepiandrosterone Sulfate is Associated With Increased Risk of Ischemic Stroke Among Women

Monik C. Jiménez, ScD; Qi Sun, MD, ScD; Markus Schürks, MD, MSc; Stephanie Chiuve, ScD; Frank B. Hu, MD, PhD; JoAnn E. Manson, MD, DrPH; Kathryn M. Rexrode, MD, MPH

Background and Purpose—Previous research suggests greater risk of coronary heart disease with lower levels of the adrenal steroid dehydroepiandrosterone sulfate (DHEAS). No studies have examined the association between DHEAS and risk of ischemic stroke. DHEAS may influence ischemic stroke risk through atherosclerotic-related mechanisms (endothelial function and smooth muscle cell proliferation) or insulin resistance.

Methods—Between 1989 and 1990, 32,826 women without prior stroke in the Nurses’ Health Study, an observational cohort, provided blood samples and were followed up for cardiovascular events. Among this sample, using a nested case–control design, 461 ischemic strokes were confirmed by medical records by 2006. Cases were matched to controls free of stroke at the time of the index case and by age, race, menopausal status, postmenopausal hormone use, smoking status, and date of sample collection. Multivariable conditional logistic regression was used.

Results—Median DHEAS levels did not differ between cases (median=58.7) and controls (median=66.0; P=0.10). Conditional on matching factors, the lowest DHEAS quartile exhibited a relative risk of 1.30 for ischemic stroke (95% confidence interval, 0.88–1.94), compared with the highest quartile and marginally unchanged when adjusted for confounders (relative risk=1.33; 95% confidence interval, 0.87–2.02). When modeled as a binary variable dichotomized at the lowest quartile, women with low DHEAS (≤the lowest quartile) had a significantly increased multivariable adjusted risk of ischemic stroke compared with those with higher levels (relative risk=1.41; 95% confidence interval, 1.03–1.92).

Conclusions—Lower DHEAS levels were associated with a greater risk of ischemic stroke, even after adjustment for potential confounders. These novel observations warrant confirmation in other populations. (Stroke. 2013;44:1784-1789.)

Key Words: dehydroepiandrosterone sulfate • ischemic stroke

Dehydroepiandrosterone sulfate (DHEAS), the most abundant circulating human steroid hormone, is secreted almost exclusively by the adrenal cortex.1 DHEAS serves as a precursor to ≥50% of androgens in adult men, 75% of active estrogens in premenopausal, and ≥100% in postmenopausal women.1 DHEAS levels decline with age, peaking at age 20 to 30 years and declining by 20% to 30% by age 70 to 80 years.2 Although no human studies have examined DHEAS and stroke, several studies have reported strong inverse associations between levels of DHEAS and risk of total mortality and cardiovascular disease (CVD).3-5 Recent results from the Women’s Ischemia Syndrome Evaluation (WISE) reported a >2-fold increased risk of CVD mortality among women in the lowest tertile of DHEAS compared with those in higher categories (hazard ratio [HR], 2.55; 95% CI, 1.19–5.45).6 The associations between DHEAS and risk of CVD among women have been inconsistent in the few studies available, in contrast to observations among men.1,4 These data suggest divergent effects by sex, underscoring the need for sex-specific analyses with comprehensive evaluation of hormonal-related factors.

The biological plausibility for an association between DHEAS and risk of stroke is provided by inverse associations between DHEAS and several stroke risk factors, including insulin resistance,7 carotid intima-media thickness,8 and hypertension.9 In addition, low DHEAS levels have been associated with cognitive impairment.1 Higher dehydroepiandrosterone (DHEA) levels have been shown to decrease atherogenic vascular remodeling post injury.10 Furthermore, higher DHEA/DHEAS levels have also been shown to improve endothelial cell function and promote insulin sensitivity11,12 in human and animal models. Importantly, DHEAS may act directly or through conversion to sex steroid hormones; hence, the influence of DHEAS on risk may vary depending on the hormonal environment (eg, sex, menopausal status, and postmenopausal hormone use).13 Given its extensive use as an over-the-counter supplement, elucidating the role of DHEAS in the pathogenesis of CVD could provide further support for randomized controlled trials evaluating its potential role in CVD prevention.

To further elucidate the relationship between DHEAS and risk of stroke, we evaluated its association with ischemic
stroke among women. We hypothesized that lower levels of DHEAS were associated with an increased risk of ischemic stroke in women. Importantly, we explored potential variation of the association between DHEAS and ischemic stroke by age, body mass index, smoking, history of diabetes mellitus, postmenopausal hormone use, and ovarian status.

Materials and Methods

Nurses’ Health Study Cohort

The Nurses’ Health Study (NHS) enrolled 121,700 female registered nurses living in 11 US states, aged 30 to 55 years, who completed a mailed questionnaire in 1976. Follow-up questionnaires have been mailed biennially, with a semiquantitative food frequency questionnaire mailed every 2 to 4 years since 1980, as previously published. Follow-up of the baseline population is >90% and mortality follow-up is >98% complete. Between 1989 and 1990, 32,826 women without prior evidence of stroke, 43 to 69 years, provided blood samples. Approximately 10 years later (2000–2001), 18,743 of these participants provided a second blood sample. Blood was drawn and shipped to our laboratory via overnight courier for processing. Samples were processed, archived, and have been stored in continuously monitored liquid nitrogen freezers as previously described.

A nested case–control study of ischemic stroke was conducted among women with available blood samples (1989–1990). Stroke cases were participants free of known prior stroke or cancer at the time of the blood collection, but who experienced an ischemic stroke during the follow-up interval. For each stroke case, 1 NHS control participant who was free of known prior stroke or cancer at the time of the blood collection and had not had a stroke event at the time of the index case event was selected; hence, cases and controls possessed the same amount of person-time at risk. Controls were individually matched to the index case at blood collection by age, race, smoking, menopausal status, hormone therapy, and date of sample collection (see the online-only Data Supplement).

Blood Sample Assay

Blood samples were collected from a subgroup of NHS participants (n=32,826) between 1989 and 1990 and a second sample was requested and received (n=18,743) 10 years later (2000–2001). Estimation of the association between DHEAS and risk of ischemic stroke used DHEAS collected at 1 time point (1989–1990); replicate samples were only used to estimate measures of reliability. Case–control pair samples were handled identically and together shipped to the laboratory in the same batch and assayed in the same run. Each batch included replicate, blinded plasma samples to assess laboratory precision and drift samples standardized to a particular level for the biomarker to track and correct for laboratory assay drift.

DHEAS was measured by competitive radioimmunoassay at Quest Diagnostics/Nichols Institute (San Juan Capistrano, CA) for all cases and controls, with a mean intra-assay coefficient of variation of 4%. Previously, DHEAS levels have shown stability for 2 to 3 years in this population of postmenopausal women (intraclass correlation coefficient, 0.88; 95% CI, 0.84–0.93); and ≤15 years stability in frozen serum in other populations. We estimated the intraclass correlation coefficient among 102 women with 2 measurements ≥10 years apart and observed good reproducibility (intraclass correlation coefficient, 0.73; 95% CI, 0.66–0.79).

Total, low-density lipoprotein, high-density lipoprotein-cholesterol, C-reactive protein, and sex-hormone–binding globulin were measured at the Clinical and Epidemiological Research Laboratory at Children’s Hospital (Boston, MA), and estradiol and testosterone were measured at the Mayo Clinic (Rochester, MN) for all cases and controls using standard laboratory assays (see the online-only Data Supplement). All CVs for low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, C-reactive protein, and sex-hormone–binding globulin were <10% and <16% for estradiol and testosterone.

Cerebrovascular Disease Assessment

We included ischemic strokes that occurred from the return of the blood sample through 2006. Nonfatal stroke was reported on biennial questionnaires and confirmed by medical records. Deaths were detected through information provided by the next of kin, postal authorities, or by systematic searches of the National Death Index. Classification of fatal stroke was confirmed by review of hospital records, autopsy, or death certificate. Women (or next of kin for decedents) reporting stroke on follow-up questionnaires were asked for permission to review medical records, which were reviewed by a physician blinded to exposure status. Stroke was classified according to the National Survey of Stroke criteria requiring evidence of a neurological deficit with sudden or rapid onset that persisted for >24 hours or until death. Strokes were classified as ischemic stroke because of thrombotic or embolic occlusion of a cerebral artery with imaging data from computed tomography or MRI or data on autopsy available for >92% of events, with high reproducibility.

Statistical Analysis

Descriptive analyses for baseline characteristics were conducted by case–control status and across quartiles of DHEAS. Spearman correlation coefficient was calculated between DHEAS levels and age.
Multivariable conditional logistic regression models estimated the association between DHEAS and risk of ischemic stroke. A priori hypotheses. Significance of the interaction was assessed using the likelihood ratio test. We examined the impact of estradiol, testosterone, and sex-hormone–binding globulin on the association between DHEAS and risk of ischemic stroke. All P values were 2-sided. Analyses were conducted with SAS for UNIX statistical software (version 9.2; SAS Institute, Cary, NC).

## Results

DHEAS levels were available for 461 complete case–control pairs. The mean age was 61 years. As expected, women who developed stroke during follow-up were more likely to be diabetic, report a history of hypertension and family history of heart disease compared with controls (Table 1) at baseline.

Age was inversely correlated with DHEAS levels (Spearman \( r = -0.32; \ P < 0.0001 \)). Women in the lowest DHEAS quartile were older and less educated but less likely to be current smokers compared with the highest quartile (Table 2). Furthermore, the lowest quartile of DHEAS was associated with a higher prevalence of hypertension, high cholesterol, and history of heart disease, as well as a higher proportion of hormone therapy use. In the lowest DHEAS quartile, the mean estradiol levels were 5.21±3.08 pg/mL among postmenopausal women not on hormone therapy and 18.00±19.00 pg/mL among hormone therapy users. For premenopausal women, there was not a clear relation between estradiol levels and DHEAS quartiles. When adjusted for matching factors, women in the lowest versus the highest quartile of DHEAS had a nonsignificant

### Table 2. Baseline Characteristics of the Population by DHEAS Quartiles in 1990

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Quartile 1 (n=259)</th>
<th>Quartile 2 (n=226)</th>
<th>Quartile 3 (n=210)</th>
<th>Quartiles 4 (n=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63±4.4</td>
<td>62±5.3</td>
<td>60±6.1</td>
<td>58±6.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4±4.4</td>
<td>25.6±5.3</td>
<td>26.4±5.4</td>
<td>25.4±4.7</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>14</td>
<td>20</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>Alcohol, g/d</td>
<td>4.9±10.4</td>
<td>5.0±10.1</td>
<td>5.4±9.6</td>
<td>7.1±11.6</td>
</tr>
<tr>
<td>Physical activity, METs/wk*</td>
<td>16.1±20.6</td>
<td>15.4±15.1</td>
<td>16.0±20.4</td>
<td>15.2±20.0</td>
</tr>
</tbody>
</table>

Values are mean±SD (medians) or percentages. BMI indicates body mass index; CRP, C-reactive protein; and DHEAS, dehydroepiandrosterone sulfate. *METs/wk: metabolic equivalents per week.

†Reference is postmenopausal nonhormone therapy use; premenopausal and dubious excluded.

#### Statement of Ethics

This study was approved by the institutional review board of Brigham and Women’s Hospital and all procedures followed were in accordance with institutional guidelines. Participants provided informed consent to participate.
greater risk of ischemic stroke (Table 3); adjustment for potential confounders did not alter results. The association was strengthened after adjusting for cardiovascular risk factors and biomarkers (HbA1c and total/high-density lipoprotein-cholesterol). When modeling binary DHEAS, women with DHEAS levels in the lowest quartile exhibited a significantly greater risk of ischemic stroke compared with higher levels in multivariable analyses (Figure; model 2); estimates were strengthened and remained statistically significant after adjustment for cardiovascular risk factors and biomarkers (Figure; model 3).

There was evidence to suggest that the association between DHEAS and risk of ischemic stroke may vary by diabetes mellitus (Pinteraction=0.03), age (Pinteraction=0.06) and smoking status (Pinteraction=0.11) (Table 4). The risk of ischemic stroke for women in the lowest DHEAS quartile compared with higher quartiles was stronger among women with diabetes mellitus than for women without diabetes mellitus; however, CIs were wide because of few events (Pinteraction=0.03). No significant interaction by time period was observed (results not shown). The association between DHEAS and risk of ischemic stroke was virtually unchanged after further adjustment for estradiol, testosterone, and sex-hormone–binding globulin (results not shown).

**Discussion**

In this nested case–control study, we observed evidence to suggest that women with DHEAS levels in the lowest quartile had a greater risk of ischemic stroke during follow-up. The association was strengthened by adjustment for cardiovascular risk factors and biomarkers and may be stronger among women with diabetes mellitus, those aged <65 years or never smokers. To our knowledge, this is the first report to evaluate DHEAS levels and risk of ischemic stroke.

Although no prior studies have examined DHEAS and stroke, several lines of evidence support a potential association. DHEAS levels have been significantly inversely associated with subclinical markers of vascular dysfunction among women: common carotid artery blood flow volume (β=0.21; P=0.05) and arterial stiffness (β=−0.27; P=0.03). An inverse association between DHEAS and CVD mortality has been suggested among men. Results from the Women’s Health and Aging Study, a population of older disabled women, reported low DHEAS levels were associated with an increased risk of CVD mortality (quartile 1 versus 3 [ref]: HR, 2.05; 95% CI, 1.27–3.32). Similarly, postmenopausal women in the Women’s Ischemia Syndrome Evaluation study with low DHEAS levels had >2-fold increased risk of CVD mortality (extreme tertiles: HR, 2.43; 95% CI, 1.06–5.56). Despite suggestive data, the association between DHEAS and risk of total CVD has been inconsistent among women: 5 studies reported null associations,20–24 2 reported that higher levels were associated with lower CVD mortality,3,4 and 1 reported that higher levels of DHEAS were associated with increased risk of CHD.6 Inconsistencies between studies may be because of variations in age, cardiovascular risk profiles, race/ethnicity, or other demographic factors, yet to be explored.3,4,6

DHEAS may influence the pathogenesis of CVD and ischemic stroke through several potential physiological mechanisms. DHEAS may act through atherosclerotic-related mechanisms, such as inhibiting the migration and proliferation of cells within the vascular wall, and increasing vascular smooth muscle cell apoptosis, thereby reducing vascular remodeling after injury. DHEA administration has also been shown to improve endothelial cell function indicated by flow-mediated dilation in vivo and increase endothelial cell proliferation independent of estrogen and androgen receptors in vitro. Furthermore, DHEAS may influence insulin resistance by affecting hepatocyte glucose production and improve peripheral tissue glucose clearance shown in animal models in vivo. Although unclear whether DHEAS acts alone or predominately through conversion to
DHEAS is a more stable marker of subclinical vascular disease.28 The underlying pathogenesis of ischemic stroke or serves as a risk marker of subclinical vascular disease. DHEAS can act independently in many cell types, including vascular endothelium.27 However, the androgenic or estrogenic activity of DHEAS may vary given a longer half-life, slower turnover rate, and it does not exhibit diurnal variation, in contrast to DHEA.

Table 4. Multivariable Association Between DHEAS* and Ischemic Stroke by Key Risk Factors

<table>
<thead>
<tr>
<th>Stratification Factor</th>
<th>Cases/Controls</th>
<th>RR (95% CI)</th>
<th>P (stratified) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>307/309</td>
<td>1.89 (1.26–2.85)</td>
<td>0.06</td>
</tr>
<tr>
<td>≥65</td>
<td>154/152</td>
<td>1.09 (0.67–1.79)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>226/254</td>
<td>1.39 (0.90–2.15)</td>
<td>0.49</td>
</tr>
<tr>
<td>≥25</td>
<td>235/207</td>
<td>1.57 (0.98–2.52)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Never</td>
<td>193/192</td>
<td>2.17 (1.32–3.56)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>186/193</td>
<td>1.30 (0.80–2.13)</td>
<td></td>
</tr>
<tr>
<td>Current†</td>
<td>82/76</td>
<td>0.85 (0.35–2.04)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus‡</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>No</td>
<td>403/433</td>
<td>1.36 (0.99–1.87)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>58/28</td>
<td>2.00 (0.51–7.86)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal hormone therapy use§</td>
<td></td>
<td>1.45 (1.07–1.98)</td>
<td>0.56</td>
</tr>
<tr>
<td>No</td>
<td>215/217</td>
<td>1.44 (1.05–1.96)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>195/194</td>
<td>1.36 (0.93–1.98)</td>
<td></td>
</tr>
<tr>
<td>Ovarian status</td>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Intact</td>
<td>334/328</td>
<td>1.36 (0.93–1.98)</td>
<td></td>
</tr>
<tr>
<td>≥1 ovary removed</td>
<td>127/133</td>
<td>1.83 (1.01–3.32)</td>
<td></td>
</tr>
</tbody>
</table>

Models adjusted for covariates in model 3 of Table 3, except stratification factor. BMI indicates body mass index; CI, confidence interval; DHEAS, dehydroepiandrosterone sulfate; and RR, relative risk.

*Lowest quartile vs quartiles 2–4.
†Current smokers not adjusted for race.
‡Adjusted for matching factors only because of sample size constraints.
§Reference is postmenopausal nonhormone therapy use; premenopausal and dubious excluded.

It is unclear whether DHEAS plays a direct role in the pathogenesis of ischemic stroke or serves as a risk marker of subclinical vascular disease. DHEAS is almost exclusively synthesized in the zona reticularis of the adrenal cortex, which is vulnerable to vascular damage; thus, DHEAS levels may be a marker of subclinical vascular disease.28 The underlying biological pathway is unclear; further research is needed to elucidate specific mechanisms.

Strengths of the current study include the nested case–control design with DHEAS levels collected before ischemic stroke events. DHEAS is a more stable marker than DHEA given a longer half-life, slower turnover rate, and it does not exhibit diurnal variation, in contrast to DHEA. However, DHEAS levels vary substantially by sex; androgenic or estrogenic activity of DHEAS may vary given a longer half-life, slower turnover rate, and it does not exhibit diurnal variation, in contrast to DHEA.

DHEAS is a more stable marker of subclinical vascular disease. Moreover, the association between DHEAS and risk of stroke did not differ by time to event. We were unable to evaluate ischemic stroke classification by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. Although cases–control pairs were not matched on hypertension status, as it may be on the causal pathway, we did adjust for hypertension in sensitivity analyses (model 3). Furthermore, the sample size was moderate and while we examined variation by key cardiovascular risk factors which have not been previously explored, analyses were underpowered and warrant further study.

Summary

In this cohort of older women, these results suggest evidence for an inverse association between DHEAS and risk of ischemic stroke, where lower levels of DHEAS were associated with an increased risk of ischemic stroke. There was an indication that an elevated risk of ischemic stroke associated with low DHEAS levels in this population may be strongest among diabetics, younger women (<65 years) and never smokers. Additional research is warranted to confirm these associations in other populations.

Acknowledgments

We thank Nurses’ Health Study participants.

Disclosures

HL088521-S1, HL34594, and Dr. Sun received grant support from K99HL098459 from the National Heart, Lung, and Blood Institute, and CA87969 and CA49449 from the National Cancer Institute of the National Institutes of Health. The other authors have no conflict to report.

References


Low Dehydroepiandrosterone Sulfate is Associated With Increased Risk of Ischemic Stroke Among Women
Monik C. Jiménez, Qi Sun, Markus Schürks, Stephanie Chiuve, Frank B. Hu, JoAnn E. Manson and Kathryn M. Rexrode

Stroke. 2013;44:1784-1789; originally published online May 23, 2013; doi: 10.1161/STROKEAHA.111.000485

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/7/1784

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2013/05/23/STROKEAHA.111.000485.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
Supplemental Methods

Nurses’ Health Study Cohort
Controls were selected from eligible participants and individually matched to the index case at blood collection by: age (2 years), ancestry (Caucasian/ African American/ Asian/ Hispanic/ Other/ Unknown), smoking (current/ past/ never), menopausal status, hormone therapy (yes/ no) and date of sample collection (+/-3 months, +/-3 years for 13 controls).

Blood Sample Assays
CRP was assayed using a latex-enhanced immunonephelometric assay on a BN II analyzer (Dade-Behring, Newark, DE) with a mean intra-assay CV of 2%. Total cholesterol was measured enzymatically, with a mean intra-assay CV 4%. HDL-C concentration was determined using a direct enzymatic colorimetric assay, with a mean intra-assay CV of 3%. LDL-C was determined by a homogenous direct method from Roche Diagnostics (Indianapolis, IN) with a mean intra-assay CV of 3%. HbA1c levels were determined on an analyzer (Hitachi, 911) based on turbidimetric immunoinhibition using packed red blood cells (Roche Diagnostics) with a mean intra-assay CV of 1.2%.

Statistical Analysis
We estimated 3 multivariable models: Model 1 adjusted for matching factors (age, menopausal status, hormone use and smoking status), Model 2 additionally adjusted for BMI (<18.5, 18.5-24.9, 25-29.9, ≥30 kg/m²), physical activity (METs/wk-tertiles), aspirin use(<1 tablet/wk, 1-5 tablets/wk, ≥6 tablets/wk), alcohol consumption (0, >0-4.9, 5-14.9, ≥15 g/d), Alternate Healthy Eating Index 2010 (aHEI 2010-score based on a diet low in trans fat, red and processed meats, sodium, and high in fruits and vegetables, nuts and legumes, polyunsaturated fats and whole grains) and Model 3 adjusted for all prior covariates in addition to history of diabetes (yes/no), high blood pressure (yes/no), and CHD or revascularization (yes/no), HbA1c, and total/HDL-C as an exploratory analysis. Physical activity, diet, hormonal status and chronic disease outcomes (hypertension, diabetes and CHD or revascularization) have been previously validated in this or similar populations.2-5
References:


