Available at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Severity and outcome of acute stroke in women: relation to adrenal sex steroid levels

Theodora Pappa[a,*], *Konstantinos Vemmos*[b], *Katerina Saltiki*[a], *Emilia Mantzou*[c], *Kimon Stamatelopoulos*[d], *Maria Alevizaki*[a]

[a] *Endocrine Unit, Department of Clinical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Greece*
[b] *Acute Stroke Unit, Department of Clinical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Greece*
[c] *Department of Endocrinology, Metabolism and Diabetes, Evgenideion Hospital, Athens University School of Medicine, Greece*
[d] *Vascular Laboratory, Department of Clinical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Greece*

ARTICLE INFO

Article history:
Received 12 February 2011
Accepted 5 June 2011

ABSTRACT

Adrenal sex steroids exert diverse metabolic and neurobiological actions. Their levels have been associated with cardiovascular disease, but data concerning cerebrovascular disease are lacking. The objective of our study was to investigate the role of adrenal sex steroids in a female population suffering an acute stroke. We addressed the question of whether their levels are associated with disease severity and prognosis. A 2-year cohort study was performed in 2 tertiary hospitals, where we prospectively studied 302 consecutive postmenopausal female patients hospitalized for an acute stroke. Neurological severity on admission was assessed by the National Institutes of Health Stroke Scale; and handicap 1 month after stroke, with the modified Rankin Scale. Δ4-androstenedione levels were positively and dehydroepiandrosterone sulfate was inversely associated with stroke severity (r = 0.142, P = .014 and r = –0.153, P = .008, respectively), and both parameters remained as significant determinants even after entering other confounders in the multivariate model (r = 0.118, P = .039 and r = –0.150, P = .011, respectively). Levels of Δ4-androstenedione were significantly associated with 1-month mortality in the multivariate analysis (odds ratio with 95% confidence intervals: 1.540 [1.107-2.138)], P = .010). Δ4-androstenedione and dehydroepiandrosterone sulfate levels were associated with poor outcome in the univariate analysis, that is, combined severe handicap (modified Rankin Scale ≥4) and death, 1 month poststroke, although this was not significant in the multivariate analysis. Adrenal sex steroids, and especially Δ4-androstenedione, are significantly associated with stroke severity on admission and short-term prognosis among female stroke subjects. Well-designed prospective studies will further clarify their role in cerebrovascular disease.

Authors' contributions: MA and KV designed the study; TP and Kat. S collected patients' data and blood samples; TP, EM, Kim. S, KV. and MA analyzed and interpreted the data and contributed in writing the manuscript. All authors read and approved the final manuscript.

* *Corresponding author*. Tel.: +30 6945280137; fax: +30 210 7704143.
E-mail address: dorispappas@hotmail.com (T. Pappa).

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.06.003

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfated ester (DHEAS) are the most abundant sex steroid products of the adrenal gland; they are converted to androgens and estrogens in the periphery. Regarding the steroidogenic pathway, DHEA can be converted to Δ4-androstenedione by 3β-hydroxysteroid dehydrogenase, a microsomal enzyme with $\Delta^5 \rightarrow \Delta^4$–isomerase activity. Δ4-androstenedione can also be produced through the hydroxylation of 17OH-progesterone by P450C17, an enzyme with dual 17α-hydroxylase and a 17,20-lyase activity [1].

The decline of DHEAS levels with aging, as well as the sexually dimorphic pattern of this decline, has been well established [2,3]. Possible explanations for the age-associated decline of DHEAS levels include the diminished 17,20-lyase activity of the P450C17 enzyme [4] and the reduction in the relative size and functional capacity of the adrenal zona reticularis [5]. Dehydroepiandrosterone sulfate concentration has been associated with indices of cardiovascular disease (CVD) [6,7], whereas data regarding Δ4-androstenedione are contradictory. In the study of Bernini et al [8], both Δ4-androstenedione and DHEAS levels were negatively associated with carotid artery intima-media thickness (IMT), an early marker of subclinical atherosclerosis. Similar results for DHEAS came from the study of Vryonidou et al in young subjects with polycystic ovary syndrome [9], whereas a possible protective role in the vasculature was suggested by the study of Dagre et al [10] and other investigators [11]. However, a positive association was shown between Δ4-androstenedione and IMT in the aforementioned study of Vryonidou et al [9]; in accordance, the study of Luque-Ramirez et al [12] identified Δ4-androstenedione as an independent determinant of carotid IMT in polycystic ovary syndrome subjects.

Dehydroepiandrosterone sulfate levels were inversely associated with left ventricular mass and septal thickness in postmenopausal hypertensive patients [13], as well as with biomarkers of disease severity in patients with chronic heart failure [14]. These data point to a protective role of DHEAS in the cardiovascular system. However, in the prospective cohort of the Rotterdam Study, radiographically detected aortic atherosclerosis was not associated with DHEAS concentrations in either sex [15]. As for Δ4-androstenedione, a definite conclusion cannot be easily drawn; Δ4-androstenedione levels were significantly lower in female patients with severe carotid atherosclerosis and CVD compared with the control population [16,17]. In addition, testosterone supplementation in female patients with chronic heart failure seemed to be safe and beneficial in terms of improved functional capacity and insulin sensitivity [18].

Clinical data investigating the association of adrenal sex steroid levels with markers of cerebrovascular disease, a major cause of mortality and disability, are scant. Female patients tend to have increased stroke severity as well as mortality after stroke compared with men, possibly because of higher incidence of cardioembolic stroke, greater age of first stroke, and thus more comorbidities compared with men [19]. Therefore, in this study, we aimed to identify potential associations between adrenal sex steroid levels and the severity as well as outcome of the disease in a female population suffering an acute stroke.

2. Materials and methods

We prospectively studied 302 consecutive postmenopausal female patients suffering an acute stroke derived from a total cohort of 806 patients (male and female) hospitalized in 2 general hospitals in Athens, Greece, over a period of 2 years (May 2008-April 2010). All study patients were admitted within 24 hours from stroke onset and treated in a total of 6 units with facilities to manage acute stroke: 2 acute stroke units, 2 internal medicine wards, and 2 neurology wards. A detailed medical history and a thorough physical examination were performed in all patients by an internist who specialized in stroke or a neurologist. Patients with transient ischemic stroke and subarachnoid hemorrhage, as well as patients on steroid therapy, were excluded from the study. We failed to recruit 23 patients (13 with a fatal stroke within 24 hours from admission and 11 not wishing to participate in the study). The study has been approved by the scientific committees of the 2 hospitals, and informed consent was obtained from all subjects.

Risk factors, including history of arterial hypertension, atrial fibrillation, previous ischemic attacks, coronary artery disease (CAD), cigarette smoking, diabetes mellitus, and hyperlipidemia, were recorded. A *history of arterial hypertension* was defined as systolic blood pressure (SBP) greater than 140 mm Hg and/or diastolic blood pressure (DBP) greater than 90 mm Hg diagnosed at least twice before stroke or if treatment of hypertension had been implemented [20]. Atrial fibrillation was diagnosed by at least one electrocardiogram during the last year before the episode. A history of previous ischemic attacks and history of coronary heart disease (myocardial infarction, angina pectoris, congestive heart failure) were assessed by questionnaire and relevant medical confirmation. Current smoking was considered present when a subject had smoked daily before the stroke and was considered absent when the subject had never smoked or had stopped smoking for at least 1 year preceding the stroke event. The *presence of diabetes mellitus* was defined as use of a blood glucose–lowering drug before the occurrence of stroke or if the fasting blood glucose level exceeded 126 mg/dL before stroke [21]. *Hyperlipidemia* was defined by history or if a total plasma cholesterol concentration of greater than 250 mg/dL was detected the day after admission [22]. *Stroke* was defined according to World Health Organization criteria [23], and pathological stroke subtype (ischemic vs hemorrhagic) was established by brain imaging.

All patients underwent an initial brain computed tomography scan and a 12-lead electrocardiogram. A second brain computed tomography or magnetic resonance imaging scan was performed within the first week to confirm the diagnosis, and additional investigations were performed as clinically indicated. Neurological impairment and stroke severity on admission were assessed using the National Institutes of Health Stroke Scale (NIHSS) [24]. The NIHSS score ranges from 0 to 34, with higher values reflecting more severe stroke. One month after stroke onset, patients' outcome and degree of handicap in daily living were evaluated with the modified Rankin Scale (mRS) by an internist who specialized in stroke or a neurologist [25]. The mRS scale ranges from 0 to 5. A score of 0 or 1 stands for full independence and means that the patient

has no or minimal symptoms and is able to carry out all usual duties and activities. A score of 4 or 5 stands for severe handicap and means that the patient is bedridden, requiring constant nursing care and attention. Twenty-one patients were lost to this follow-up. All stroke patients were treated following the European Stroke Organisation Guidelines [26].

Besides basic biochemical investigation and lipid profile testing, a hormonal panel, including DHEAS, Δ4-androstenedione, testosterone, and sex-hormone binding globulin (SHBG), was performed in all subjects 2 to 3 days after stroke onset. Phlebotomy was performed between 8:00 and 9:00 AM under fasting conditions. A 2-site chemiluminescent immunometric assay was used in all hormonal analyses (Siemens Healthcare Diagnostics Products, Llanberis, UK). The reference range was 13 to 200 μg/dL for DHEAS, 0.3 to 3.3 ng/mL for Δ4-androstenedione, 0.1 to 1.2 ng/mL for testosterone, and 18 to 114 nmol/L for SHBG.

2.1. Statistical analysis

The Statistical Package for Social Sciences (SPSS version 17.0 for Windows; SPSS, Chicago, IL) was used for the statistical analysis. Categorical variables were compared with the χ^2 test; continuous variables were compared with the Student *t* test when the distribution was normal and with the Mann-Whitney test when not. For nonparametric correlations between continuous variables, the Spearman rank correlation coefficient was calculated. To identify the variables that can serve as independent determinants of the parameter NIHSS, univariate and multivariate stepwise linear regression analyses were performed. Parameters significantly correlating ($P < .05$) with the dependent variable in the univariate regression analysis were inserted in the regression models. As the parameter NIHSS was not normally distributed, the natural logarithm (logNIHSS) was used in the regression analyses. Univariate and multivariate binary logistic stepwise regression models were used to identify independent factors for mortality and poor outcome, including severe handicap, that is, mRS of at least 4, and death 1 month after stroke. Colinearity among variables incorporated as independent terms in linear and logistic regression models was examined by the variance inflation factor values, whereas parameters correlating with a coefficient of variation (*r*) equal or more than 0.8 were not inserted in the same model. In all multivariate models, no significant colinearity was observed (variance inflation factor <2). Finally, analysis of covariance was performed to adjust for possible confounders for differences found in sex hormone levels between specific subgroups. Statistical significance in all analyses was reached if $P < .05$. Variables with *P* values < .05 in univariate regression analyses were inserted in the multivariate models.

3. Results

Our study population had a mean age of 73.60 ± 12.92 years and body mass index (BMI) of 28.69 ± 5.66 kg/m^2. The prevalence of arterial hypertension was 77.75%, and that of diabetes mellitus 25.16%. One hundred sixty-one patients (53.31%) had poor outcome 1 month after stroke, including severe handicap, that is, mRS of at least 4, and death. Only 5 patients underwent thrombolysis. Our patients' demographic characteristics and hormonal parameters are cumulatively depicted in Table 1. The DHEAS levels were inversely correlated with patients' age ($r = –0.328$, $P < .001$) and positively correlated with total cholesterol and high-density lipoprotein (HDL) concentration ($r = 0.139$, $P = .017$ and $r = 0.178$, $P = .020$, respectively). A positive correlation was found between DHEAS levels and BMI ($r = 0.134$, $P = .022$). Smokers had significantly higher DHEAS levels compared with nonsmokers (63.07 ± 56.03 vs 46.42 ± 40.30 μg/dL, respectively; $P = .039$), in accordance with previous reports [27]. Patients with prior stroke had significantly lower DHEAS levels compared with first-ever stroke patients (38.89 ± 32.49 vs 53.39 ± 47.06 μg/dL, respectively; $P = .027$), and the same trend was documented in subjects with a history of atrial fibrillation (40.92 ± 37.11 and 55.78 ± 47.66 μg/dL, respectively; $P = .008$). However, it should be mentioned that these findings could be biased by the younger age of smokers and the greater age of patients with previous stroke and those with atrial fibrillation ($P < .05$ in all cases). Indeed, after adjusting for age, the differences in DHEAS observed between those with and without history of previous stroke and atrial fibrillation were significantly attenuated ($P = .110$ and $P = .077$, respectively).

Table 1 – Patients' characteristics and hormonal parameters

Variables	
Age (years)	73.60 (12.92)
BMI (kg/m^2)	28.69 (5.66)
Waist to hip ratio	0.95 (0.05)
Risk factors	
Arterial hypertension	234 (77.75)
Diabetes mellitus	76 (25.16)
Current smoking	57 (18.87)
Hyperlipidemia	113 (37.42)
Atrial fibrillation	124 (41.06)
Known CAD	37 (12.25)
Previous stroke	79 (26.16)
Type of stroke and severity	
Ischemic stroke	261 (86.42)
Hemorrhagic stroke	41 (13.57)
Stroke severity [a]	10.71 (7.39)
SBP (mm Hg)	142.64 (21.95)
DBP (mm Hg)	82.51 (12.68)
Cholesterol (mg/dL)	189.38 (59.99)
HDL (mg/dL)	46.70 (16.77)
LDL (mg/dL)	116.63 (35.67)
Triglycerides (mg/dL)	117.75 (54.78)
DHEAS (μg/dL)	49.48 (44.11)
Δ4-androstenedione (ng/mL)	1.85 (1.58)
Testosterone (ng/mL)	0.50 (0.38)
SHBG (nmol/L)	52.88 (26.99) [b]

Variables are presented as mean for continuous or number for nominal variables. Numbers in parentheses indicate standard deviation for continuous or percentages for nominal variables.
[a] Stroke severity on admission as assessed by the NIHSS.
[b] To convert DHEAS concentration to micromoles per liter, multiply by 0.02714; to convert Δ4-androstenedione to nanomoles per liter, multiply by 3.492; and to convert testosterone to nanomoles per liter, multiply by 3.467.

Table 2 – Univariate and multivariate linear regression analyses showing the independent determinants of logNIHSS

	Univariate		Multivariate			
Independent Variables	β	*P* value univariate analysis	*P* value multivariate analysis	β	Model R^2 value	Model *P* value
Dependent variable: logNIHSS					0.418	<.001
Δ4-androstenedione	0.142	**.014**	.039	0.118		
SHBG	–0.068	.244				
Testosterone	0.115	**.048**				
DHEAS	–0.153	**.008**	.011	–0.150		
Age	0.071	.220				
BMI	0.050	.392				
Waist to hip ratio	0.023	.695				
Known CAD	–0.506	.332				
Previous stroke	0.062	.283				
Arterial hypertension	0.044	.452				
Diabetes mellitus	–0.113	**.050**				
Current smoking	–0.114	**.048**				
Atrial fibrillation	0.122	**.035**	.006	0.155		
SBP	0.152	**.008**	.015	0.134		
DBP	0.081	.159				
Cholesterol	–0.130	**.025**				
HDL	–0.202	**<.001**	<.001	–0.201		
LDL	–0.080	.167				
Triglycerides	0.110	.058				
Hemorrhagic stroke	0.233	**<.001**	<.001	0.221		

Variables with *P* values ≤ .05 (in bold) in univariate regression analysis were inserted in the multivariate model. Only significant *P* values are shown in multivariate analysis.

Table 3 – Univariate and multivariate binary logistic regression analyses showing the independent determinants of mortality 1 month after the episode

	Univariate		Multivariate			
Independent Variables	OR (95% CI)	*P* value univariate analysis	*P* value multivariate analysis	OR (95% CI)	Model χ^2	Model *P* value
Dependent variable: 1-mo mortality					40.863	<0.001
Δ4-androstenedione	1.240 (1.034-1.488)	**.021**	.010	1.540 (1.107-2.138)		
SHBG	0.997 (0.981-1.013)	.672				
Testosterone	2.774 (1.179-6.525)	**.019**				
DHEAS	0.996 (0.985-1.006)	.432				
Age	1.027 (0.989-1.066)	.170				
BMI	1.021 (0.954-1.093)	.546				
Waist to hip ratio	0.012 (0.000-65.188)	.312				
Known CAD	0.629 (0.142-2.790)	.541				
Previous stroke	2.601 (1.113-6.074)	**.027**				
Arterial hypertension	0.363 (0.153-0.859)	**.021**				
Diabetes mellitus	0.569 (0.188-1.722)	.319				
Hyperlipidemia	0.308 (0.103-0.926)	**.036**				
Current smoking	0.845 (0.277-2.576)	.768				
Atrial fibrillation	1.228 (0.531-2.839)	.631				
NIHSS	1.180 (1.106-1.259)	**<.001**	<.001	1.174 (1.097-1.256)		
SBP	0.999 (0.981-1.018)	.954				
DBP	0.974 (0.942-1.007)	.126				
Cholesterol	1.000 (0.994-1.007)	.932				
HDL	0.973 (0.942-1.005)	.093				
LDL	0.988 (0.976-1.000)	.058				
Triglycerides	0.993 (0.983-1.003)	.175				
Hemorrhagic stroke	2.183 (0.816-5.840)	.120				

Variables with *P* values ≤ .05 (in bold) in univariate regression analysis were inserted in the multivariate model. Only significant *P* values are shown in multivariate analysis.

An inverse association was found between DHEAS levels and stroke severity on admission, as expressed by logNIHSS (r = –0.153, P = .008), whereas positive associations were evident between Δ4-androstenedione and testosterone levels and stroke severity (r = 0.142, P = .014 and r = 0.115, P = .048, respectively). Using univariate linear regression analysis, as shown in Table 2, other factors associated with logNIHSS were the SBP, total cholesterol, and HDL levels. After entering all possible confounders in a multivariate linear regression model, Δ4-androstenedione and DHEAS levels remained as the only hormonal parameters independently associated with stroke severity along with the history of atrial fibrillation, SBP, and HDL levels, as well as a hemorrhagic stroke type (Table 2).

When applying the univariate binary logistic regression analysis to identify the factors associated with mortality 1 month after stroke, Δ4-androstenedione and testosterone levels attained statistical significance, as shown in Table 3. We failed to demonstrate a significant difference in DHEAS levels between survivors and nonsurvivors (50.09 ± 44.79 vs 42.82 ± 35.92 μg/dL, respectively; P = .431). In the multivariate binary logistic regression model, only Δ4-androstenedione could be considered an independent determinant of 1-month mortality along with stroke severity on admission as expressed by NIHSS (odds ratio [OR] with 95% confidence intervals [CI] and P value: 1.540 [1.107-2.138], P = .010 and 1.174 [1.097-1.256], P < .001, respectively) (Table 3).

When analyzing patients' outcome 1 month after stroke, it was shown that patients with poor outcome, including severe handicap, that is, mRS of at least 4, and death, had significantly lower DHEAS levels compared with patients with mild or moderate handicap, that is, mRS from 0 to 3, (43.77 ± 38.35 vs 55.95 ± 49.19 μg/dL, respectively; P = .019). In the univariate binary logistic regression analysis, DHEAS and Δ4-androstenedione levels were both associated with worse outcome. However, this was nonsignificant in the multivariate model; and the only independent determinants of poor outcome that remained were patients' age, stroke severity on admission expressed by NIHSS, and hemorrhagic stroke type (OR with 95% CI and P value: 1.053 [1.013-1.095], P = .010; 1.721 [1.504-1.968], P < .001; and 6.402 [1.632-25.165], P = .008 respectively). Similar results were observed when poor outcome was defined as mRS of at least 4 without including death. By multivariate analysis, the independent determinants of this outcome were age, stroke severity on admission expressed by NIHSS, and hemorrhagic stroke type (OR with 95% CI and P value: 1.044 [1.003-1.087], P = .035; 1.781 [1.538-2.062], P < .001; and 8.606 [2.071-35.720], P = .003, respectively) (Table 4).

4. Discussion

The most interesting finding of our study was the positive association of Δ4-androstenedione levels with neurological impairment and stroke severity on admission, as well as with 1-month mortality, a finding that has not been reported before in a clinical setting. These associations were independent of

Table 4 – Univariate and multivariate binary logistic regression analyses showing the independent determinants of poor outcome, that is, mRS 4 to 5, excluding death, 1 month after the episode

	Univariate		Multivariate			
Independent Variables	OR (95% CI)	P value univariate analysis	P value multivariate analysis	OR (95% CI)	Model χ^2	Model P value
Dependent variable: poor outcome 1 mo after stroke (mRS 4-5)					235.542	<0.001
Δ4-androstenedione	1.128 (0.959-1.328)	.146				
SHBG	0.998 (0.989-1.006)	.607				
Testosterone	1.306 (0.667-2.557)	.436				
DHEAS	0.994 (0.988-0.999)	**.029**				
Age	1.024 (1.004-1.045)	**.016**	.035	1.044 (1.003-1.087)		
BMI	1.030 (0.986-1.076)	.178				
Waist to hip ratio	1.032 (0.986-1.075)	.151				
Known CAD	0.856 (0.420-1.743)	.669				
Previous stroke	1.326 (0.766-2.296)	.314				
Arterial hypertension	1.561 (0.864-2.821)	.140				
Diabetes mellitus	0.618 (0.358-1.065)	.083				
Hyperlipidemia	0.633 (0.389-1.029)	.065				
Current smoking	0.564 (0.305-1.043)	.068				
Atrial fibrillation	1.774 (1.093-2.879)	**.020**				
NIHSS	1.667 (1.474-1.886)	**<.001**	<.001	1.781 (1.538-2.062)		
SBP	1.018 (1.006-1.030)	**.003**				
DBP	1.013 (0.993-1.032)	.208				
Cholesterol	0.997 (0.992-1.003)	.331				
HDL	0.987 (0.971-1.002)	.089				
LDL	0.998 (0.992-1.005)	.594				
Triglycerides	1.002 (0.998-1.006)	.408				
Hemorrhagic stroke	6.098 (2.443-15.224)	**<.001**	.003	8.606 (2.071-35.720)		

Variables with P values ≤ .05 (in bold) in univariate regression analysis were inserted in the multivariate model. Only significant P values are shown in multivariate analysis.

other confounding factors. Although the role of adrenal sex steroids in cardiovascular disease has been extensively studied, data on its significance in subjects–let alone female patients—with cerebrovascular disease are lacking.

Indeed, several studies have examined the association between endogenous androgens and the clustering of metabolic abnormalities and cardiovascular risk profile; a close link appears to exists between endogenous androgens and indices of the metabolic syndrome, like leptin and adiponectin concentrations, insulin resistance, and visceral adiposity [28-34]. Still, the existing literature on the role of androgens in CVD shows conflicting results. The Rancho Bernardo Study did not show an important role of testosterone on CVD mortality in women [35], whereas in the study of Sievers et al [36], the contrary was shown, that is, that low testosterone levels were associated with higher all-cause mortality and cardiovascular events. Naessen et al [37] suggest that such associations may mirror the result rather than indicate a causative factor of atherosclerosis. On the other hand, a recent study demonstrated a strong association of biochemical hyperandrogenemia with severity of CAD and worse CVD-free survival in women [38].

According to animal experimental models, the sensitivity to cerebral ischemia is sex specific and partly attributed to the protective action of female sex hormones, whereas few data exist on the role of androgens [39,40]. However, the net effect of sex hormones is probably more complicated considering the local conversion of androgens to estrogen in the brain via aromatase expressed in the astrocytes, suggesting a pivotal role of brain aromatization in neuroprotection [41]. This should be taken into consideration when trying to interpret sex hormones' actions based on their circulating levels.

Another interesting finding of our study was the independent inverse association between DHEAS and neurological severity on admission, as well as short-term handicap and outcome of stroke patients 1 month after the event, although this did not remain significant in the multivariate model. Several studies, some of them very recent, have provided evidence of an association between low DHEAS levels and increased cardiovascular and all-cause mortality, mainly in the male population [42-46]. The Rancho Bernardo Study found an association between DHEAS levels and mortality only in men and not in women [27]. More recently, in the Women's Health and Aging Study, it was shown that women with lower DHEAS had greater CVD mortality [47]. Our findings appear to agree with the latter. In the same direction were the findings of Shufelt et al [48] who found an association of low DHEAS and cardiovascular and all-cause mortality in women undergoing coronary angiography, whereas a cohort study reported an association of DHEAS levels with longevity in men but not in women [49].

With respect to the metabolic and neurobiological role of this hormone, a large body of data has accumulated from clinical supplementation studies [50-53]. Dehydroepiandrosterone sulfate has been reported to be a vasoactive hormone with genomic and nongenomic actions on the vasculature leading to an increase of nitric oxide production [54]. Dehydroepiandrosterone also appears to act as an antiapoptotic factor on vascular endothelial cells [55]. Furthermore, DHEAS is a neurosteroid with a prominent neuroprotective role [56]. It acts as a positive allosteric modulator of the N-methyl-D-aspartic acid receptor and as a $\sigma 1$ receptor agonist [57]. Its positive neuronal effect has been attributed in part to an antiglucocorticoid action [58,59]. In rat models, DHEA supplementation inhibited neuronal injury and could represent a putative neuroprotective strategy for the treatment of cerebral ischemia [60]. However, DHEA administration during ischemia and early reperfusion state exerted a neurotoxic effect. This observation may indicate that the putative "therapeutic" time window for DHEA may be narrow [61].

The strengths of our study include the appreciable sample of female stroke patients, as well as the consideration of a variety of anthropometric, biochemical, and hormonal parameters. Furthermore, widely accepted neurological scales were applied to allow a quantified estimation of the neurological severity and outcome of the disease. However, our study also has certain limitations that need to be addressed. We should point out that our study only included female patients; comparison of data in female and male subjects might add useful information about the sexually dimorphic pattern already established in stroke. However, the initial design of our study focused specifically on the analysis of the role of endogenous androgens in women, who represent a growing and not thoroughly studied body of stroke patients. We cannot exclude the possibility that the timing of hormone testing (2-3 days poststroke) could have interfered with the analysis and resulted in altered sex steroids measurements. This represents a drawback of our study; and it is possible that stroke itself results in deranged steroid levels in proportion to the extent of the neurological damage, this change being the epiphenomenon of the primary disease or a reflection of medical manipulations and other confounding factors. Ideally, this could be resolved by consecutive hormonal measurements after the onset of stroke. It should be mentioned that Marklund et al [62], in their study focusing on the importance of cortisol levels in acute ischemic stroke patients, have reported a decline of DHEAS levels between days 1 and 4 in a subgroup of 29 patients. The DHEAS levels had no association with patients' outcome in that study; however, one has to note that the sample was very small. A limiting factor in our study was the short follow-up period, mainly due to difficulties in keeping track of stroke patients after discharge. Longer follow-up would provide more accurate data on their functional performance after rehabilitation. In addition, the current study sample is inhomogeneous in terms of stroke etiopathogenesis and degree of underlying vascular disease, which should be kept into consideration when trying to interpret statistical results and emerging associations.

Clearly, our clinical observation does not imply a causative relation between adrenal sex steroids and neurological severity of acute stroke. However, the link between $\Delta 4$-androstenedione and mortality 1 month after stroke appears intriguing; and it is plausible that this hormone might represent a useful biomarker to identify female stroke patients with an increased mortality risk. It should be pointed out that, compared with patients with cardiovascular disease, stroke subjects are less meticulously studied. Given that they represent a growing body of population suffering from a disease with increased handicap and mortality, it would be of interest to clarify the degree of adrenal hormones effect—if

any—on the severity and outcome of cerebrovascular incidents. Larger-scale studies including both sexes with a longer follow-up period could provide more definite answers, whereas data from basic experimental studies are expected to assert the exact role of adrenal sex steroids in the complex pathophysiologic mechanisms of acute stroke.

In conclusion, Δ4-androstenedione levels are an independent determinant of 1-month mortality among female stroke patients. Δ4-androstenedione and DHEAS levels are associated with the severity of stroke on admission, as well as with their short-term handicap. The association of Δ4-androstenedione and DHEAS levels with stroke severity was independent of other confounding factors. Our clinical study addresses a potential role for Δ4-androstenedione as a surrogate marker of stroke severity and short-term mortality; clearly, well-designed prospective clinical studies, as well as experimental models of cerebral ischemia, will broaden our knowledge on the role of adrenal sex steroids in cerebrovascular disease and provide deeper insight in their neurobiological properties.

Conflict of Interest

The authors have nothing to declare.

REFERENCES

[1] Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev 2011;32:81-151.

[2] Orentreich N, Brind JL, Rizer RL, et al. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 1984;59:551-5.

[3] Laughlin GA, Barrett-Connor E. Sexual dimorphism in the influence of advanced aging on adrenal hormone levels: the Rancho Bernardo Study. J Clin Endocrinol Metab 2000;85:3561-8.

[4] Liu CH, Laughlin GA, Fischer UG, et al. Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: evidence for a reduced 17,20-desmolase enzymatic activity. J Clin Endocrinol Metab 1990;71:900-6.

[5] Parker Jr CR, Mixon RL, Brissie RM, et al. Aging alters zonation in the adrenal cortex of men. J Clin Endocrinol Metab 1997;82: 3898-901.

[6] Sanders JL, Boudreau RM, Cappola AR, et al. Cardiovascular disease is associated with greater incident dehydroepiandrosterone sulfate decline in the oldest old: the cardiovascular health study all stars study. J Am Geriatr Soc 2010;58:421-6.

[7] Fukui M, Ose H, Kitagawa Y, et al. Relationship between low serum endogenous androgen concentrations and arterial stiffness in men with type 2 diabetes mellitus. Metabolism 2007;56:1167-73.

[8] Bernini GP, Sgro' M, Moretti A, et al. Endogenous androgens and carotid intimal-medial thickness in women. J Clin Endocrinol Metab 1999;84:2008-12.

[9] Vryonidou A, Papatheodorou A, Tavridou A, et al. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90:2740-6.

[10] Dagre A, Lekakis J, Mihas C, et al. Association of dehydroepiandrosterone-sulfate with endothelial function in young women with polycystic ovary syndrome. Eur J Endocrinol 2006;154:883-90.

[11] Yoshida S, Aihara K, Azuma H, et al. Dehydroepiandrosterone sulfate is inversely associated with sex-dependent diverse carotid atherosclerosis regardless of endothelial function. Atherosclerosis 2010;212:310-5.

[12] Luque-Ramírez M, Mendieta-Azcona C, Alvarez-Blasco F, et al. Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome. Hum Reprod 2007;22:3197-203.

[13] Szathmári M, Treszl A, Vásárhelyi B. Left ventricular mass index and ventricular septum thickness are associated with serum dehydroepiandrosterone-sulphate levels in hypertensive women. Clin Endocrinol (Oxf) 2003;59:110-4.

[14] Moriyama Y, Yasue H, Yoshimura M, et al. The plasma levels of dehydroepiandrosterone sulfate are decreased in patients with chronic heart failure in proportion to the severity. J Clin Endocrinol Metab 2000;85:1834-40.

[15] Hak AE, Witteman JC, de Jong FH, et al. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. J Clin Endocrinol Metab 2002;87:3632-9.

[16] Debing E, Peeters E, Duquet W, et al. Endogenous sex hormone levels in postmenopausal women undergoing carotid artery endarterectomy. Eur J Endocrinol 2007;156:687-93.

[17] Khatibi A, Agardh CD, Shakir YA, et al. Could androgens protect middle-aged women from cardiovascular events? A population-based study of Swedish women: The Women's Health in the Lund Area (WHILA) Study. Climacteric 2007;10: 386-92.

[18] Iellamo F, Volterrani M, Caminiti G, et al. Testosterone therapy in women with chronic heart failure: a pilot double-blind, randomized, placebo-controlled study. J Am Coll Cardiol 2010;56:1310-6.

[19] Rosamond W, Flegal K, Friday G, et al. Heart disease and stroke statistics—2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2007;115:e69-171.

[20] Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI). Arch Intern Med 1997;157:2413-46.

[21] World Health Organisation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Part 1. Diabetes mellitus—diagnosis. Geneva: World Health Organisation/ International Diabetes Federation; 2006.

[22] Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143.

[23] Hatano S. Experience from a multicentre stroke register: a preliminary report. Bull World Health Organisation 1976;54: 541-53.

[24] Brott T, Adams Jr HP, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864-70.

[25] Rankin J. Cerebral vascular accidents in patient over the age of 60. II. Prognosis. Scott Med J 1957;2:200-15.

[26] European Stroke Organisation (ESO) Executive Committee; ESO Writing Committee. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. Cerebrovasc Dis 2008;25:457-507.

[27] Barrett-Connor E, Goodman-Gruen D. Dehydroepiandrosterone sulfate does not predict cardiovascular death in postmenopausal women. The Rancho Bernardo Study. Circulation 1995;91:1757-60.

[28] Van Beek AP, de Ruijter-Heijstek FC, Jansen H, et al. Sex steroids and plasma lipoprotein levels in healthy women: the importance of androgens in the estrogen-deficient state. Metabolism 2004;53:187-92.
[29] Korytkowski MT, Krug EI, Daly MA, et al. Does androgen excess contribute to the cardiovascular risk profile in postmenopausal women with type 2 diabetes? Metabolism 2005;54:1626-31.
[30] Weinberg ME, Manson JE, Buring JE, et al. Bottom of form low sex hormone-binding globulin is associated with the metabolic syndrome in postmenopausal women. Metabolism 2006;55:1473-80.
[31] Rolland YM, Perry 3rd HM, Patrick P, et al. Leptin and adiponectin levels in middle-aged postmenopausal women: associations with lifestyle habits, hormones, and inflammatory markers—a cross-sectional study. Metabolism 2006;55:1630-6.
[32] Maturana MA, Breda V, Lhullier F, et al. Relationship between endogenous testosterone and cardiovascular risk in early postmenopausal women. Metabolism 2008;57:961-5.
[33] Phillips GB, Jing T, Heymsfield SB. Does insulin resistance, visceral adiposity, or a sex hormone alteration underlie the metabolic syndrome? Studies in women. Metabolism 2008;57:838-44.
[34] Merki-Feld GS, Imthurn B, Rosselli M, et al. Serum concentrations of high-molecular weight adiponectin and their association with sex steroids in premenopausal women. Metabolism 2011;60:180-5.
[35] Barrett-Connor E, Goodman-Gruen D. Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. BMJ 1995;311:1193-6.
[36] Sievers C, Klotsche J, Pieper L, et al. Low testosterone levels predict all-cause mortality and cardiovascular events in women: a prospective cohort study in German primary care patients. Eur J Endocrinol 2010;163:699-708.
[37] Naessen T, Sjogren U, Bergquist J, et al. Endogenous steroids measured by high-specificity liquid chromatography-tandem mass spectrometry and prevalent cardiovascular disease in 70-year-old men and women. J Clin Endocrinol Metab 2010;95:1889-97.
[38] Shaw LJ, Bairey Merz CN, Azziz R, et al. Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health–National Heart, Lung, and Blood Institute sponsored Women's Ischemia Syndrome Evaluation. J Clin Endocrinol Metab 2008;93:1276-84.
[39] Alkayed NJ, Harukuni I, Kimes AS, et al. Gender-linked brain injury in experimental stroke. Stroke 1998;29:159-65.
[40] Krause DN, Duckles SP, Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. J Appl Physiol 2006;101:1252-61.
[41] McCullough LD, Blizzard K, Simpson ER, et al. Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. J Neurosci 2003;23:8701-5.
[42] Barrett-Connor E, Khaw KT, Yen SS. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. N Engl J Med 1986;315:1519-24.
[43] LaCroix AZ, Yano K, Reed DM. Dehydroepiandrosterone sulfate, incidence of myocardial infarction, and extent of atherosclerosis in men. Circulation 1992;86:1529-35.
[44] Trivedi DP, Khaw KT. Dehydroepiandrosterone sulfate and mortality in elderly men and women. J Clin Endocrinol Metab 2001;86:4171-7.
[45] Philips A, Carroll D, Gale C, et al. Cortisol, dehydroepiandrosterone sulphate, their ratio and all-cause and cause-specific mortality in the Vietnam Experience Study. Eur J Endocrinol 2010;163:285-92.
[46] Ohlsson C, Labrie F, Barrett-Connor E, et al. Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men. J Clin Endocrinol Metab 2010;95:4406-14.
[47] Cappola AR, Xue QL, Walston JD, et al. DHEAS levels and mortality in disabled older women: the Women's Health and Aging Study I. J Gerontol A Biol Sci Med Sci 2006;61:957-62.
[48] Shufelt C, Bretsky P, Almeida CM, et al. DHEA-S levels and cardiovascular disease mortality in postmenopausal women: results from the National Institutes of Health–National Heart, Lung, and Blood Institute (NHLBI)–sponsored Women's Ischemia Syndrome Evaluation (WISE). J Clin Endocrinol Metab 2010;95:4985-92.
[49] Enomoto M, Adachi H, Fukami A, et al. Serum dehydroepiandrosterone sulfate levels predict longevity in men: 27-year follow-up study in a community-based cohort (Tanushimaru study). J Am Geriatr Soc 2008;56:994-8.
[50] Kawano H, Yasue H, Kitagawa A, et al. Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. J Clin Endocrinol Metab 2003;88:3190-5.
[51] Arlt W, Callies F, van Vlijmen JC, et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 1999;341:1013-20.
[52] Hunt PJ, Gurnell EM, Huppert FA, et al. Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. J Clin Endocrinol Metab 2000;85:4650-6.
[53] Boxer RS, Kleppinger A, Brindisi J, et al. Effects of dehydroepiandrosterone (DHEA) on cardiovascular risk factors in older women with frailty characteristics. Age Ageing 2010;39:451-8.
[54] Simoncini T, Mannella P, Fornari L, et al. Dehydroepiandrosterone modulates endothelial nitric oxide synthesis via direct genomic and nongenomic mechanisms. Endocrinology 2003;144:3449-55.
[55] Liu D, Si H, Reynolds KA, et al. Dehydroepiandrosterone protects vascular endothelial cells against apoptosis through a Galphai protein-dependent activation of phosphatidylinositol 3-kinase/Akt and regulation of antiapoptotic Bcl-2 expression. Endocrinology 2007;148:3068-76.
[56] Lapchak PA, Chapman DF, Nunez SY, et al. Dehydroepiandrosterone sulfate is neuroprotective in a reversible spinal cord ischemia model: possible involvement of GABA(A) receptors. Stroke 2000;31:1953-6.
[57] Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol 2000;21:1-56.
[58] Kimonides VG, Khatibi NH, Svendsen CN, et al. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc Natl Acad Sci USA 1998;95:1852-7.
[59] Apostolova G, Schweizer RA, Balazs Z, et al. Dehydroepiandrosterone inhibits the amplification of glucocorticoid action in adipose tissue. Am J Physiol Endocrinol Metab 2005;288:E957-64.
[60] Li H, Klein G, Sun P, et al. Dehydroepiandrosterone (DHEA) reduces neuronal injury in a rat model of global cerebral ischemia. Brain Res 2001;888:263-6.
[61] Li Z, Cui S, Zhang Z, et al. DHEA-neuroprotection and -neurotoxicity after transient cerebral ischemia in rats. J Cereb Blood Flow Metab 2009;29:287-96.
[62] Marklund N, Peltonen M, Nilsson TK, et al. Low and high circulating cortisol levels predict mortality and cognitive dysfunction early after stroke. J Intern Med 2004;256:15-21.