Original Article

Association of Plasma Dehydroepiandrosterone-Sulfate Levels with Endothelial Function in Postmenopausal Women with Coronary Risk Factors

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Age-related decline of plasma dehydroepiandrosterone-sulfate (DHEA-S) levels may be associated with the risk of cardiovascular disease in women. We investigated whether plasma DHEA-S levels are related to endothelial function in postmenopausal women with coronary risk factors. One hundred and fifteen postmenopausal women (mean age $\pm$ SD: 57 $\pm$ 5 years; range: 48–65 years) who underwent measurement of flow-mediated vasodilation (FMD) of the brachial artery using ultrasonography were enrolled. Plasma hormone levels were determined in the morning after a 14-h fast, and the relationship between hormone levels and FMD was analyzed. DHEA-S was significantly correlated with %FMD ($r=0.392$, $p<0.001$), while estradiol, total testosterone and cortisol were not. %FMD in the highest quartile of DHEA-S was 1.8-fold higher than that in the lowest quartile (5.3 $\pm$ 1.3 vs. 2.9 $\pm$ 2.0 [means $\pm$ SD], $p<0.01$). Multiple regression analysis revealed that DHEA-S was related to %FMD independent of age, body mass index, hypertension, hyperlipidemia, diabetes mellitus and smoking ($\beta=0.344$, $p<0.01$), and was itself independent of age, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, fasting plasma glucose and smoking ($\beta=0.291$, $p<0.05$). In conclusion, plasma DHEA-S levels were weakly but significantly related to endothelial function in postmenopausal women independent of other coronary risk factors, suggesting a protective effect of DHEA on the endothelium. (*Hypertens Res* 2008; 31: 69–74)

Key Words: endothelium, vasodilation, risk factor, man, nitric oxide

Introduction

Plasma levels of dehydroepiandrosterone-sulfate (DHEA-S), the most abundant circulating steroid that is secreted from the adrenal cortex, decline with advancing age in men and women (1, 2). The age-related decrease in DHEA-S concentrations has often been associated with the pathological processes of aging, such as osteoporosis, depression and

dementia (3, 4). A number of studies have investigated the link between DHEA-S and cardiovascular disease, though with inconsistent results (5–7). Among them, Haffner *et al.* have shown that low DHEA-S levels predicted ischemic heart disease mortality in diabetic women (8). The association of low DHEA-S levels with carotid artery atherosclerosis (9), obesity (10) and decreased diurnal blood pressure variability (11) also suggests a vasoprotective role of DHEA(-S) in women. Furthermore, experimental studies showing endothe-

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Table 1. Characteristics of Study Subjects (n=115)

Age, years	57±5	[48–65]
Body mass index, kg/m ²	23.3±4.0	[17.6–35.0]
Risk factors		
Hypertension, n (%)	35 (30)	
Hyperlipidemia, n (%)	71 (62)	
Diabetes mellitus, n (%)	20 (17)	
Current smoker, n (%)	17 (15)	
Hemodynamic and vascular measurements		
Systolic blood pressure, mmHg	123±17	[93–170]
Diastolic blood pressure, mmHg	70±10	[52–100]
%FMD	4.8±2.4	[0.0–12.7]
%NTG	14.0±4.5	[4.1–22.5]
Carotid IMT, mm	0.92±0.22	[0.46–1.45]
Blood chemistry and hormones		
Total cholesterol, mmol/L	5.72±1.01	[3.73–8.96]
HDL cholesterol, mmol/L	1.66±0.42	[0.77–2.87]
Triglycerides, mmol/L	1.18±0.65	[0.36–3.49]
Fasting plasma glucose, mmol/L	5.42±1.22	[4.00–10.43]
Hemoglobin A1c, %	5.5±1.0	[4.3–9.6]
Estradiol, pmol/L	35±26	[18–160]
Testosterone, nmol/L	0.85±0.43	[0.21–2.01]
DHEA-S, µmol/L	2.28±1.07	[0.37–5.02]
Cortisol, nmol/L	316±121	[110–728]

Values except risk factors are expressed as mean±SD [range]. %FMD, percent flow-mediated dilation of brachial artery; %NTG, percent nitroglycerin-induced dilation of brachial artery; IMT, intima-media thickness of common carotid artery; HDL, high-density lipoprotein; DHEA-S, dehydroepiandrosterone-sulfate.

lium-dependent (12) and -independent (13) vasodilating effects of DHEA(-S) led us to hypothesize that postmenopausal women with low plasma DHEA-S levels would have impaired vasomotor function.

To test this hypothesis, we conducted a cross-sectional survey of 115 postmenopausal women by examining flow-mediated dilation (FMD) of the brachial artery and plasma sex hormones, and showed that low DHEA-S levels were associated with endothelial dysfunction.

Methods

Subjects

One hundred and fifteen postmenopausal women who underwent examination of vasomotor function of the brachial artery and intima-media thickness (IMT) of the carotid artery in our department were enrolled. The subjects were referred to our department to check their cardiovascular disease or risks. All of them were in chronic stable condition. A history was taken, and physical examination and laboratory tests were performed in all subjects. Subjects with a history of cardiovascu-

lar disease, including stroke, coronary heart disease, congestive heart failure and peripheral arterial disease, malignancy, overt endocrine disease or administration of steroid hormones, were excluded. The postmenopausal status of each subject was confirmed by the fact that at least 12 months had passed since her last menses and by the measurement of follicular stimulating hormone and estradiol. The characteristics of the study subjects are shown in Table 1.

Seventy-three percent of the subjects had one or more classical coronary risk factors, such as hypertension, hyperlipidemia, diabetes mellitus or current smoking. Hypertension, hyperlipidemia and diabetes mellitus were considered to be present based on the published diagnostic criteria (14–16) or if the subjects were taking any medications for these diseases. Eighty-six percent of the hypertensive subjects were treated: 75% with calcium antagonists, 19% with angiotensin-converting enzyme inhibitors, 10% with diuretics and 7% with β blockers. Eighty-six percent of the hyperlipidemic subjects were treated with statins, and 75% of the diabetic subjects were treated with oral hypoglycemic agents. None of the study subjects were taking nitrates. Each subject gave written informed consent before enrollment in this study. The study protocol was approved by the ethics committee of the Graduate School of Medicine, the University of Tokyo.

Vascular Measurement

Vasomotor function of the brachial artery was evaluated using an ultrasound machine according to the method described previously (17). Briefly, endothelium-dependent flow-mediated vasodilation (%FMD) was measured as the maximal percent change of the vessel diameter after reactive hyperemia. The subjects were examined in the morning after a 14-h overnight fast with no medication. Subsequently, endothelium-independent nitroglycerin-induced vasodilation (%NTG) was measured as the maximal percent change of the vessel diameter after sublingual administration of nitroglycerin spray (0.3 mg; Toa Eiyo Co., Tokyo). Carotid IMT was evaluated using an ultrasound machine as described previously (17).

Plasma Hormones

Blood sampling was performed in the morning of the vascular measurement after a 14-h overnight fast, and plasma was stored at -80°C until assay. Plasma estradiol, testosterone (total testosterone), DHEA-S and cortisol concentrations were determined using sensitive radioimmunoassays by a commercial laboratory (SRL Inc., Tokyo, Japan). The intra-assay coefficients of variation for these measurements were less than 5%.

Because plasma was deep-frozen for 3–7 years, we checked the change in titers using the stored samples, which had been measured at sampling 5–7 years before. The Pearson's correlation coefficients between the two measurements were 0.965

Table 2. Pearson's Correlation Coefficients between Age, Vascular Measurements and Plasma Hormones

	Age	%FMD	Carotid IMT
Estradiol	-0.108	0.041	-0.136
Testosterone	0.061	0.019	-0.088
DHEA-S	-0.198*	0.392 [†]	-0.187*
Cortisol	0.074	0.140	0.064

%FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery. [†] $p < 0.001$, * $p < 0.05$.

for estradiol ($n=34$), 0.976 for testosterone ($n=20$), 0.991 for DHEA-S ($n=15$) and 0.937 for cortisol ($n=16$), indicating that there was no significant change in plasma titers in our frozen samples.

Data Analysis

Pearson's simple correlation coefficients between age, vascular measurements and plasma hormones were determined. Standardized regression coefficients from multiple regression analysis of vascular measurements in relation to age, coronary risk factors and plasma hormones were determined. Differences between the groups were analyzed using one-factor ANOVA, followed by Newman-Keuls' test. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using the SPSS ver. 11.0 software.

Results

Changes in Plasma Hormones and Vascular Measurements According to Age and Coronary Risk Factors

Plasma levels of DHEA-S declined with age, while those of estradiol, testosterone and cortisol did not significantly change according to age (Table 2). %FMD decreased slightly but not significantly ($r = -0.166$, $p = 0.08$), while carotid IMT increased significantly ($r = 0.337$, $p < 0.01$) with advancing age.

The subjects with hypertension, hyperlipidemia or diabetes showed impaired %FMD compared to those without these diseases (hypertension, 4.0 ± 2.4 vs. 5.1 ± 2.2 ; hyperlipidemia, 4.3 ± 2.3 vs. 5.6 ± 2.2 ; diabetes mellitus, 4.0 ± 2.8 vs. 5.2 ± 2.2 ; $p < 0.05$). %FMD in the patients taking antihypertensive agents, statins or hypoglycemic agents was comparable to or smaller than that in the patients without medical agents (hypertension, 4.0 ± 2.5 vs. 4.3 ± 2.3 , n.s.; hyperlipidemia, 4.1 ± 2.2 vs. 5.1 ± 2.4 , n.s.; diabetes, 3.5 ± 2.7 vs. 6.5 ± 1.5 , $p < 0.05$), suggesting that the favorable effects of medical treatment on endothelial function, if present, might have been lost in patients with a long history of coronary risk factors. In contrast, none of the plasma hormones were significantly

Table 3. Age-Adjusted Regression Coefficients between Vascular Measurements and Plasma Hormones

	%FMD	Carotid IMT
Estradiol	0.023	-0.098
Testosterone	0.029	-0.117
DHEA-S	0.366 [§]	-0.146
Cortisol	0.156	-0.062

%FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery. Standardized regression coefficients by multiple regression analyses with %FMD or carotid IMT as a dependent variable and age and each of the hormones as independent variables are shown. [§] $p < 0.01$.

changed according to coronary risk factors or medications (data not shown).

Relationship between Plasma Hormones and Vascular Measurements

First, simple correlation coefficients between plasma hormones and vascular measurements were determined. As shown in Table 2, %FMD was positively correlated with DHEA-S, whereas carotid IMT was negatively correlated with DHEA-S. There was no significant correlation between vascular measurements and other steroid hormones.

Next, age-adjusted regression coefficients were determined, because age was correlated with some of the hormones and vascular measurements, as mentioned above. The results showed that none of the hormones were significantly related to carotid IMT (Table 3). In contrast, DHEA-S was significantly related to %FMD, independent of age. Furthermore, the age-adjusted regression coefficients between DHEA-S and %FMD were 0.374 in the subjects with no coronary risk factor or medication ($p < 0.05$, $n = 31$) and 0.399 in those with coronary risk factors ($p < 0.05$, $n = 84$), most of whom were taking some medications.

Finally, multiple regression analyses were performed to exclude the influence of coronary risk factors on the relationship between hormones and %FMD. As shown in Table 4, DHEA-S was related to %FMD, independent of age, body mass index, hypertension, hyperlipidemia, diabetes mellitus and current smoking (Model 1), and were independent of age, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, fasting plasma glucose and current smoking (Model 2). Furthermore, the relationship between DHEA-S and %FMD was significant after addition of carotid IMT to the model (Model 3), suggesting that the relationship was not attributable to the effect of DHEA(-S) on the development of subclinical atherosclerosis. Also, the statistical result was unchanged after addition of nitroglycerin-induced dilation of the brachial artery to the model (Model 4), indicating that DHEA-S is related to endothelial function independent of arterial compliance. Estradiol,

Table 4. Regression Coefficients between %FMD and Plasma Hormones Adjusted for Coronary Risk Factors

	Model 1	Model 2	Model 3	Model 4
Estradiol	0.065	0.042	0.025	0.094
Testosterone	0.036	0.052	0.028	0.148
DHEA-S	0.344 [§]	0.291*	0.263*	0.250*
Cortisol	0.150	0.127	0.099	0.110

Standardized regression coefficients by multiple regression analyses with %FMD as a dependent variable and coronary risk factors (covariates used in each analysis are listed below) and each of the hormones as independent variables are shown. [§] $p < 0.01$, * $p < 0.05$. Model 1: age, body mass index, hypertension, hyperlipidemia, diabetes mellitus, and current smoking. Model 2: age, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose and current smoking. Model 3: Model 2 plus carotid intima-media thickness. Model 4: Model 2 plus percent nitroglycerin-induced dilation of brachial artery. %FMD, percent flow-mediated dilation of brachial artery.

testosterone and cortisol were not significantly related to %FMD in similar multivariate analyses (Table 4). As shown in Fig. 1, %FMD showed a stepwise increment according to quartiles of DHEA-S, and %FMD in the highest quartile of free testosterone was 1.8-fold higher than that in the lowest quartile (5.3 ± 1.3 vs. 2.9 ± 2.0 , $p < 0.01$).

All the variables except hormones were mandatorily incorporated into the multiple regression models shown in Table 4, because they are classical coronary risk factors. In forward and backward stepwise models including age, body mass index, hypertension, hyperlipidemia, diabetes mellitus, smoking and the four steroid hormones, DHEA-S, hypertension and hyperlipidemia ($\beta = 0.390$, -0.224 and -0.295 , respectively) were selected as significant variables. Next, in forward and backward stepwise models including age, body mass index, systolic blood pressure, total cholesterol, HDL cholesterol, fasting plasma glucose, smoking and the four steroid hormones, only DHEA-S and glucose were selected as significant variables ($\beta = 0.330$ and -0.325 , respectively). Taken together, these results show that DHEA-S was consistently related to %FMD in the multivariate analyses.

Discussion

In this cross-sectional study, plasma DHEA-S levels were positively correlated with %FMD, a surrogate marker of clinical atherosclerosis that reflects endothelial function (18, 19). Adjustment for potential confounders such as age, coronary risk factors and nitroglycerin-induced dilation of the brachial artery had little influence on the results. These results suggest that endogenous DHEA(-S) plays a vasoprotective role in postmenopausal women.

The majority of DHEA exists as the sulfated form (DHEA-S) in circulation, and its concentration is reported to be 100-

Fig. 1. Percent flow-mediated dilation of the brachial artery (%FMD) according to quartiles of serum dehydroepiandrosterone-sulfate (DHEA-S). Values are expressed as the means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. Q1.

to 500-fold higher than that of testosterone and 1,000- to 10,000-fold higher than that of estradiol (4), suggesting an important physiologic role of DHEA(-S). Plasma DHEA-S levels do not drop during the menopause transition, but decline with aging (1, 2, 20). A recent study has shown a positive correlation between DHEA-S levels and the duration of reactive hyperemia, another less specific marker of endothelial function compared to FMD, in young women (26 $\pm$ 6 years old, $n = 27$) with polycystic ovary syndrome (21). In postmenopausal women, however, a correlation between DHEA-S levels and endothelial vasomotor function has not yet been reported. On the other hand, two studies have reported the effects of DHEA supplementation on FMD in postmenopausal women (12, 22). Williams *et al.* (12) showed a significant increase in FMD after the 12-week administration of DHEA (50 mg/day) in healthy postmenopausal women, while Silvestri *et al.* (22) observed no effect using the same dose of DHEA for 4 weeks in postmenopausal women with increased cardiovascular risk. In middle-aged men, it has been reported that low-dose DHEA supplementation (25 mg/day) for 12 weeks improved FMD (23). Experimental studies have also demonstrated the effects of DHEA on endothelial proliferation (12) and endothelial nitric oxide synthase (24), further supporting the notion that DHEA(-S) has a protective effect on the endothelium.

At this moment, intrinsic receptors for DHEA(-S) have not been identified. Thus, DHEA(-S) may exert its activity after conversion to androgens or estrogens *via* androgen or estrogen receptors, although in previous reports neither an androgen receptor antagonist nor an estrogen receptor antagonist blocked some of the *in vitro* effects of DHEA on endothelial cells (12, 24). Testosterone supplementation in men (25, 26) and estrogen replacement in postmenopausal women (27, 28) improves endothelial vasomotor function. Activation of endothelial nitric oxide synthase and stimulation of nitric oxide production *via* androgen and estrogen receptors (29) might be attributable to the effect of DHEA(-S) on FMD. For

this reason, we added testosterone and estradiol into the analysis. Plasma testosterone and estradiol levels, however, were correlated neither with %FMD nor with DHEA-S (data not shown), although tissue conversion of DHEA(-S) into androgens or estrogens might play a role. Further studies are needed to clarify the molecular mechanism underlying the association between DHEA-S and FMD.

The incidence of cardiovascular disease is lower in premenopausal women than in men of the same age, and increases after menopause (30), indicating the cardioprotective effect of endogenous estrogen in premenopausal women. Accordingly, estrogen replacement therapy had been expected to prevent cardiovascular disease in postmenopausal women, with disappointing results in randomized controlled trials (31). Alternatively, there has been a resurgence of interest in DHEA as an anti-aging hormone, and DHEA has been widely used in this context in the USA despite the dearth of information on its physiologic and pharmacologic effects (4). Although the present study indicated a cardioprotective effect of endogenous DHEA(-S), we do not suggest that DHEA be prescribed for postmenopausal women with coronary risk factors until the efficacy and safety of DHEA supplementation has been established. At present, life-style modification such as exercise is preferable, because exercise can increase both DHEA levels (32) and nitric oxide production (33) in elderly women.

The results of this study do not imply that DHEA(-S) has favorable effects on endothelial function in men. In fact, the plasma DHEA-S level was not related to FMD in middle-aged men after adjustments for age and coronary risk factors (our unpublished observation). Consequently, the meaning of DHEA-S in association with endothelial function may be different between men and women. Gender differences in other steroid hormones and steroid hormone receptor expression in arteries (34, 35) might play a mechanistic role.

This study had some limitations. First, since this was a cross-sectional study, the causal relationship between DHEA-S and vasomotor function could not be determined. Endothelial dysfunction might be associated with a reduction in blood flow of the adrenal glands, leading to decreased hormone production. Longitudinal studies following the subjects might add some information in this regard. Secondly, a population bias was possible. Three-quarters of the study subjects had one or more of the coronary risk factors, and most of them were taking medications. Consequently, the results might have been different if subjects homogeneous in terms of health status and medications had been studied, although the association of DHEA-S with %FMD was consistent in the subjects with or without coronary risk factors and medications, as mentioned above. The subjects with a history of cardiovascular disease were excluded from the study, because they might have low plasma DHEA-S levels as a result of advanced atherosclerosis and reduced blood flow of the adrenal glands, although the inclusion of those subjects did not fundamentally alter the statistical results (data not shown).

In summary, low plasma DHEA-S levels were associated with endothelial dysfunction in postmenopausal women independent of other risk factors, suggesting a protective effect of DHEA(-S) on the endothelium. This finding provides a mechanistic insight into the role of endogenous DHEA in the development of cardiovascular disease in postmenopausal women.

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