

Serum DHEA-S Level Is Associated with the Presence of Atherosclerosis in Postmenopausal Women with Type 2 Diabetes Mellitus

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Abstract. We investigated the relationship between serum dehydroepiandrosterone-sulfate (DHEA-S) and insulin-like growth factor-I (IGF-I) to various parameters for atherosclerosis in type 2 diabetes. The levels of DHEA-S and IGF-I are known to decrease with aging and thereby might be associated with an increased risk of cardiovascular disease. One hundred forty-eight men and 106 postmenopausal women with type 2 diabetes were assessed in a cross-sectional study. Serum DHEA-S and IGF-I concentrations were measured and brachial-ankle pulse wave velocity (baPWV) and ultrasonographically-evaluated intima-media thickness (IMT) were assessed. Although simple regression analysis showed that log(DHEA-S) and IGF-I in men and log(DHEA-S) in women were significantly and inversely correlated with baPWV and IMT, only log(DHEA-S) in women was still significantly and inversely correlated with these atherosclerotic parameters after multiple regression analysis was adjusted for age, duration of diabetes, BMI, HbA_{1c}, systolic blood pressure, LDL-Cholesterol (C), serum creatinine, and smoking (Brinkman index). Serum DHEA-S level seemed to be associated with atherosclerosis in diabetic postmenopausal women independent of age, body stature, diabetic status, and other atherosclerotic risk factors, and might be a useful addition to other parameters for assessing the risk of atherosclerosis in this population.

Key words: DHEA-S, IGF-I, Type 2 diabetes mellitus, Atherosclerosis, Postmenopausal women

(Endocrine Journal 55: 667–675, 2008)

AGING hormones have recently attracted widespread attention due to their beneficial anti-aging effects. Both dehydroepiandrosterone (DHEA) and growth hormone (GH)-insulin-like growth factor (IGF)-I axis are known to decrease during aging, called adrenopause and somatopause, respectively. Accumulating evidence has shown that DHEA and IGF-I themselves seem to exert protective effects against atherosclerosis [1–4], diabetes [5, 6], and osteoporosis [7, 8], which become prevalent during aging. Thus, maintaining DHEA and IGF-I in the circulation at sufficient levels might be important for preventing these aging-related

diseases.

Recent studies have indicated that decreases in circulating DHEA and IGF-I were related to atherosclerosis and plaque progression, resulting in cerebro- and cardiovascular diseases (CVD). DHEA-sulfate (S), which is converted to the active form DHEA in a linear manner, represents the circulating hormonal pool of DHEA and is a good marker for DHEA availability. Decreased serum levels of DHEA-S have been found to be associated with coronary heart disease [9], and an inverse correlation between DHEA-S and cardiovascular mortality has been reported in men and postmenopausal women [3, 4], while other studies failed to find these associations [10]. Produced by the adrenal gland DHEA is known to be a weak androgen contributing to androgenicity mainly after peripheral conversion to more potent androgens, testosterone and dihydrotestosterone. In postmenopausal women, adre-

Received: November 21, 2007

Accepted: March 4, 2008

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nal DHEA functions as a sex hormone in compensation for a decline in estradiol, and is more potently associated with CVD than in men and premenopausal women [11]. On the other hand, previous studies have demonstrated that serum IGF-I level was decreased in patients with CVD [12, 13], while other studies have found no correlation [14]. IGF-I seems to exert complex effects on vascular cells. IGF-I stimulates vascular smooth muscle cell (VSMC) proliferation [15] as well as migration to promote neointimal formation [16], whereas IGF-I might serve to protect against plaque instability and rupture by suppressing VSMC apoptosis and increasing VSMC elastogenesis [17].

DHEA and IGF-I are also involved in glucose metabolism. DHEA is known to improve insulin resistance [6] and to increase insulin secretion from the pancreas [18]. Several reports have indicated an association between diabetic control and the level of DHEA(-S) [19, 20]. On the other hand, IGF-I is the second most powerful naturally-occurring peptide with glucose-lowering effects after insulin [21]. IGF-I increases insulin sensitivity and peripheral glucose uptake, decreases hepatic glucose production, and improves the lipid profile [5, 22]. Lowered serum IGF-I level was suggested to predict worsening of insulin-mediated glucose uptake in older people [23]. In patients with type 2 diabetes, serum IGF-I levels were dependent on the degree of glucose control, with near normal IGF-I levels in well-controlled diabetes, whereas they tended to decrease in poorly controlled diabetes [24, 25]. Thus, circulating DHEA and IGF-I levels seem to be affected by diabetic status and might be associated with atherosclerosis in diabetic patients differently than in non-diabetic subjects.

In this study, in order to address roles of DHEA and IGF-I in atherosclerosis of diabetic patients, we measured serum levels of DHEA-S and IGF-I in Japanese men and postmenopausal women with type 2 diabetes, and investigated the association of these hormone levels to atherosclerosis and other cardiovascular risk factors, as well as the presence of cardiovascular diseases (CVD) and microvascular complications. Atherosclerosis was assessed by brachial-ankle pulse wave velocity (baPWV) and ultrasonographically-evaluated intima-media thickness (IMT).

Subjects and Methods

Subjects

The subjects in this study were 148 Japanese men and 106 postmenopausal Japanese women with type 2 diabetes aged 20–82 and 47–85 years (mean 59 and 63 years, respectively), respectively. Subjects visited Shimane University Hospital for an education, evaluation or treatment of diabetes. Clinical characteristics of subjects are shown in Table 1. Subjects agreed to participate in the study and gave their informed consent. This study was approved by the review board of our institution. All women had been without spontaneous menses for more than one year. None of the subjects had hepatic or renal dysfunction or nutritional derangements that might cause changes in hormonal metabolism. Subjects exhibiting macroalbuminuria

Table 1. Clinical characteristics of patients

characteristic	Male	Female
n	148	106
Age (years)	59 ± 14	65 ± 10*
Duration of diabetes (years)	10.7 ± 9.2	12.3 ± 10.1
Height (cm)	165.4 ± 7.6	150.8 ± 5.9*
Weight (kg)	65.9 ± 14.8	56.5 ± 11.5*
BMI (kg/m ²)	24.0 ± 4.2	24.9 ± 4.7
SBP (mmHg)	130 ± 17	128 ± 21
DBP (mmHg)	78 ± 11	74 ± 13*
FPG (mg/dl)	169 ± 63	170 ± 61
HbA _{1c} (%)	9.0 ± 2.7	9.1 ± 2.4
C-peptide (ng/ml)	1.7 ± 1.0	1.6 ± 0.7
Total Cholesterol (mg/dl)	196 ± 44	200 ± 41
Triglyceride (mg/dl)	138 ± 98	119 ± 63*
HDL-Cholesterol (mg/dl)	51 ± 15	58 ± 17
LDL-Cholesterol (mg/dl)	118 ± 36	119 ± 35
BUN (mg/dl)	14.9 ± 4.0	15.5 ± 4.1
Creatinine (mg/dl)	0.77 ± 0.16	0.62 ± 0.16
Urine Albumin (mg/day)	57.8 ± 146.1	43.1 ± 97.8
DHEA-S (ng/ml)	1347 ± 989	699 ± 384*
IGF-I (ng/ml)	154 ± 54	128 ± 48*
baPWV (m/s)	14.3 ± 2.5	14.9 ± 2.5
IMT-Max (mm)	2.1 ± 1.2	1.9 ± 0.9
IMT-mean (mm)	1.3 ± 0.4	1.2 ± 0.4
Brinkman index	614 ± 673	40 ± 98

BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, FPG; fasting plasma glucose, BUN; blood urea nitrogen, DHEA-S; dehydroepiandrosterone sulfate, IGF-I; insulin-like growth factor-I, baPWV; brachial-ankle pulse wave velocity, IMT; intima-media thickness. *, $p < 0.05$ compared with male.

(>300 mg/day) were excluded from this study. Forty-six patients had received insulin treatment, 143 patients had taken oral hypoglycemic agents, and 62 patients had taken HMG-CoA reductase inhibitors for dyslipidemia treatment. Seventy-seven patients had taken calcium antagonists, 59 patients had taken angiotensin converting enzyme inhibitors or angiotensin II receptor blockers for hypertension treatment, and 41 patients have taken aspirin for atherosclerosis treatment. All subjects were free of drugs known to influence sex hormone concentrations until the time of the present study. Neuropathy was diagnosed by the chief physicians with subjective symptoms, the attenuation of Achilles' tendon reflex, and the sensory disturbance of foot. Retinopathy was evaluated by ophthalmologists at our hospital. Nephropathy was estimated by daily urine albumin (>30 mg/day). CVD was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history.

Arterial stiffness measurement

PWV was measured using the VaSera VS-1000 (Fukuda Denshi, Tokyo, Japan), an automated recording device that calculates the time delay between two pulse waves recorded simultaneously. Briefly, the subject was examined in the supine position, with a volume plethysmographic sensor in cuffs on both the brachia and ankles. After 15 minutes of rest, the subject's volume pulse form was detected, and time intervals (T) between the wave front of the heart and that of the ankles were calculated. The distance (L) between the heart and sampling points was calculated automatically according to the subject's height. baPWV was calculated according to the following formula: $\text{baPWV} = L/T$ ($L = L_a - L_b$, where L_a is the path length from the heart to ankle, and L_b is the path length from the heart to brachium) [26]. In the present study, the measurement of baPWV was performed on a different occasion from the blood collection so that the participant would be mentally relaxed. The mean of the right and left baPWV were used during analysis because there was a strong significant correlation between right and left baPWV ($r = 0.944$, $p < 0.0001$).

Ultrasonographic measurement of carotid intima-media thickness

B-mode ultrasonographic imaging of the carotid

artery was performed using HDI 5000 (Philips, Tokyo, Japan), a high-resolution, real-time ultrasonograph with a 7.5-MHz transducer. All scans were performed by two trained sonographers who remained unaware of each other's data. The scanning of extracranial carotid arteries in the neck was conducted bilaterally at longitudinal projections and at the transverse projection for measurement of IMT. Each carotid wall and segment was explored to identify the thickest intima-medial sites. IMT was measured as the distance between the lumen-intima interface and the media-adventitia interface on the B-mode image. Four segments were identified on each side: the distal 1.5 cm of the common carotid proximal to the bifurcation (S1), the bifurcation itself (S2), the proximal 1.5 cm of the internal carotid artery (S3), and more proximal 1.5 cm of S3 (S4). To quantify carotid artery wall thickness, the following measures were chosen: mean of the maximum wall thickness of 8 sites (S1–4; far wall of the left and right sides) for IMT-mean; the maximum of 8 sites IMT for IMT-Max.

Biochemical measurements

After overnight fasting, serum was collected. Biochemical markers were measured by standard biochemical methods. Serum IGF-I and DHEA-S were measured by radioimmunoassay (RIA). Hemoglobin A1c (HbA1c) was determined by high performance liquid chromatography (HPLC). As a marker of diabetic nephropathy, albumin excretion was measured in 24 h urine. Serum C-peptide was measured by enzyme linked immunosorbent assay (ELISA). Total cholesterol (TC), triglyceride (TG), and HDL-Cholesterol (C) were evaluated using an enzymatic method. LDL-C was calculated by the formula $[\text{TC} - (\text{HDL-C} + \text{TG}/5)]$.

Statistical analysis

Data were expressed as mean $\pm$ SD. Since serum DHEA-S level showed a markedly skewed distribution, DHEA-S was transformed to its logarithms before simple or multiple regression analysis. Simple, multiple, and logistic regression analysis were performed using the statistical computer program Stat-View (Abacus Concepts, Berkeley, CA). $P < 0.05$ was considered to be significant.

Results

Serum levels of DHEA-S and IGF-I with various variables

Simple regression analysis between the logarithm of serum DHEA-S level [log(DHEA-S)] or serum IGF-I levels versus various demographic variables is summarized in Table 2. Log(DHEA-S) was significantly affected by age, duration of diabetes, and body mass index (BMI) in both sexes, and serum IGF-I level was significantly correlated with age in men. Thus, the relationships between log(DHEA-S) or serum IGF-I level versus blood pressure and biochemical parameters were examined by multiple regression analysis adjusted for age, duration of diabetes, and BMI (Table 3). In male patients, log(DHEA-S) was significantly and positively correlated with LDL-C, and IGF-I was significantly and inversely correlated with HDL-C, FPG,

and HbA1C, and positively with LDL-C. In female patients, log(DHEA-S) was not significantly correlated with any variables, while IGF-I was significantly and inversely correlated with diastolic blood pressure (DBP) and fasting plasma glucose (FPG), and positively with HDL-C.

Relationship between serum levels of DHEA-S and IGF-I

Simple regression analysis showed that log(DHEA-S) was significantly correlated with IGF-I in both men and women ($r = 0.510$, $p < 0.0001$ and $r = 0.276$, $p = 0.0059$, respectively). Also, multiple regression analysis adjusted for age, duration of diabetes, and BMI also revealed these correlations ($r = 0.385$, $p < 0.0001$ and $r = 0.251$, $p = 0.0342$, respectively).

Table 2. The correlations between serum DHEA-S or IGF-I and demographic variables

variable	Male				Female			
	log(DHEA-S)		IGF-I		log(DHEA-S)		IGF-I	
	r	p	r	p	r	p	r	p
Age	-0.581	<0.0001	-0.438	<0.0001	-0.385	<0.0001	-0.172	0.0778
Duration of diabetes	-0.257	0.0031	-0.125	0.1400	-0.385	0.0001	-0.100	0.3233
Height	0.414	<0.0001	0.291	0.0004	0.173	0.0884	0.108	0.2709
Weight	0.401	<0.0001	0.231	0.0054	0.268	0.0075	0.188	0.0538
BMI	0.293	0.0006	0.153	0.0673	0.227	0.0248	0.163	0.0949

Table 3. The correlations between serum DHEA-S or IGF-I versus blood pressure and biochemical parameters

variable	Male				Female			
	log(DHEA-S)		IGF-I		log(DHEA-S)		IGF-I	
	r	p	r	p	r	p	r	p
SBP	0.027	0.7165	-0.091	0.2422	-0.102	0.2865	-0.078	0.4427
DBP	-0.019	0.7993	0.057	0.4704	-0.013	0.8946	-0.221	0.0318
T-cho	0.142	0.0828	0.108	0.1995	0.087	0.3776	0.106	0.3156
TG	0.070	0.3974	0.045	0.6032	-0.175	0.0772	-0.095	0.3692
HDL-C	-0.115	0.1328	-0.196	0.0130	0.192	0.0513	0.240	0.0196
LDL-C	0.172	0.0269	0.185	0.0206	0.067	0.4974	0.034	0.7497
BUN	0.110	0.1424	0.145	0.0636	-0.080	0.4507	0.164	0.1457
Crea	0.133	0.0761	0.074	0.3448	-0.121	0.2469	0.116	0.2943
uAlb	-0.144	0.0577	-0.050	0.5296	-0.127	0.2054	-0.125	0.2548
FPG	-0.101	0.1768	-0.192	0.0131	-0.047	0.6252	-0.264	0.0113
HbA _{1c}	-0.124	0.1123	-0.220	0.0063	-0.117	0.2144	-0.141	0.1617
C-peptide	0.065	0.4646	0.107	0.2589	0.016	0.8908	0.138	0.2434

Multiple regression analysis adjusted for age, duration of diabetes and BMI was performed

Relationship between serum levels of DHEA-S or IGF-I versus baPWV and IMT

Simple regression analysis between baPWV and IMT versus log(DHEA-S) or IGF-I as well as other risk factors are shown in Table 4. baPWV, IMT-Max, and IMT-mean were significantly and inversely correlated with both log(DHEA-S) and IGF-I in male patients, and with log(DHEA-S) but not with IGF-I in female patients. These atherosclerotic parameters were also significantly associated with established cardiovascular risk factors such as age, duration of diabetes, BMI, HbA_{1c}, systolic blood pressure (SBP), LDL-C, serum creatinine, and smoking (Brinkman

index). Next, in order to investigate whether or not log(DHEA-S) and IGF-I were related to baPWV and IMT independent of these cardiovascular risk factors, multiple regression analysis adjusted for these risk factors were performed between them (Table 5). In postmenopausal women, log(DHEA-S) but not IGF-I was significantly and inversely correlated with IMT-Max and IMT-mean, and tended to be correlated with baPWV independent of cardiovascular risk factors. Log(DHEA-S) was still significantly and inversely correlated with IMT-mean, and tended to be correlated with baPWV and IMT-Max after additional adjustment for serum IGF-I (baPWV; $r = -0.146$, $p = 0.1166$, IMT-Max; $r = -0.220$, $p = 0.0619$; IMT-mean;

Table 4. The correlations between baPWV or IMT and demographic variables

variable	Male						Female					
	baPWV		IMTmax		IMTmean		baPWV		IMTmax		IMTmean	
	r	p	r	p	r	p	r	p	r	p	r	p
Age	0.565	<0.0001	0.503	<0.0001	0.540	<0.0001	0.559	<0.0001	0.390	<0.0001	0.436	<0.0001
Duration of diabetes	0.322	<0.0001	0.361	<0.0001	0.388	<0.0001	0.286	0.0045	0.315	0.0014	0.279	0.0074
Height	-0.294	0.0003	-0.265	0.0011	-0.311	0.0002	-0.345	0.0004	-0.225	0.0206	-0.074	0.8189
Weight	-0.193	0.0191	-0.303	0.0002	-0.274	0.0013	-0.215	0.0298	-0.244	0.0119	-0.022	0.9471
BMI	-0.101	0.2229	-0.251	0.0021	-0.191	0.0258	-0.081	0.4174	-0.172	0.0786	-0.0067	0.5145
SBP	0.474	<0.0001	0.259	0.0015	0.253	0.0030	0.333	0.0006	-0.151	0.1215	-0.059	0.5632
DBP	0.124	0.1337	0.029	0.7304	-0.071	0.4097	0.113	0.2564	0.031	0.7509	0.087	0.3966
FPG	0.069	0.4065	-0.094	0.2570	-0.126	0.1442	0.162	0.1046	0.079	0.4221	0.201	0.5307
HbA _{1c}	-0.007	0.9283	-0.052	0.5276	-0.073	0.3998	0.251	0.0119	0.134	0.1749	0.213	0.0382
C-peptide	0.000	0.9962	-0.232	0.0058	-0.182	0.0388	-0.041	0.6853	0.049	0.6229	-0.224	0.4844
Total Cholesterol	-0.155	0.0607	-0.109	0.1861	-0.021	0.8040	-0.193	0.0524	-0.168	0.0849	-0.432	0.1606
Triglyceride	-0.136	0.0998	-0.199	0.0155	-0.199	0.0359	-0.081	0.4186	-0.024	0.8069	-0.088	0.7863
HDL-Cholesterol	0.041	0.1240	-0.069	0.4032	-0.069	0.4224	0.054	0.5871	-0.065	0.5057	-0.130	0.2044
LDL-Cholesterol	-0.128	0.1763	0.002	0.9788	0.105	0.2230	-0.224	0.0236	-0.224	0.1151	-0.082	0.4266
BUN	0.095	0.2523	0.150	0.0680	0.189	0.0278	0.197	0.0474	0.310	0.0012	0.301	0.3417
Creatinine	0.185	0.0247	0.082	0.3213	0.138	0.1102	0.199	0.0444	0.240	0.0130	0.224	0.0273
Urine Albumin	0.114	0.1763	-0.003	0.9670	-0.005	0.9591	0.026	0.7952	0.060	0.5491	-0.050	0.6295
Brinkman index	-0.099	0.2356	0.210	0.0108	0.153	0.0775	-0.093	0.3526	-0.003	0.9782	0.050	0.6234
Log(DHEA-S)	-0.264	0.0021	-0.243	0.0047	-0.244	0.0059	-0.460	<0.0001	-0.375	0.0001	-0.378	0.0002
IGF-I	-0.290	0.0004	-0.233	0.0049	-0.221	0.0110	-0.174	0.0797	-0.135	0.1674	-0.128	0.2117

Table 5. The correlations between serum DHEA-S or IGF-I versus baPWV and IMT

	Male				Female			
	log(DHEA-S)		IGF-I		log(DHEA-S)		IGF-I	
	r	p	r	p	r	p	r	p
baPWV	0.114	0.1346	-0.001	0.9885	-0.140	0.1211	0.004	0.9625
IMT-Max	0.099	0.2751	-0.011	0.8924	-0.227	0.0485	-0.081	0.3972
IMT-mean	0.111	0.2335	0.048	0.5645	-0.278	0.0175	-0.076	0.4558

Multiple regression analysis was performed between log(DHEA-S) or IGF-I versus baPWV and IMT adjusted for age, duration of diabetes, BMI, HbA_{1c}, SBP, LDL-C, serum creatinine, and Brinkman index.

Table 6. Serum levels of DHEA-S and IGF-I compared between subjects with and without cardiovascular disease and diabetic microvascular complications.

		n	DHEA-S		IGF-I	
		Yes/No	Yes	No	Yes	No
Male	Cardiovascular disease	24/124	973.8 ± 864.4	1416.0 ± 999.0	133.3 ± 47.3	157.5 ± 55.0*
	Myocardial infarction	14/134	859.0 ± 717.9	1399.1 ± 1002.4	126.9 ± 46.8	156.4 ± 54.5
	Cerebral infarction	13/135	1023.8 ± 1008.8	1375.5 ± 986.7	130.1 ± 51.8	155.8 ± 54.3
	Neuropathy	86/62	1148.9 ± 832.4	1613.8 ± 1121.6*	148.2 ± 52.5	161.5 ± 56.6
	Retinopathy	51/97	1232.7 ± 953.9	1400.5 ± 1006.4	143.2 ± 48.4	158.8 ± 57.0
	Nephropathy	49/99	1546.8 ± 880.8	1255.3 ± 1026.7	157.4 ± 55.4	151.6 ± 54.1
Female	Cardiovascular disease	8/98	624.5 ± 352.7	703.4 ± 387.6	119.6 ± 34.0	128.4 ± 49.4
	Myocardial infarction	4/102	595.7 ± 349.7	701.9 ± 386.7	117.8 ± 35.4	128.1 ± 48.9
	Cerebral infarction	4/102	653.3 ± 431.6	700.0 ± 385.3	121.5 ± 37.8	127.9 ± 48.8
	Neuropathy	71/35	639.3 ± 354.3	820.9 ± 419.7*	125.2 ± 48.3	132.8 ± 48.7
	Retinopathy	43/63	565.1 ± 294.1	789.2 ± 416.0*	124.5 ± 53.1	129.3 ± 42.9
	Nephropathy	24/82	602.2 ± 329.9	728.2 ± 396.9	125.9 ± 51.3	128.2 ± 47.7

* significance of difference between the presence of complications, $p < 0.05$.

$r = -0.272$, $p = 0.0233$, respectively). On the other hand, there were no significant correlations between either of the hormones and any atherosclerotic parameters in men.

Comparison of serum levels of DHEA-S and IGF-I between patients with and without CVD or diabetic microvascular complications

Next, we compared serum levels of DHEA-S and IGF-I between patients with and without CVD or diabetic microvascular complications (Table 6). Serum IGF-I level was lower in men with CVD than without CVD ($p = 0.0461$), and serum DHEA-S level was lower in men with neuropathy than without neuropathy ($p = 0.0067$). Serum DHEA-S level was lower in postmenopausal women with neuropathy than without neuropathy ($p = 0.0275$). However, after being adjusted for age, duration of diabetes, BMI, HbA1C, SBP, LDL-C, serum creatinine and Brinkman index, logistic regression analysis revealed no significant association of these hormone levels with the presence of CVD or microvascular complications (data not shown).

Discussion

The incidence of CVD is approximately two or three times as high in patients with type 2 diabetes as in age-matched controls without diabetes [27, 28], and the risk of CVD increases with the duration of diabetes

and with age [29–31]. However, the overall outcome of the UKPDS has revealed that CVD could not be completely prevented by the common methods of blood glucose control [32], suggesting that other conventional cardiovascular risk factors such as plasma lipids, lipoproteins and hypertension are also involved in this excessive risk of developing atherosclerosis and CVD in individuals with type 2 diabetes [31, 33].

Increased arterial stiffness and carotid-artery intima-media thickness are known to be correlated with atherosclerosis and have recently been shown to be an independent marker of cardiovascular mortality [34–37]. Therefore, the measurement of PWV and the carotid artery IMT are clinically useful for predicting cardiovascular diseases by noninvasive methods. The present study showed that serum levels of DHEA-S were significantly and inversely correlated with baPWV and carotid artery IMT in postmenopausal women with type 2 diabetes independent of age, BMI, diabetic status, and other cardiovascular risk factors. Thus, decreasing serum DHEA-S would be specifically linked to atherosclerosis in diabetic postmenopausal women.

Bernini *et al.* have also showed that serum DHEA-S was inversely correlated with IMT in women, but this relationship was dependent on age [2]. The difference in age-dependency between their study and ours may be because about half of their subjects comprised premenopausal younger women. It is well known that the hormonal status of the women dramatically changes after menopause, where the relative contribution of the adrenal gland to total C19 steroid production is

known to increase in compensation for a loss of ovarian follicular androgen synthesis. Thus, the clinical effect of impaired adrenal androgen synthesis might be more profound in postmenopausal women than in younger women or men. On the other hand, diabetes patients have significantly lower plasma concentrations of DHEA than non-diabetic controls because of their low 17,20-lyase activity in the adrenal steroidogenic enzymes [19, 20]. Thus, the negative correlation between serum DHEA-S level and atherosclerosis found in this study might be more likely to occur in diabetic postmenopausal women than in any other population.

Fukui *et al.* reported that serum DHEA-S concentration was negatively associated with urinary albumin excretion [38] and carotid atherosclerosis determined by measurement of IMT [39] in men with type 2 diabetes, suggesting that the hormone concentration may partly explain higher CVD mortality in them. The results from our simple regression analysis (Tables 3 and 4) also corroborate their findings.

Our findings suggested that serum IGF-I itself was not involved in either atherosclerosis in patients with type 2 diabetes or the significant inverse correlation of serum DHEA-S level with atherosclerosis in diabetic postmenopausal women. However, previous studies have demonstrated that in type 2 diabetes IGF-I therapy as an adjunct to insulin could decrease cardiovascular events by approximately 22% during 12 months of follow-up after coronary balloon angioplasty [40]. Thus, further prospective studies are necessary to estimate the effect of IGF-I on CVD in diabetic patients.

This study has some limitations. First, the sample size was not large enough to make any definite conclusions. Although we demonstrated a significant inverse correlation of serum DHEA-S level with baPWV and IMT in diabetic postmenopausal women, we found no significant association of the hormone level with the presence of CVD in the group. This may be because only eight patients with CVD history were included in

our study. Second, we analyzed only subjects who visited Shimane University Hospital, a tertiary center, for evaluation or treatment of diabetes mellitus. Therefore, the patients enrolled in this study might have relatively severe states of the disorder, as shown in the moderately high background HbA1C level, and might not be representative of Japanese men and postmenopausal women with the disorder. Third, many subjects in this study had taken drugs for the treatments of diabetes, hypertension, and dyslipidemia. Previous studies indicated that insulin, metformin, or amlodipine could affect serum DHEA-S levels [41–43]. Although serum DHEA-S level was not significantly different between subjects with and without treatments in this study (data not shown), we have to consider these effects when evaluating the hormonal levels. Fourth, although we carefully eliminated the influence of diabetic status with statistical methods, we did not carry out a study on non-diabetic controls. Thus, it is unclear whether the usefulness of serum DHEA-S level for assessing the risk of atherosclerosis is specific to diabetic postmenopausal women, or is also applicable to their non-diabetic counterpart. Consequently, the assessment of larger numbers of patients is necessary to determine that the measurement of serum DHEA-S level can be used as a suitable tool for the diagnosis and therapeutic monitoring of atherosclerosis and CVD in diabetic postmenopausal women.

In conclusion, our study showed that serum DHEA-S level was significantly and inversely associated with atherosclerosis in postmenopausal women with type 2 diabetes, and that this finding was independent of age, body stature, diabetic status, cardiovascular risk factors, or serum IGF-I level. Several studies have shown that DHEA administration greatly improved a variety of endocrinologic, metabolic, and psychological parameters in both genders [44–46]. Prospective studies are needed to investigate whether or not DHEA supplementation clinically improves atherosclerosis in postmenopausal women with type 2 diabetes.

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