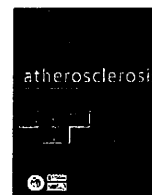


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Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors

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ABSTRACT

Objective: Recent epidemiological studies have found that testosterone deficiency is associated with higher mortality largely due to cardiovascular (CV) disease in community-dwelling older men. We investigated whether a low plasma testosterone level could predict cardiovascular events in middle-aged Japanese men with coronary risk factors.

Methods: One hundred and seventy-one male outpatients (30–69 years old, mean \pm SD = 48 \pm 13 years) who had any coronary risk factor (hypertension, diabetes, dyslipidemia, smoking, and obesity) without a previous history of CV disease were followed up. At baseline, the subjects underwent examination of coronary risk factors, measurement of flow-mediated dilation (FMD) of the brachial artery as an indicator of vascular endothelial function and assays of plasma total testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol and cortisol.

Results: During the mean follow-up period of 77 months, a total of 20 CV events occurred. Kaplan–Meier survival analysis by tertile of plasma hormone levels revealed that the subjects with the lowest testosterone tertile were more likely to develop CV events than those with the highest tertile ($P < 0.01$ by log-rank test). Cox proportional hazards models showed that the subjects with the lowest tertile of plasma testosterone (< 14.2 nmol/L) had an approximately 4-fold higher CV event risk compared to those with the higher testosterone tertiles after adjustment for coronary risk factors including medication and FMD (unadjusted hazard ratio, 3.61; 95% CI, 1.47–8.86; multivariate-adjusted hazard ratio, 4.61; 95% CI, 1.02–21.04). Multivariate analysis did not show any significant association of DHEA-S, estradiol or cortisol with CV events.

Conclusions: A low plasma testosterone level is associated with CV events in middle-aged Japanese men, independent of coronary risk factors and endothelial function. This is the first report to show the relationship between endogenous testosterone and CV events in Asian population.

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1. Introduction

Plasma testosterone level declines with advancing age in men [1]. Testosterone deficiency is often associated with age-related diseases such as erectile dysfunction, osteoporosis, depressed mood, cognitive impairment and frailty [2,3]. Furthermore, a number of studies suggest that testosterone deficiency is related to cardiovascular (CV) disease and its risk factors in men. Inverse relations between testosterone level and coronary risk factors including obesity [4,5], hypertension [5,6], dyslipidemia [4,5], and diabetes [5,7] have been reported. In addition, we and others have

shown that a low testosterone level is associated with markers of atherosclerosis such as impaired endothelial vasomotor function [8], increased carotid intima-media thickness [9] and aortic calcification [4]. Although these data do not indicate a causal relationship between endogenous testosterone and CV disease, recent epidemiological studies have demonstrated that community-dwelling older men with a low testosterone level are more likely to die [10–12], largely due to CV disease [11,12]. However, this issue remains unknown in Asian population.

Based on these backgrounds, we tested the hypothesis that a low testosterone level is an independent risk factor for CV disease even in middle-aged Japanese men with coronary risk factors. For this purpose, we conducted a survey of 171 male patients by using baseline clinical information and by measuring sex hormone levels in stored plasma.

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2. Methods

2.1. Subjects

Male subjects aged 30–69 years at baseline, who were referred to our department to check for CV disease and undergo examination of vasomotor function of the brachial artery in 1996–2000, and had any of the classical coronary risk factors including hypertension, dyslipidemia, diabetes mellitus and current smoking, were eligible. Hypertension, dyslipidemia and diabetes mellitus were defined according to diagnostic criteria [13–15] or if the subject was taking any medication for these diseases. Subjects with a history of CV disease, including stroke, coronary heart disease, congestive heart failure and peripheral arterial disease, were excluded. Malignancy, overt endocrine disease and use of steroid hormones were also excluded, because these conditions may have a significant influence on both plasma sex hormones and clinical course.

Of the 188 eligible subjects whose plasma was stored, written informed consent was obtained from 171 subjects; 1 subject refused and 16 subjects were lost to follow-up. Then, plasma hormone levels were measured and follow-up data were obtained in 171 subjects. The study protocol was approved by the ethics committee of the Graduate School of Medicine, The University of Tokyo. Each subject or a family member, if the subject had died, gave written informed consent for enrollment in this study.

2.2. Clinical measurements

Clinical information was collected at baseline when each patient attended our department. Blood sampling and measurement of height, weight, blood pressure and vasomotor function were performed in the morning after a 14-h overnight fast. Blood pressure was measured at least twice using an automated, digital electro-sphygmomanometer (Omron Healthcare Co., Ltd., Kyoto, Japan) on the nondominant arm in a sitting position, and the average was used for analysis.

Serum total cholesterol and triglyceride concentrations were measured enzymatically, and serum high-density lipoprotein (HDL) cholesterol concentration was measured by the heparin- $\text{Ca}^{2+}\text{Ni}^{2+}$ precipitation method. Plasma glucose concentration was assayed by the glucose oxidase method, and hemoglobin A1c level was measured by high-performance liquid chromatography.

Plasma concentrations of total testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol and cortisol were determined using sensitive radioimmunoassays by a commercial laboratory (SRL, Inc., Tokyo, Japan). Because the plasma used for hormone assays was deep-frozen (-80°C) for up to 7 years, we checked the change in titers using the stored samples, which had been measured at sampling 5–7 years before. Pearson's correlation coefficient between the two measurements was 0.965 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. The intra-assay coefficients of variation for the measurements were less than 5%.

Vasomotor function of the brachial artery was evaluated using an ultrasound machine according to the method described previously [16]. Briefly, endothelium-dependent flow-mediated vasodilation (%FMD) was measured as the maximal percent change in the vessel diameter after reactive hyperemia. Subsequently, endothelium-independent nitroglycerin-induced vasodilation was measured as the maximal percent change in the vessel diameter after sublingual administration of nitroglycerin spray (0.3 mg; Toa Eiyo Co., Tokyo). The same examiner (M.H.) performed the measurements of FMD throughout this study.

2.3. Follow-up

The subjects were followed in 2006–2007 by mail and/or visits to our clinic. Each subject or a family member completed the questionnaire on CV disease and health status. CV events analyzed as the endpoints of this study included stroke, coronary artery disease, sudden cardiac death, and peripheral arterial disease. If CV events were reported on the questionnaire, we attempted to confirm the diagnosis of each event by medical records and/or interview by research doctors who were unaware of the patient's plasma hormone levels. Finally, after thorough examination, 20 cases were determined as CV events. Eighteen cases were ascertained by medical records which included clinical course, physical examination, laboratory tests and imagings. Because medical records were not available on other two cases of self-reported ischemic stroke, they were diagnosed according to the phone interview to each patient.

2.4. Data analysis

Values are expressed as mean \pm SD in the text unless otherwise stated. Differences between the groups were analyzed using ANOVA for continuous variables and Chi-squared test for categorical variables. Survival was analyzed using Kaplan–Meier plots and log-rank tests. Hazard ratios (HRs) for CV events were analyzed using Cox proportional hazards regression. A value of $P < 0.05$ was considered statistically significant. Data were analyzed using SPSS (Ver. 17.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of subjects according to plasma testosterone level

Table 1 shows the baseline characteristics of the subjects by tertile of plasma testosterone. As reported previously [4–8], subjects with the lowest testosterone tertile tended to be obese, hypertensive, dyslipidemic, diabetic, and to have impaired endothelial vasomotor function compared to those with higher testosterone tertiles. Age and smoking status were not different between the groups.

3.2. CV events and hormones

During the mean follow-up period of 77 ± 46 months (median = 54 months), a total of 20 CV events occurred (Table 2). Eleven cases of coronary artery disease included three of myocardial infarction, three of medically treated angina pectoris, four of percutaneous coronary intervention, and one of coronary artery bypass grafting. All of the five cases of stroke were due to cerebral infarction.

As shown in Fig. 1, Kaplan–Meier survival analysis by tertile of plasma testosterone level revealed that low testosterone was associated with CV events. Cox proportional hazards models showed that the subjects with the lowest tertile of plasma testosterone, but not those with the middle tertile, had significantly increased risk for CV events compared to those with the highest tertile (Table 2). Adjustment for age and body mass index did not attenuate the effect.

Then, HRs for the lowest tertile of plasma testosterone vs. the higher (middle and highest) tertiles were analyzed. The subjects with the lowest tertile (<14.2 nmol/L) showed an unadjusted HR of 3.61 (95% CI, 1.47–8.86), and an adjusted HR of 4.24 (95% CI, 1.67–10.78) for age, body mass index, and current smoking. The HR was 4.61 (95% CI, 1.02–21.04) after adjustment for age, body mass index, current smoking, systolic blood pressure, HDL cholesterol, non-HDL cholesterol, hemoglobin A1c, %FMD,

Table 1
Baseline characteristics of subjects by tertile group of plasma testosterone.

	Tertile 1 <14.2 nmol/L (n = 57)	Tertile 2 14.2–19.4 nmol/L (n = 57)	Tertile 3 >19.4 nmol/L (n = 57)	p for trend
Testosterone (nmol/L)	11.0 ± 3.0	17.0 ± 1.6	24.0 ± 3.0	<0.001
(ng/dL)	(318 ± 86)	(490 ± 45)	(693 ± 86)	
DHEA-S (μmol/L)	4.94 ± 2.68	4.55 ± 2.25	4.83 ± 2.64	0.81
Estradiol (pmol/L)	115 ± 30	116 ± 31	133 ± 30	0.004
Cortisol (nmol/L)	386 ± 138	378 ± 142	361 ± 120	0.67
Age (years)	47 ± 13	45 ± 13	50 ± 14	0.24
Body mass index (kg/m ²)	27.6 ± 5.5	25.6 ± 4.3	24.1 ± 3.6	<0.001
Systolic blood pressure (mmHg)	131 ± 18	125 ± 16	123 ± 12	0.01
Diastolic blood pressure (mmHg)	79 ± 15	74 ± 11	74 ± 9	0.04
Non-HDL cholesterol (mmol/L)	4.19 ± 1.27	3.91 ± 1.06	3.74 ± 1.01	0.10
HDL cholesterol (mmol/L)	1.20 ± 0.36	1.23 ± 0.41	1.44 ± 0.48	0.005
Triglycerides (mmol/L)	2.04 ± 2.12	1.91 ± 1.85	1.46 ± 1.28	0.18
Fasting plasma glucose (mmol/L)	6.00 ± 1.18	5.73 ± 0.92	5.73 ± 1.28	0.34
Hemoglobin A1c (%)	5.9 ± 1.7	5.2 ± 0.8	5.5 ± 1.2	0.03
%FMD	4.2 ± 2.7	5.7 ± 4.2	6.1 ± 3.8	0.01
%NTG	12.8 ± 4.3	14.2 ± 5.4	13.2 ± 5.0	0.30
Hypertension, n (%)	30 (53)	20 (35)	20 (35)	0.09
Dyslipidemia, n (%)	33 (58)	35 (61)	24 (42)	0.09
Diabetes mellitus, n (%)	15 (26)	7 (12)	9 (16)	0.13
Current smoker, n (%)	28 (49)	25 (44)	29 (51)	0.74

DHEA-S, dehydroepiandrosterone-sulfate; HDL, high-density lipoprotein; %FMD, percent flow-mediated dilation of brachial artery; %NTG, percent nitroglycerine-induced dilation of brachial artery.

Values are expressed as mean ± SD. Continuous variables were compared by ANOVA and categorical variables by Chi-squared test.

Table 2
Cardiovascular events by tertile of plasma testosterone.

	Tertile 1 <14.2 nmol/L (n = 57)	Tertile 2 14.2–19.4 nmol/L (n = 57)	Tertile 3 >19.4 nmol/L (n = 57)	Total (n = 57)
Number of events				
Stroke	2	3	0	5
Coronary artery disease	7	2	2	11
Sudden cardiac death	2	0	0	2
Peripheral arterial disease	1	0	1	2
Total cardiovascular events	12	5	3	20
HRs (95% CI) for total cardiovascular events				
Unadjusted	4.82 (1.36, 17.12)	1.67 (0.40, 6.99)	1(Ref)	
Adjusted for age	6.36 (1.78, 22.80)	1.82 (0.43, 7.71)	1(Ref)	
Adjusted for age and BMI	7.01 (1.94, 25.34)	1.86 (0.44, 7.86)	1(Ref)	

BMI, body mass index. HRs (Hazard ratios) were analyzed using Cox proportional hazards regression.

medications (antihypertensives, statins, hypoglycemic agents and antiplatelet agents), estradiol and DHEA-S. In addition to testosterone, age (HR per year, 1.12; 95% CI, 1.05–1.20), %FMD (HR per 1% increase, 0.80; 95% CI, 0.64–0.99) and HDL cholesterol (HR per 1 mg/dL, 0.88; 95% CI, 0.81–0.95) were independently asso-

ciated with CV events, but other variables were not in this final model. Further inclusion of other hormones and nitroglycerin-induced endothelium-independent vasodilation into the model did not influence the statistical results (data not shown).

Two subjects with the lowest tertile of plasma testosterone suffered CV events within 6 months of follow-up; a case of sudden cardiac death and a case of coronary artery bypass grafting. Accordingly, similar statistical analyses were performed excluding these two cases. The results were essentially unchanged, although the HRs were slightly smaller (unadjusted HR, 3.06; 95% CI, 1.21–7.78; multivariate-adjusted HR, 3.80; 95% CI, 1.06–13.52).

Among other hormones examined, only DHEA-S was associated with increased risk for CV events, but was canceled by adjustment for age (data not shown). Further multivariate analysis did not show any significant association of DHEA-S, estradiol or cortisol with CV events.

4. Discussion

In this follow-up study of middle-aged Japanese men with coronary risk factors, a low plasma testosterone level was associated with CV events. Although the subjects with lower testosterone levels had worse profiles of coronary risk factors [4–7,11,12] and endothelial function [8] at baseline, as reported previously, adjustment for these confounders including age and cardiovascu-

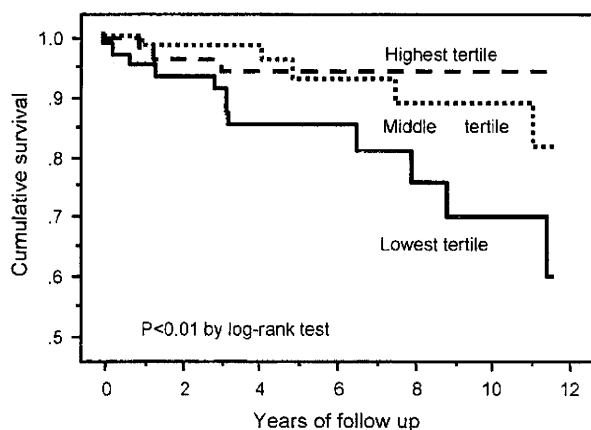


Fig. 1. Survival curves for cardiovascular events by tertile group of plasma concentration of testosterone. Cut-offs of the tertiles for testosterone were 14.2 and 19.4 nmol/L (410 and 560 ng/dL).

lar medication indicated that low testosterone was an independent risk factor for CV events. In contrast, DHEA-S, estradiol and cortisol levels were not related to CV events.

A number of cross-sectional studies have shown an association between low testosterone level and CV disease [17,18], but have not provided evidence of a causal relationship between them. In recent years, longitudinal follow-up studies have demonstrated that community-dwelling older men (around 70 years on average) with lower testosterone levels are more likely to die from CV disease [11,12]. In contrast, a low testosterone level was not associated with CV deaths [19] or events [20] in community-dwelling middle-aged men (early 50s on average). These different findings might arise from the characteristics of the populations such as age and coronary risk factors, duration of follow-up and/or cut-off level of plasma testosterone at baseline. In any case, since all the above-mentioned studies were achieved in Caucasians, our study is the first to investigate the relationship between endogenous testosterone and CV events in Asians. Also, the present study showed a positive association between low testosterone level and CV events in middle-aged men with coronary risk factors, implying the clinical importance of measuring plasma testosterone in patients at risk, even if they are not old.

Unlike the previous reports showing an association of CV events with low levels of DHEA-S [21] and estradiol [22], and with a high cortisol:testosterone ratio [20], the present study did not show any significant association of CV events with estradiol, cortisol or cortisol:testosterone ratio (data not shown). The association between low DHEA-S and CV events was abolished by statistical adjustment for age, suggesting that the age-dependent decline of DHEA-S (Pearson's correlation coefficient between age and DHEA-S: -0.588 ; $P < 0.001$) might have eliminated the association with CV events if present. Taking together with the Cox regression model including all hormones, it is suggested that testosterone is the strongest among four steroid hormones that could be predictive of CV events in this population.

There could be several mechanisms by which endogenous testosterone protects men from CV disease. Consistent with the present study, observational studies [4–8,11,12] suggest that testosterone might prevent risk factors such as obesity, hypertension, dyslipidemia, diabetes and endothelial dysfunction. Supplementary studies support the beneficial effects of testosterone on adiposity [23] and endothelial vasomotor function [24]. Based on these findings, risk markers and endothelial vasomotor function were entered into the multivariate models. Although statistical adjustment may have been insufficient to exclude the interaction between testosterone and these risk factors, testosterone remained a significant predictor of CV events in the present study. Testosterone has been reported to inhibit vascular smooth muscle cell proliferation and neointima formation [25], suggesting the direct action of testosterone on the vasculature. Also, the effects of testosterone on inflammation, hemostasis and cardiac ischemia [26] might be involved in the final process leading to CV events. The precise mechanisms, including the role of the androgen receptor and aromatization to estrogen, should be addressed in the future.

The finding of this study should not be extended to men without coronary risk factors. Our preliminary data of 47 middle-aged men without coronary risk factors showed that no subject suffered CV events during the mean follow-up period of 102 months, although a quarter of them had plasma testosterone level below the cut-off of this study (<14.2 nmol/L). Thus, the relationship between plasma testosterone and CV outcomes might be totally different in middle-aged Japanese men without coronary risk factors.

This study has several limitations. First, the number of CV events was too small to reach a clear conclusion with strong statistical power, due primarily to the small sample size and secondarily to the low incidence of CV events (approximately 2%/year). Second,

the largely retrospective design (the protocol had been approved a few years before the final data collection) reduced the quality of the study and compelled us to lose many plasma samples and 16 subjects in the follow-up. Third, not all the CV events were confirmed by medical recordings. Two cases (a case in the lowest tertile and another in the middle tertile of plasma testosterone level) were determined according to the phone interview to each patient. Although the exclusion of these two cases did not significantly influence the statistical results (data not shown), self-reported outcomes limit the accuracy of this study. Fourth, the potential influence of medication on plasma testosterone level and on CV events cannot be excluded, although statistical adjustment for each class of drugs did not affect the results. For instance, beta-blockers have been reported to decrease plasma testosterone [27], but were taken by only nine subjects and were not related to testosterone level in our population (data not shown). Fifth, active forms of testosterone such as bioavailable and calculated free testosterone were not measured, because a direct assay of bioavailable testosterone or an assay of sex hormone binding globulin, which is necessary for free testosterone calculation, is not available in Japan. However, since previous longitudinal studies [11,12] have shown an association of total testosterone with CV mortality, the fundamental findings might not have differed if active forms of testosterone had been analyzed.

In summary, a low plasma testosterone level was associated with CV events in middle-aged Japanese men, independent of coronary risk factors and endothelial function. This study is the first to show the relationship between endogenous testosterone and CV events in Asian population, and provides evidence supporting the protective role of endogenous testosterone in the development of CV disease in men.

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Supplemental Material

BrdU Incorporation Assay

BrdU incorporation was analyzed using a commercial kit (Roche, Indianapolis, USA).

Telomerase Assay

Telomerase activity was measured with 2 μ g protein using a telomerase PCR-ELISA kit according to the manufacturer's instructions (Chemicon, Temecula, CA, USA).

Supplementary Figure Legends

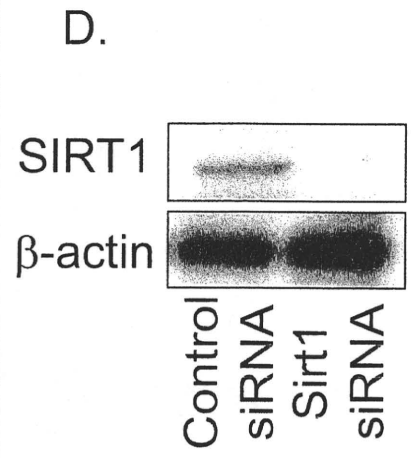
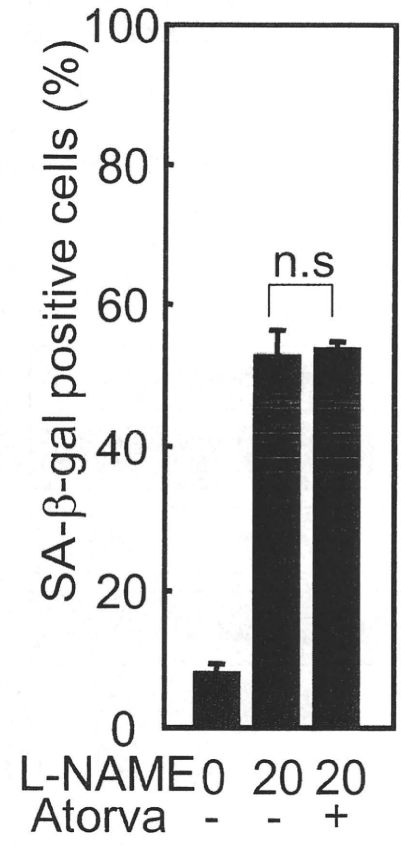
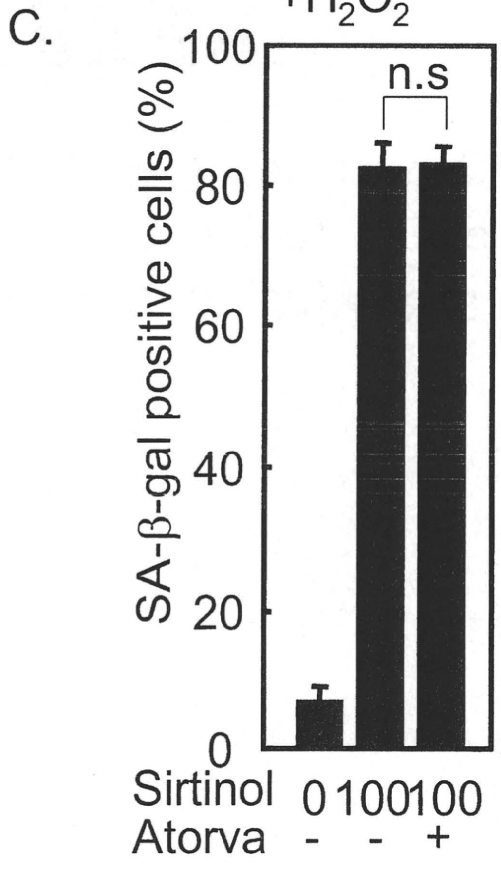
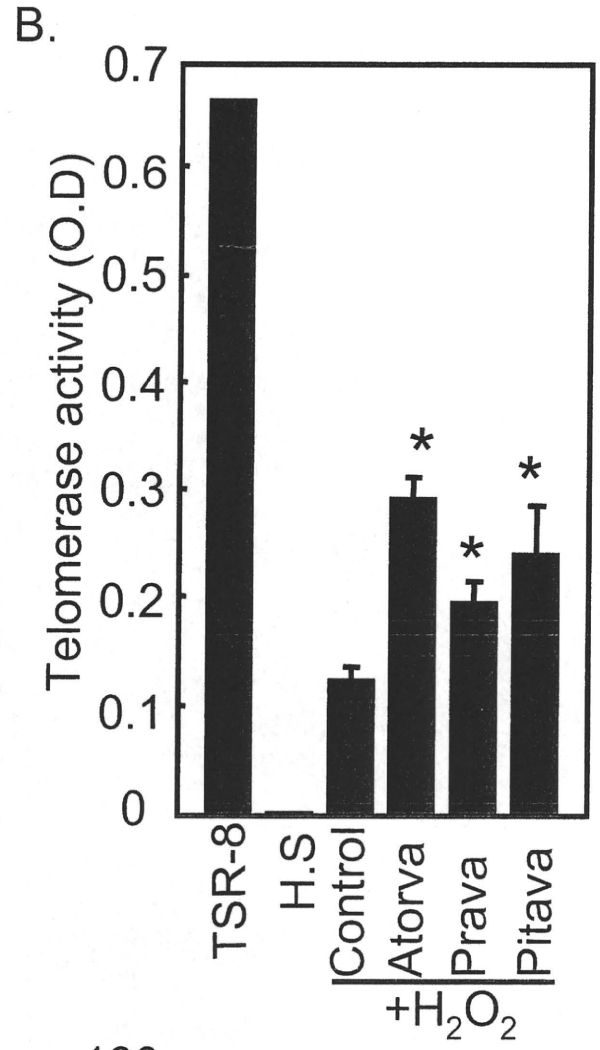
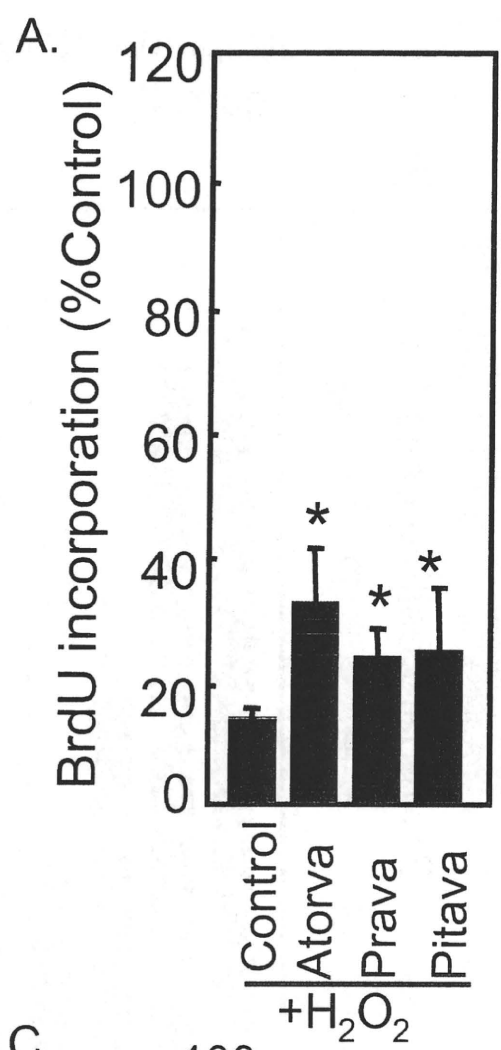
Supplementary Figure I. Atorvastatin (atorva), pravastatin (prava), and pitavastatin (pitava) (50, 100 nmol/L) inhibited H₂O₂ (100 μ mol/L)-induced endothelial senescence as judged by BrdU incorporation (**A**) and telomerase activity (**B**) at 10 days after addition of H₂O₂ (**p*<0.05, N=3). **C.** Atorvastatin (100 nmol/L) did not inhibit sirtinol (100 μ mol/L) or L-NAME (20 μ mol/L)-induced endothelial senescence as judged by SA- β gal staining (**p*<0.05, N=3), n.s: not significant. **D.** Knock down of SIRT1 by siRNA was confirmed by Western blotting analysis.

Supplementary Figure II. Overexpression of SIRT1 (10 μ g) and eNOS (10 μ g) inhibited oxidative-stress induced senescence-like phenotype in HEK293 cells. Expression of SIRT1 and eNOS were detected by western blotting analysis (**A**). For detection of a senescence-like phenotype, senescent morphological appearance and SA β -gal staining (**B**) (**p*<0.05, N=3) were used. **C.** Expression of SIRT1, eNOS, and catalase were detected by western blotting analysis. Treatment with atorvastatin

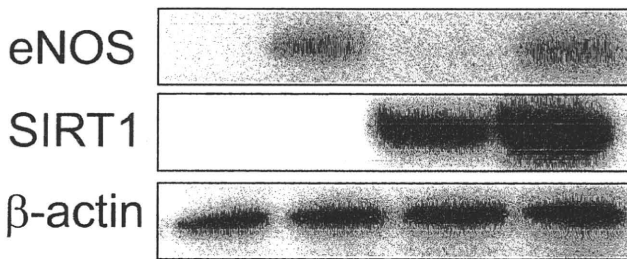
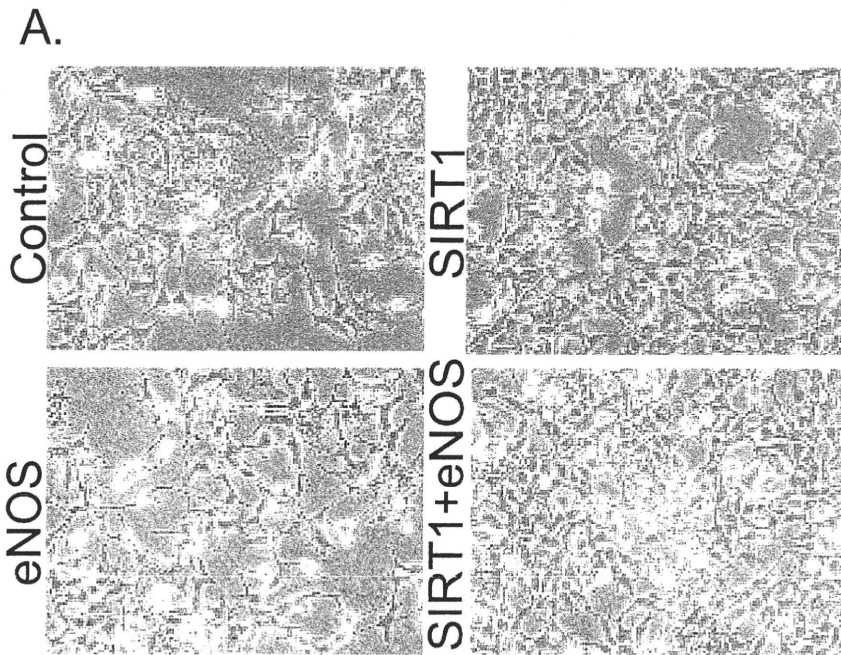
increased SIRT1, eNOS, and catalase expression for 6, 12, and 24 hrs, respectively in HUVEC.

Supplementary Figure III. **A.** Treatment with resveratrol (10, 30, 100 $\mu\text{mol/L}$) increased SIRT1 and eNOS expression. **B.** Treatment with resveratrol (res) (100 $\mu\text{mol/L}$) increased eNOS activity (* $p < 0.05$, $N=3$). **C.** Knockdown of PGC-1 α and catalase reversed the inhibitory effect on senescence of resveratrol (100 nmol/L , * $p < 0.05$, $N=3$). **D.** The molecular mechanism of anti-senescence by which statin treatment upregulates eNOS (2), SIRT1 (3), and catalase expression through phosphorylation of Akt (1).

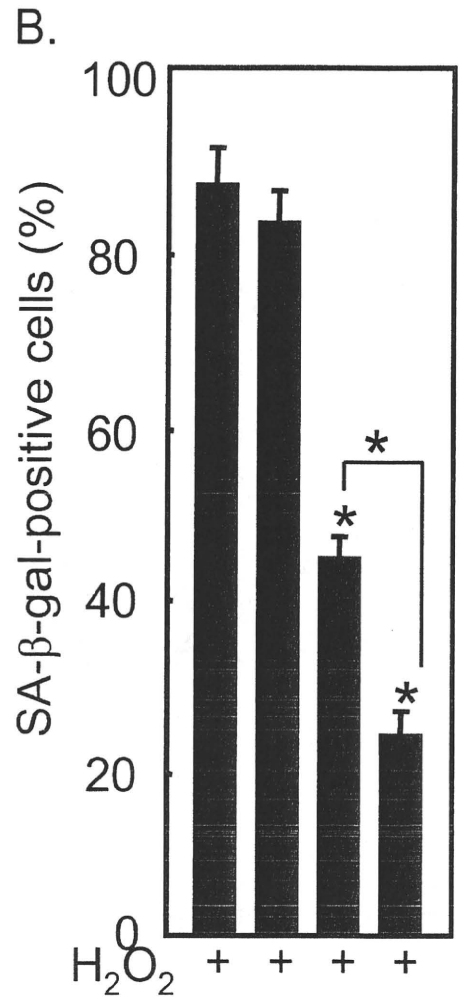
Supplementary Figure IV. **A.** Plasma glucose and plasma insulin levels in streptozotocin (STZ)-diabetic mice (* $p < 0.05$, $N=3$). **B.** Body weight (BW), blood pressure (BP), and pulse rate (PR) of STZ-diabetic mice with and without pitavastatin (3 mg/kg/day). **C.** *Sirt1* KO (+/-) mice did not show a senescent phenotype of aorta without treatment with STZ.



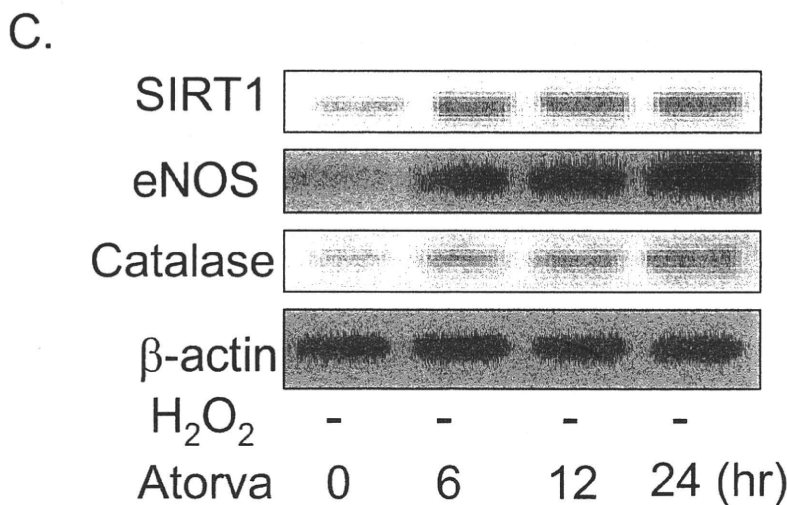
Supplementary
Figure II.



Control (μg)	10	5	5	0
eNOS (μg)	0	5	0	5
SIRT1 (μg)	0	0	5	5

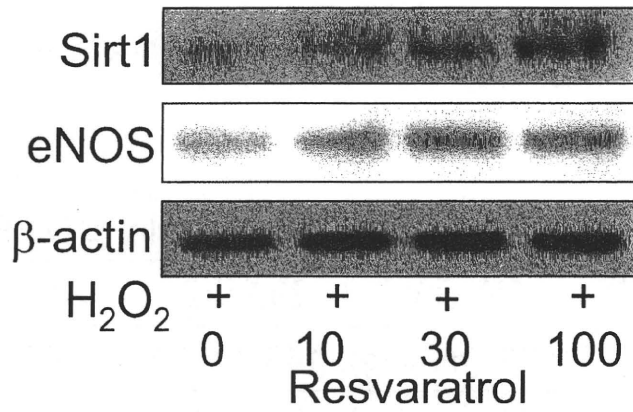


Control (μg)	10	5	5	0
eNOS (μg)	0	5	0	5
SIRT1 (μg)	0	0	5	5

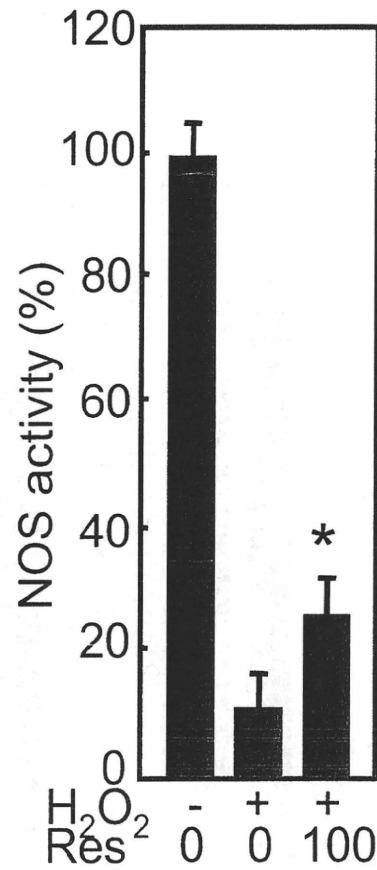


Supplementary Figure III.

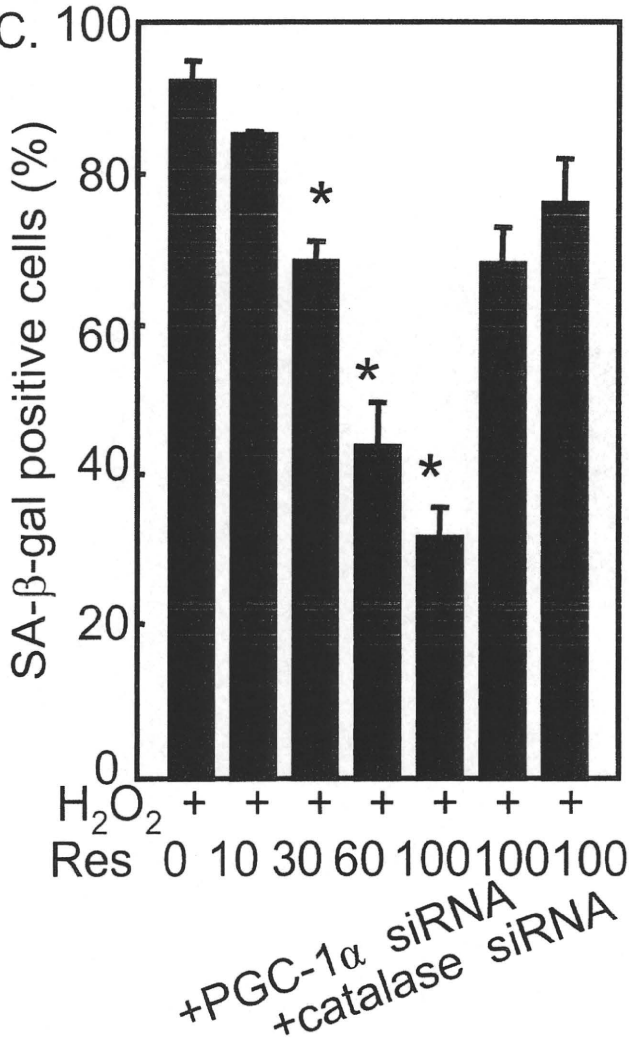
A.



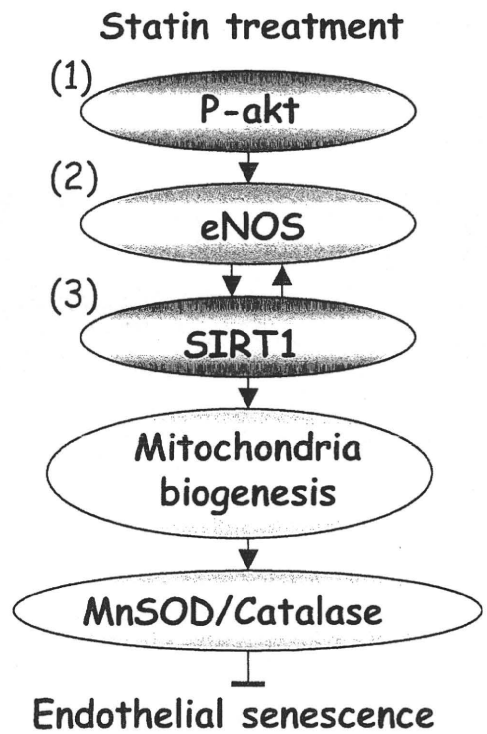
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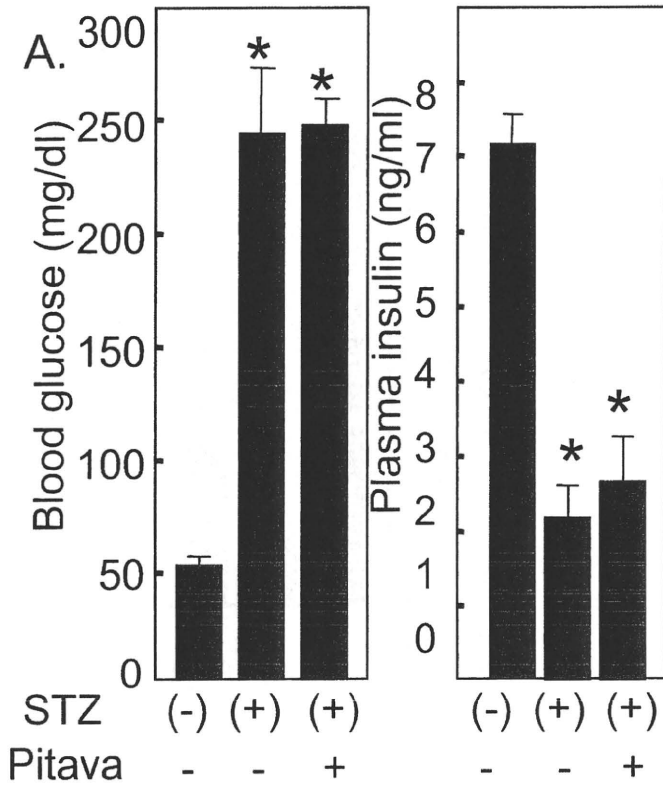
C.



D.



Supplementary Figure IV.



B.

Mouse Group	STZ+Vehicle	STZ+Pitava
BW (g)	29.9±2.1	28.7±2.2
sBP (mmHg)	146.3±9.5	147.2±6.3
dBP (mmHg)	61.4±6.4	64.8±4.9
PR (/min)	463.1±35.5	475.5±42.6

C.



STZ (-)

ORIGINAL ARTICLE: EPIDEMIOLOGY,
CLINICAL PRACTICE AND HEALTH

Effects of dehydroepiandrosterone supplementation on cognitive function and activities of daily living in older women with mild to moderate cognitive impairment

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Aim: There is little evidence that dehydroepiandrosterone (DHEA) has beneficial effects on physical and psychological functions in older women. We investigated the effect of DHEA supplementation on cognitive function and ADL in older women with cognitive impairment.

Methods: A total of 27 women aged 65–90 years (mean \pm standard deviation, 83 ± 6) with mild to moderate cognitive impairment (Mini-Mental State Examination, MMSE; 10–28/30 points), receiving long-term care at a facility in Japan were enrolled. Twelve women were assigned to receive DHEA 25 mg/day p.o. for 6 months. The control group ($n = 15$) matched for age and cognitive function was followed without hormone replacement. Cognitive function was assessed by MMSE and Hasegawa Dementia Scale-Revised (HDS-R), and basic activities of daily living (ADL) by Barthel Index at baseline, 3 and 6 months. Plasma hormone levels including testosterone, DHEA, DHEA-sulfate and estradiol were also followed up.

Results: After 6 months, DHEA treatment significantly increased plasma testosterone, DHEA and DHEA-sulfate levels by 2–3-fold but not estradiol level compared to baseline. DHEA administration increased cognitive scores and maintained basic ADL score, while cognition and basic ADL deteriorated in the control group (6-month change in DHEA group vs control group; MMSE, $+0.6 \pm 3.2$ vs -2.1 ± 2.2 , $P < 0.05$; HDS-R, $+2.8 \pm 2.8$ vs -0.3 ± 4.1 , $P < 0.05$; Barthel Index, $+3.7 \pm 7.1$ vs -2.7 ± 4.6 , $P = 0.05$). Among the cognitive domains, DHEA treatment improved verbal fluency ($P < 0.05$).

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Conclusion: DHEA supplementation in older women with cognitive impairment may have beneficial effects on cognitive function and ADL. *Geriatr Gerontol Int* 2010; 10: 280–287.

Keywords: activities of daily living, cognitive function, dehydroepiandrosterone.

Introduction

Dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are the most abundant circulating steroids mainly produced by the adrenal zona reticularis in both sexes.¹ Their circulating levels decline with advancing age,^{1–4} and there has been growing public interest in DHEA supplementation to prevent age-associated physical and cognitive impairment. DHEA is considered a crucial precursor of human sex steroid biosynthesis, and to exert indirect androgenic and estrogenic effects following conversion into smaller amounts of testosterone and estradiol.^{5,6} While this conversion contributes to a part of testosterone production in men, its role may be much more significant in postmenopausal women whose ovarian production of androgen and estrogen has waned. Importantly, postmenopausal women with intact ovaries continue to produce androgens; DHEA(-S), testosterone and androstenedione, while their production of estradiol is minimal.⁷ However, the role of androgens in older women's health is not fully understood.

Clinical trials of the effects of estrogen replacement therapy on cognitive function have shown a lack of efficacy in postmenopausal women initiating hormone replacement therapy after the age of 65 years.^{8,9} On the other hand, previous reports have suggested that DHEA may have neuroprotective effects, and the age-associated DHEA(-S) decline is associated with cognitive impairment in older women.^{2,10–12} One longitudinal study observed lower DHEA-S levels in patients who subsequently developed Alzheimer's disease.¹³ However, controlled trials with DHEA supplementation have failed to show beneficial effects on cognition in healthy middle-aged to older women.^{14–16} In these studies, the participants were limited to those who did not have cognitive impairment; therefore, it is reasonable to hypothesize that DHEA supplementation may be effective in much older women with cognitive decline as well as lower DHEA levels.

Dehydroepiandrosterone deficiency is also considered to be involved in the development of physical frailty.¹⁷ Clinical experience with DHEA supplementation in older women is limited, and the few clinical trials examining its effect on physical function and activity of daily living (ADL) have yielded inconsistent results.^{18–20} Evidence is lacking for much older women in whom physical impairment becomes more apparent and is

accompanied by an age-associated DHEA decline. In our previous study, plasma DHEA and DHEA-S levels, but not estradiol level, were independently related to higher basic ADL in older women aged 70–93 years with functional decline receiving long-term care.²¹ We hypothesized that in older women, DHEA replacement could be effective for the age-related decline of physical as well as psychological function.

This study therefore examined the effect of relatively low-dose (25 mg daily) p.o. DHEA supplementation for 6 months on cognitive function and ADL in older women with cognitive impairment.

Methods

Subjects and study design

In this open, non-randomized controlled study, 27 women aged 65 years or older who attended a health service facility for the elderly (a facility that provides nursing care and rehabilitation services to elderly people with disability, Mahoroba-no-Sato, located in Nagano Prefecture, Japan) were enrolled. The participants were in a chronic stable condition and receiving Long-term Care Insurance service either for admission to the facility or day-care services. The principal inclusion criteria were mild to moderate cognitive decline; both Mini-Mental State Examination (MMSE)²² and Hasegawa Dementia Scale-Revised (HDS-R)²³ scores were between 10 and 28. The subjects were diagnosed as having a mild cognitive impairment²⁴ or Alzheimer's disease according to the Diagnostic and Statistical Manual of Mental Disorders IV.²⁵ The participants had never been treated with hormone replacement therapy, and plasma DHEA-S concentration was less than 3.0 $\mu\text{mol/L}$. The exclusion criteria were history of stroke, extremely low ADL status (Barthel Index²⁶ <50), malnutrition (serum albumin <3.5 mg/dL), malignancy, acute inflammation (fever, white blood cell count >10 000/ μL , or other signs of infection within 4 weeks before enrollment) and overt endocrine diseases, because these diseases may affect both plasma sex hormone levels and functions. None of the subjects were taking a cholinesterase inhibitor (donepezil hydrochloride) or glucocorticoid, opiate or hormone supplement.

Twelve women were assigned to receive DHEA capsule (25 mg/day, Athena Clinics International,

Honolulu, HI, USA) and 15 women were followed up without any additive medication. Medications that could influence cognitive function and plasma hormone levels were not changed during the study period. Outcome measures were cognitive function, ADL, plasma hormone levels, blood cell counts, blood chemical parameters and subjective adverse events. They were assessed at baseline, and after 3 and 6 months. The institutional review board of Mahoroba-no-Sato approved the study protocol, and all participants or their families gave written informed consent.

Hormone measurements

Blood samples were obtained from the participants in the morning after an overnight fast, and plasma hormone levels in addition to blood cell counts and blood chemical parameters were determined by a commercial laboratory (Health Sciences Research Institute, Yokohama, Japan). DHEA and DHEA-S were assayed using sensitive radioimmunoassays with minimum detection limits of 0.04 ng/mL (0.14 nmol/L) and 2.0 µg/dL (0.05 µmol/L), respectively. Total testosterone and estradiol were assayed using chemiluminescent immunoassays with minimum detection limits of 7 ng/dL (0.2 nmol/L) and 4 pg/mL (14.7 pmol/L), respectively. The intra-assay coefficients of variation for these measurements were less than 5%.

Cognitive function

Trained examiners administered two standardized cognitive function tests, MMSE²² and HDS-R,²³ to assess multiple, diverse aspects of cognitive function at baseline and at the 3- and 6-month visits. Both scores range 0–30, with higher scores indicating better performance. HDS-R includes questions about the subject's age, orientation, immediate recall, serial subtraction of 7 s, reciting digits backward, recalling three words, recalling five objects and word fluency (generating names of vegetables). MMSE evaluates five aspects of cognition: (i) orientation; (ii) registration; (iii) attention and calculation; (iv) recall; and (v) comprehension of spoken language (naming objects, spoken language ability, following commands). MMSE, but not HDS-R, includes four performance tests: (i) three-stage command; (ii) reading and following a command; (iii) writing; and (iv) construction drawing). Based on the results of HDS-R and MMSE, we evaluated seven cognitive domains (points) as follows: (i) orientation (10); (ii) verbal memory (9); (iii) attention and calculation (5); (iv) visual memory (5); (v) spoken-language comprehension (9); (vi) verbal fluency (5); and (vii) performance (7).

Other functional parameters and anthropometric measures

Trained nurses and physical therapists visited the participants at the facility and performed the assessments. Basic ADL was assessed by Barthel Index,²⁶ mood by Geriatric Depression Scale (GDS, 15 items),²⁷ and ADL-related vitality by Vitality Index (10-point scale).²⁸ Higher GDS scores indicate a more marked self-reported depressive status, while higher Vitality Index scores indicate greater willingness.

Adverse events

Information regarding adverse events was obtained by questioning or examining the subjects. At each visit during the treatment period, all new complaints and symptoms were recorded. The safety of DHEA supplementation was assessed from the symptoms and by measuring blood chemical parameters including liver and kidney function, electrolyte levels and hematological parameters. Preexisting complaints or symptoms that increased in intensity or frequency during the treatment period also were examined.

Statistical analysis

Data were analyzed using SPSS statistical software ver. 17.0. Changes in outcome measures at 3 and 6 months were calculated by comparing the values at baseline with those at each measurement. Within each group, the significance of the change from baseline to 6 months was tested using paired Student's *t*-test. Repeated-measures ANOVA was used to test the statistical significance of the effects of DHEA versus control. Significance tests were two-sided, with an α -level of 0.05.

Results

Hormone changes and adverse effects

Characteristics and hormone levels at baseline according to treatment groups are shown in Table 1. There were no significant differences between the DHEA group and the control group in age, length of education, nutritional parameters, functional parameters and plasma hormone levels. DHEA supplementation was well tolerated, with high adherence, and there were no detectable adverse events and none of the subjects dropped out during the study. Measures of liver function, kidney function, electrolyte levels and hemoglobin level were not significantly altered by treatment with DHEA (data not shown). Body mass index remained unchanged in both groups.

Subjects in the DHEA group showed a significant increase from baseline to 3 and 6 months in levels of

Table 1 Participant characteristics at baseline

	DHEA	Control
No. of subjects	12	15
Age, years	82 ± 6 (69–90)	83 ± 6 (65–89)
Education, years	8 ± 2	8 ± 2
Nutritional parameters		
Body mass index, kg/m ²	22.0 ± 2.4 (18.8–26.4)	22.4 ± 3.2 (17.6–27.1)
Albumin, g/dL	4.4 ± 0.3 (3.7–4.9)	4.3 ± 3.2 (3.8–4.7)
Total cholesterol, mg/dL	227 ± 39 (166–294)	203 ± 22 (173–250)
Functional parameters		
MMSE	24.0 ± 4.2 (18–28)	23.4 ± 4.4 (14–28)
HDS-R	19.9 ± 5.8 (10–28)	21.7 ± 5.6 (10–28)
Barthel Index	89.6 ± 9.4 (55–100)	89.7 ± 6.4 (75–100)
Vitality Index	9.8 ± 0.6 (8–10)	9.9 ± 0.3 (9–10)
GDS	7.0 ± 4.4 (1–15)	7.0 ± 4.0 (1–13)
Hormones		
DHEA-S, μmol/L	1.8 ± 0.6 (0.7–2.4)	1.6 ± 0.8 (0.3–2.9)
DHEA, nmol/L	7.6 ± 4.7 (2.4–19.1)	6.6 ± 3.1 (2.1–11.5)
Testosterone, nmol/L	1.4 ± 0.4 (0.9–2.3)	1.3 ± 0.9 (0.2–3.8)
Estradiol, pmol/L	88 ± 52 (15–187)	70 ± 26 (45–115)

Values are shown as mean ± standard deviation (range). HDS-R, Hasegawa Dementia Scale-Revised; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone. There was no significant difference in each parameter between the groups.

circulating DHEA, DHEA-S and testosterone, with levels reaching approximately 2–3-fold higher than those at baseline, whereas the increase in estradiol level was not significant (Table 2). Subjects in the control group showed no significant change in hormone levels.

Changes in cognitive function and ADL

The changes in functional parameters in each group from baseline to 6 months are shown in Table 2. After 6 months, mean HDS-R score significantly improved in the DHEA group while it remained unchanged in the control group. Mean MMSE score significantly declined in the control group while it remained unchanged in the DHEA group. As a result, significant differences were found in these scores between the groups. DHEA treatment maintained Barthel Index score, whereas the score deteriorated significantly during 6 months in the control group, although the between-group difference at 6 months was not statistically significant. Regarding the components of Barthel Index, in the control group, the sum score of mobility deteriorated significantly after 6 months compared to baseline, while no significant change was observed in the sum score of self care (Table 3). Neither Vitality Index nor GDS changed significantly in both groups.

Table 4 shows the cognitive domain scores at baseline and at 3- and 6-month follow up. Among the seven cognitive domains, DHEA treatment improved verbal fluency ($P < 0.05$), resulting in a significant difference at 6 months between the groups. Verbal memory showed a non-significant trend towards improvement in the DHEA group. Performance test scores significantly declined over time in both groups. There were no differences between the groups in the scores of orientation, attention and calculation, visual memory and spoken-language comprehension.

Discussion

Daily administration of DHEA 25 mg for 6 months in elderly women with mild to moderate cognitive impairment improved cognitive function and maintained basic ADL, compared to the control group. Among the cognitive domains, DHEA significantly improved verbal fluency. At baseline, DHEA and DHEA-S levels were lower than those reported in healthy postmenopausal women in both groups,^{2,4} and DHEA treatment increased DHEA, DHEA-S and testosterone levels by 2–3-fold to the mid-normal range for premenopausal

Table 2 Changes in hormone levels and functional parameters by treatment group

	DHEA				Control			P	
	Baseline	3 months	6 months	0-6-month difference	Baseline	3 months	6 months		0-6-month difference
Hormones									
DHEA-S, $\mu\text{mol/L}$	1.8 \pm 0.6	4.5 \pm 1.3*	5.6 \pm 2.9*	3.8 \pm 2.8	1.6 \pm 0.8	1.8 \pm 1.0	1.7 \pm 0.8	-0.02 \pm 0.4	<0.01
DHEA, nmol/L	7.6 \pm 4.7	12.2 \pm 4.8*	13.7 \pm 7.7*	6.1 \pm 8.2	6.6 \pm 3.1	7.3 \pm 3.7	7.4 \pm 4.5	0.9 \pm 2.8	0.04
Testosterone, nmol/L	1.4 \pm 0.4	2.3 \pm 0.7*	2.3 \pm 0.8*	0.9 \pm 0.8	1.4 \pm 0.7	1.4 \pm 0.7	1.6 \pm 0.8	0.2 \pm 0.5	<0.01
Estradiol, pmol/L	88 \pm 52	92 \pm 48	101 \pm 37	13 \pm 51	70 \pm 26	68 \pm 20	67 \pm 42	-4.0 \pm 38	0.17
Functional parameters									
MMSE	24.0 \pm 4.2	24.1 \pm 4.6	24.6 \pm 4.3	0.6 \pm 3.2	23.4 \pm 4.4	23.1 \pm 5.4	21.3 \pm 5.0**	-2.1 \pm 2.2	0.04
HDS-R	19.9 \pm 5.8	20.5 \pm 7.3	22.7 \pm 6.3**	2.8 \pm 2.8	21.7 \pm 5.6	22.1 \pm 5.6	21.3 \pm 6.4	-0.3 \pm 4.1	0.04
Barthel Index	89.6 \pm 9.4	92.7 \pm 6.5	93.3 \pm 6.8	3.7 \pm 7.1	89.7 \pm 6.4	86.9 \pm 7.2	87.0 \pm 6.7*	-2.7 \pm 4.6	0.04
Vitality Index	9.8 \pm 0.6	9.7 \pm 0.5	9.7 \pm 0.7	-0.1 \pm 1.0	9.9 \pm 0.3	9.8 \pm 0.5	9.7 \pm 1.0	-0.3 \pm 1.0	0.80
GDS	7.0 \pm 4.4	6.2 \pm 3.4	6.6 \pm 3.7	-0.4 \pm 1.7	7.0 \pm 4.0	8.3 \pm 3.9	7.5 \pm 3.5	0.5 \pm 3.3	0.60

Values are shown as mean \pm standard deviation (range). P-values are for repeated-measure ANOVA over all three time points. DHEA, dehydroepiandrosterone; HDS-R, Hasegawa Dementia Scale-Revised; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone. **P < 0.01 compared to baseline, *P < 0.05 compared to baseline.

women.² No detectable adverse effects were observed throughout the study.

According to the previous trials, DHEA supplementation of 50 mg or more daily does not provide beneficial effects on cognition in healthy middle-aged to elderly women without cognitive impairment.¹⁴⁻¹⁶ However, in a small-scale randomized double-blind placebo-controlled study, DHEA transiently improved cognition (after 3 months) in subjects with Alzheimer's disease while the improvement was not significant at 6 months.²⁹ Preliminary analysis of the small number of subjects in the present study suggested that DHEA treatment was no less effective in subjects with low baseline cognitive function than those with higher cognitive function (data not shown). Whether the effects of DHEA might be influenced by baseline cognitive function should be further investigated.

It is noteworthy that the 6-month effect of donepezil hydrochloride (5 or 10 mg), the only cholinesterase inhibitor used in Japan, in patients with Alzheimer's disease ranged from no change to less than 1 point improvement in MMSE score,²⁹⁻³³ which is not so different from the effect of DHEA observed in the present study.

In the present study, not only the participants' cognitive function was impaired, but baseline plasma DHEA(-S) level was also low compared to that in postmenopausal or perimenopausal women.^{2,4,10} Regarding DHEA-S levels, according to a report in which healthy pre- and postmenopausal women were studied, DHEA-S levels in women aged 35-44 years and 45-55 years were as follows: 4.31 \pm 2.11, 3.90 (mean \pm standard deviation) and 3.42 \pm 2.01 $\mu\text{mol/L}$.² In this study, DHEA-S was measured using chemiluminescent enzyme immunometric assay; although the measurements by this method and those by radioimmunoassay have been reported to be comparable. In our study, DHEA treatment increased DHEA-S levels to the mid-normal range for premenopausal women.² Also, the subjects with lower baseline DHEA-S levels showed non-significant trend towards more improvement in cognitive scores (data not shown). Thus, future studies are needed to explore whether the effects of DHEA might be influenced by baseline DHEA levels.

Because the DHEA receptor has not been identified, DHEA may act after conversion to testosterone and subsequently estradiol through estrogen receptors and androgen receptors, both of which are found in the hippocampus and frontal lobes and subserve verbal memory and working memory in women.^{34,35} Further, hippocampal volume and perfusion have been shown to correlate with serum DHEA-S level in demented patients.^{36,37} It has also been suggested that estrogenic and androgenic derivatives of DHEA might have different effects on cognitive functions.³⁸ However, the mechanism by which DHEA improves cognitive

Table 3 Changes in mobility and self-care scores in Barthel Index during the study

Domains (points)	Mean \pm SD				<i>P</i>
	Baseline	3 months	6 months	Change (0–6 months)	
Mobility (55)					
DHEA	46.9 \pm 9.2	48.2 \pm 6.0	49.2 \pm 5.2	2.3 \pm 5.4	0.01
Control	47.5 \pm 5.4	46.2 \pm 5.5	45.0 \pm 4.3*	-3.7 \pm 3.9	
Self care (45)					
DHEA	42.7 \pm 6.1	44.5 \pm 1.5	43.1 \pm 2.5	0.4 \pm 6.9	0.96
Control	41.8 \pm 4.2	42.5 \pm 3.4	41.2 \pm 4.3	0.7 \pm 3.2	

Mobility is the sum score of five domains: (i) transfer (moving from a bed to a wheelchair and back); (ii) walking on a level surface; (iii) propelling a wheel chair; (iv) ascending and descending stairs; and (v) bathing and toilet use. Self care includes feeding, grooming, dressing, bowels and bladder. *P*-values are for repeated-measure ANOVA over all three time points. **P* < 0.05 compared to baseline. SD, standard deviation.

Table 4 Changes in cognitive domain scores during study

Domains (points)	Mean \pm SD				<i>P</i>
	Baseline	3 months	6 months	Change (0–6 months)	
Orientation (10)					
DHEA	8.3 \pm 1.9	8.0 \pm 2.7	7.5 \pm 3.0	-0.1 \pm 1.2	0.28
Control	8.3 \pm 1.9	8.0 \pm 2.8	7.5 \pm 2.9	-0.7 \pm 1.7	
Verbal memory (9)					
DHEA	5.7 \pm 2.1	6.5 \pm 2.3	6.7 \pm 2.5†	1.0 \pm 1.9	0.79
Control	6.5 \pm 1.7	7.5 \pm 1.8	7.0 \pm 1.9	0.5 \pm 1.7	
Attention and calculation (5)					
DHEA	2.3 \pm 1.9	2.8 \pm 2.0	2.7 \pm 1.8	0 \pm 2.3	0.79
Control	2.0 \pm 1.7	1.9 \pm 1.2	1.8 \pm 1.5	-0.5 \pm 1.4	
Visual memory (5)					
DHEA	3.6 \pm 0.9	3.6 \pm 1.3	3.8 \pm 1.2	0.3 \pm 1.1	0.91
Control	3.6 \pm 1.3	3.9 \pm 0.9	3.9 \pm 1.0	0.5 \pm 1.1	
Language comprehension (9)					
DHEA	8.5 \pm 0.8	7.8 \pm 2.5	8.7 \pm 0.7	0.1 \pm 0.3	0.12
Control	8.5 \pm 0.8	8.5 \pm 0.8	8.4 \pm 1.1	-0.1 \pm 0.9	
Verbal fluency (5)					
DHEA	2.8 \pm 3.3	2.5 \pm 2.0	4.3 \pm 1.1*	1.5 \pm 1.7	0.01
Control	3.2 \pm 1.9	3.8 \pm 1.6	3.3 \pm 1.9	0.1 \pm 2.1	
Performance (7)					
DHEA	5.7 \pm 0.7	5.5 \pm 0.7	4.8 \pm 0.4**	-0.8 \pm 0.6	0.36
Control	5.6 \pm 0.6	5.1 \pm 0.6	4.5 \pm 0.9**	-1.1 \pm 0.8	

Change refers to score change during 0–6 months for each parameter in each treatment group. *P*-values are for repeated-measure ANOVA over all three time points. DHEA, dehydroepiandrosterone. **P* < 0.05, ***P* < 0.01, †*P* < 0.1 vs baseline. SD, standard deviation.

function is unknown. In the present study, plasma estradiol level was not significantly increased after DHEA treatment, implying that its beneficial effects on cognition might be androgen-dependent. Unfortunately, free testosterone levels were not measured, because they were considered to be undetectable in many cases in older women. In addition, sex hormone-binding globulin (SHBG) measurement was not available; however, it has

been reported that DHEA 50 mg treatment for 3 months in postmenopausal women did not significantly change SHBG levels,³⁹ suggesting that the change in SHBG-bound hormone levels after DHEA treatment might be minimal. Given the local aromatization of androgen to estradiol in the brain, the effect of DHEA on cognition might be indirect, complex and heterogeneous. The molecular mechanism underlying the association

between DHEA and cognitive function needs to be clarified, and active forms of testosterone and estradiol should also be examined to investigate whether they would change after DHEA administration.

In our previous study, plasma DHEA and DHEA-S levels were independently related to higher basic ADL in older women aged 70–93 years with functional decline,²¹ and other reports have shown a correlation between DHEA level and muscle mass, strength and physical performance.^{40,41} In the present study, DHEA treatment maintained the Barthel Index score, while the score deteriorated significantly in the control group. Regarding body composition and strength, DHEA administration in postmenopausal older women aged up to 80 years did not alter body composition, physical performance or strength.^{18–20} However, in one small-scale open-label trial, DHEA treatment for 4 weeks improved ADL in three out of seven patients (both men and women) with multi-infarct dementia.⁴² All these studies are preliminary, and large-scale and long-term studies are required to ascertain whether DHEA could have a beneficial effect on ADL in older women.

In the present study, no effect of DHEA on depressive mood or vitality was observed, consistent with most clinical trials in older women.^{15,43,44} This might be attributable to the participants' relatively low depressive status and high vitality status, namely, ceiling effects.

The limitations of our study should be acknowledged. First, this study was neither blinded nor randomized. Second, the number of participants was too small to confirm the results. Thus, results need to be confirmed by large-scale randomized trials to exclude possible selection bias. Third, considering the sensitivity and accuracy, a standard test like the Alzheimer's Disease Assessment Scale should be used in clinical trials to ascertain the effect of DHEA. Finally, our study duration was 6 months so it does not provide any information on the effects of longer-term DHEA supplementation.

In summary, this small study showed that supplementation of DHEA 25 mg for 6 months to older women with mild to moderate cognitive impairment improved cognitive scores and maintained basic ADL. The results should be confirmed in large-scale randomized trials.

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