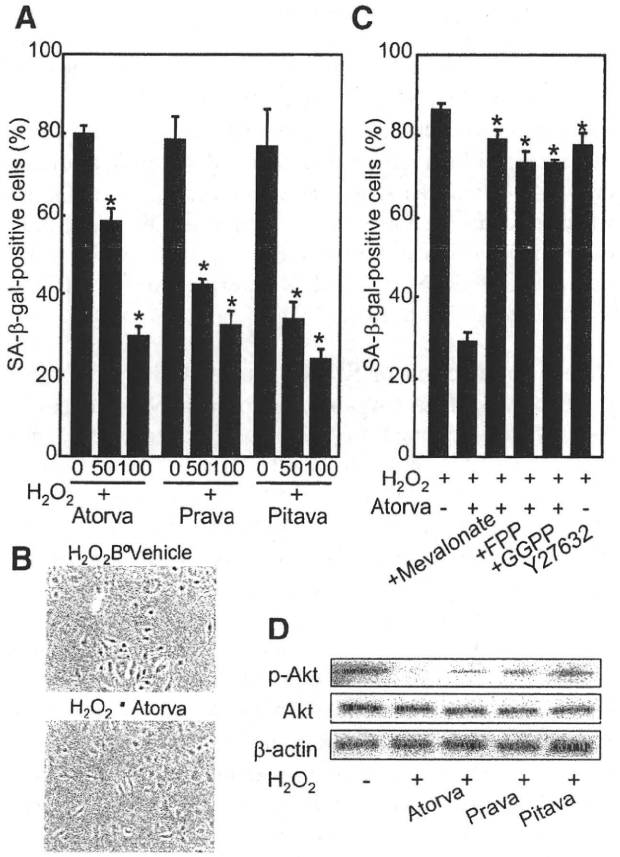

Figure 1. A and B, Atorvastatin (atorva), pravastatin (prava), and pitavastatin (pitava) (50 and 100 nmol/L) inhibited H_2O_2 (100 μmol/L)-induced endothelial senescence as judged by SA-βgal staining (A) (*$P<0.05$, n=3) and morphological changes (B) at 10 days after addition of H_2O_2. C, Atorvastatin (100 nmol/L)-treated cells coincubated with mevalonate (300 μmol/L), FPP (10 μmol/L), or GGPP (10 μmol/L) and Y27632 (10 μmol/L)-treated cells as judged by SA-βgal staining (*$P<0.05$, n=3). D, Treatment with atorvastatin, pravastatin, or pitavastatin (100 nmol/L) increased phosphorylation of Akt at Ser473. p-Akt indicates phospho-Akt.

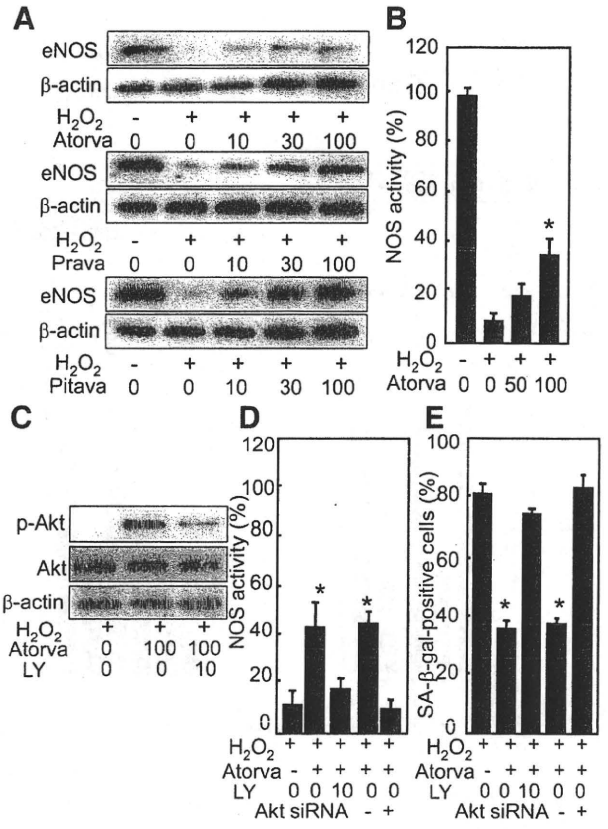

Figure 2. A, Expression of eNOS in atorvastatin, pravastatin, and pitavastatin-treated (10, 30, or 100 nmol/L) cells. B, NOS activity was measured after treatment with atorvastatin (atorva) (*$P<0.05$, n=3). C, Treatment with LY294002 (LY) (10 μmol/L) for 6 hours inhibited phosphorylation of Akt at Ser473. D, NOS activity was measured after treatment with atorvastatin+LY294002 or Akt siRNA (*$P<0.05$, n=3). E, LY294002 or Akt siRNA reversed the effect of atorvatatin (100 nmol/L) as judged by SA-βgal staining (*$P<0.05$, n=3).

As shown in Figure 1C, the addition of mevalonate, FPP, or GGPP completely reversed atorvastatin-induced inhibition of endothelial senescence. In contrast, the senescent phenotype was not altered by treatment with Y27632, a pharmacological inhibitor of Rho kinase (Figure 1C). These results indicate that statins influence endothelial senescence through isoprenylation but independently of Rho kinase. Next, we investigated the phosphorylation of Akt because several studies have demonstrated that statins stimulate the Akt pathway,[22] which is known to regulate senescence of endothelial cells.[23] As shown in Figure 1D, treatment with atorvastatin, pravastatin, or pitavastatin increased the phosphorylation of Akt at Ser473.

Treatment With Atorvastatin, Pravastatin, and Pitavastatin Increases eNOS Activity and Expression Through Akt Pathway

Recent studies have demonstrated that statins stimulate the phosphatidylinositol 3-kinase/Akt pathway, which is known to regulate eNOS activity.[24] As shown in Figure 1D, these statins increased the phosphorylation of Akt at Ser473. To confirm the influence of treatment with these statins on eNOS activity, we examined the expression and activity of eNOS. In the presence of H_2O_2, treatment with atorvastatin, pravastatin, and pitavastatin increased eNOS expression dose dependently (Figure 2A). In parallel with eNOS expression, activity of eNOS was increased by treatment with atorvastatin (Figure 2B), pravastatin, and pitavastatin (data not shown). To confirm whether a direct target of statin treatment is phosphorylation of Akt at Ser473, we treated mice with the phosphatidylinositol 3-kinase inhibitor LY294002 (10 μmol/L) for 6 hours or siRNA specifically for Akt in atorvastatin-treated endothelial cells. Treatment with LY294002 inhibited phosphorylation of Akt at Ser473 and abrogated the eNOS activation (Figure 2C and 2D). Treatment with Akt siRNA also abrogated the eNOS activation (Figure 2D). After treatment with Ly294002 or Akt siRNA, many senescent cells were observed in the presence of statins (Figure 2E). These results indicate that the antisenescent property of statins and increased eNOS activation are attributable to a direct stimulation of the Akt pathway.

Treatment With Atorvastatin, Pravastatin, and Pitavastatin Increases SIRT1 Expression Through Activation of eNOS

In our previous study, we found that after treatment with either an NO donor (such as DETA-NO or SNAP), a cAMP

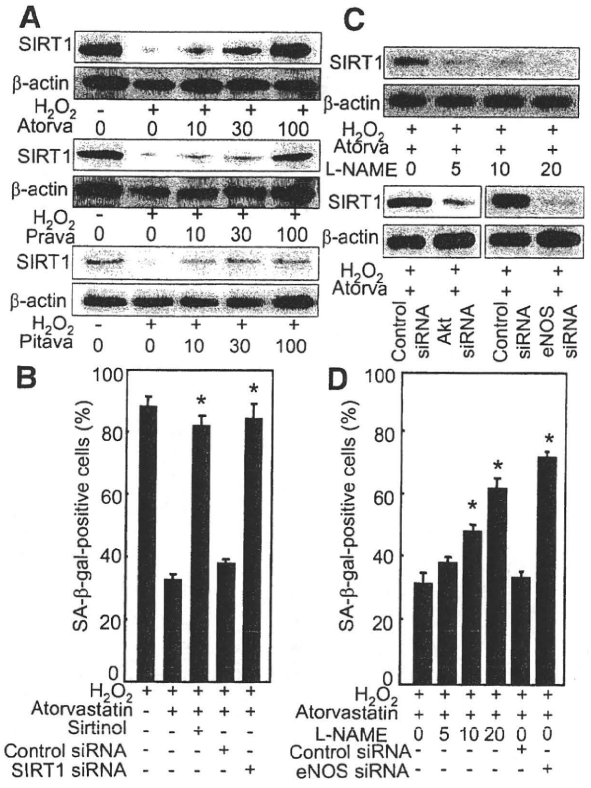

Figure 3. A, SIRT1 expression was dose-dependently increased by treatment with atorvastatin, pravastatin, or pitavastatin (10, 30, 100 nmol/L). B, Inhibition of SIRT1 by sirtinol or SIRT1 siRNA reversed the atorvastatin (100 nmol/L)-induced reduction of SA-βgal-positive cells (*$P<0.05$, n=3). C and D, Effect of L-NAME (5, 10, 20 μmol/L) for 24 hours, eNOS siRNA, or Akt siRNA on SIRT1 expression (C) and endothelial senescence (D) in atorvastatin (100 nmol/L)-treated cells (*$P<0.05$, n=3).

analog (8-Br-cAMP), or a cGMP analog (8-bromo [Br]-cGMP), expression of SIRT1 protein was markedly higher than that in untreated HUVEC.[25] Therefore, we hypothesized that an increase in eNOS activation caused by statins could promote the longevity gene, SIRT1. We found that atorvastatin, pravastatin, and pitavastatin significantly increased SIRT1 expression in a concentration-dependent manner for 10 days after treatment with H_2O_2 (Figure 3A). To determine the role of endogenous SIRT1 in premature senescence, HUVEC were treated with a SIRT1 chemical inhibitor, sirtinol, or SIRT1 siRNA. Knockdown of SIRT1 with siRNA was confirmed by Western blotting (Supplemental Figure ID). As shown in Figure 3B, SIRT1 inhibition abrogated the effect of atorvastatin on SA-βgal activity and specific senescent morphological changes (data not shown). Furthermore, to clarify the involvement of Akt/eNOS in the effect of statins, we examined the effect of Akt siRNA, eNOS siRNA, or an eNOS inhibitor, L-NAME, on SIRT1 expression and endothelial senescence. As shown in Figure 3C, treatment with Akt siRNA decreased SIRT1 expression. As shown in Figure 3C and 3D, treatment with eNOS siRNA or L-NAME decreased SIRT1 expression and the inhibitory effect of statins on senescence. These results indicate that SIRT1 could play an important role in the protective effect of statins against a senescent phenotype and that the Akt pathway is a direct target of statins to increase SIRT1 expression through eNOS activation. As previously reported, sirtinol or SIRT1 siRNA itself promotes endothelial senescence,[14] and we investigated whether statin treatment rescues the senescence induced by sirtinol alone or L-NAME alone. As shown in Supplemental Figure IC, atorvastatin did not reverse sirtinol- or L-NAME-induced senescence. In addition, we examined whether statin treatment itself affects eNOS and SIRT1 expression without oxidative stress. Treatment with atorvastatin increased eNOS and SIRT1 expression in HUVEC (Supplemental Figure IIC).

Direct Interaction of SIRT1 and eNOS Increases the Protective Effect Against Endothelial Senescence

As previously reported,[26] SIRT1 binds to, deacetylates, and activates eNOS directly in endothelial cells. To investigate whether the interaction of SIRT1 and eNOS contributes to the protective effect against cellular senescence, we examined the effect of overexpression of SIRT1 and eNOS on the senescence-like phenotype in HEK293 cells. Because HEK 293 cells lack an endogenous eNOS, we used HEK 293 cells to estimate accurate exogenous eNOS function. As shown in Supplemental Figure IIA and IIB, overexpression of eNOS alone did not inhibit SA-βgal activity or the senescent morphological appearance. In contrast, overexpression of SIRT1 inhibited the senescence-like phenotype. Furthermore, co-overexpression of SIRT1 and eNOS significantly inhibited the senescence-like phenotype. To confirm whether SIRT1 associates closely with eNOS, we performed coimmunoprecipitation of SIRT1 and eNOS. Coimmunoprecipitation showed that SIRT1 and eNOS associated with each other in human endothelial cells (Figure 4A). In addition, double immunofluorescent staining showed that endogenous SIRT1 and eNOS colocalized in the nucleus and perinuclear cytoplasm (Figure 4B). Moreover, to verify that eNOS is a substrate of SIRT1, we induced SIRT1 expression by treatment with DETA-NO, and immunoprecipitates of eNOS protein were immunoblotted with anti-acetyllysine antibody. Induction of SIRT1 by DETA-NO decreased the acetylation status of eNOS, and SIRT1 inhibition by sirtinol or SIRT1 siRNA reversed this (Figure 4C). Likewise, we found that treatment with atorvastatin had a similar effect, decreasing eNOS acetylation (Figure 4D). These results indicate that SIRT1 and eNOS interact with each other and accelerate the protective effect against a senescent phenotype (Figure 4E).

Statins Increase Mitochondria Biogenesis and Expression of Catalase Through Upregulation of SIRT1

Next, to clarify the molecular mechanisms of the antioxidative effect of SIRT1 induced by statins, we evaluated mitochondria biogenesis. As shown in Figure 5A, we found that senescent endothelial cells induced by H_2O_2 had decreased MitoTracker Red fluorescence compared with untreated cells. In contrast, treatment with atorvastatin partially restored the MitoTracker Red fluorescence. Inhibition of SIRT1 by siRNA abrogated the effect of atorvastatin. Moreover, Akt and eNOS siRNA also abrogated the effect of atorvastatin (Figure 5A). To address whether mitochondrial transcription was increased, mRNA levels of TFAM (the principal tran-

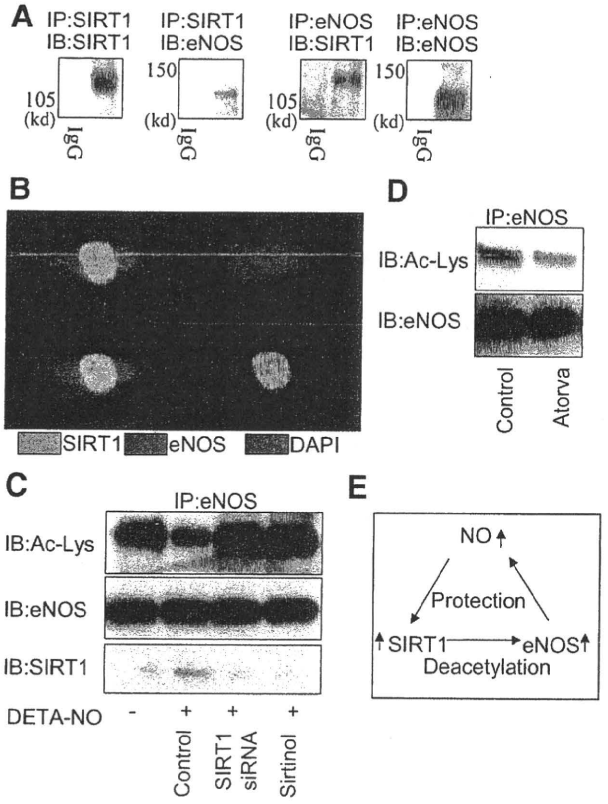

Figure 4. A, Coimmunoprecipitation of eNOS and SIRT1 in HUVEC. SIRT1 and eNOS were overexpressed, and whole-cell lysates were immunoprecipitated (IP) with anti-SIRT1 or anti-eNOS antibodies. Immunoprecipitates were immunoblotted (IB) with anti-SIRT1 and anti-eNOS antibodies. B, Double immunofluorescence for endogenous SIRT1 (green) and eNOS (red) in HUVEC. 4′,6-Diamidino-2-phenylindole (DAPI, blue) shows nuclear staining. C, SIRT1 expression was induced by treatment with DETA-NO (100 μmol/L) for 6 hours in the absence or presence of sirtinol (100 μmol/L) or SIRT1 siRNA, and immunoprecipitates of eNOS protein were immunoblotted with anti-acetyllysine antibody. D, Atorvastatin-treated (100 nmol/L) cells were lysed, and immunoprecipitates of eNOS protein were immunoblotted with anti-acetyllysine antibody. E, The SIRT1-eNOS axis modulates the protective effect of statins against endothelial senescence.

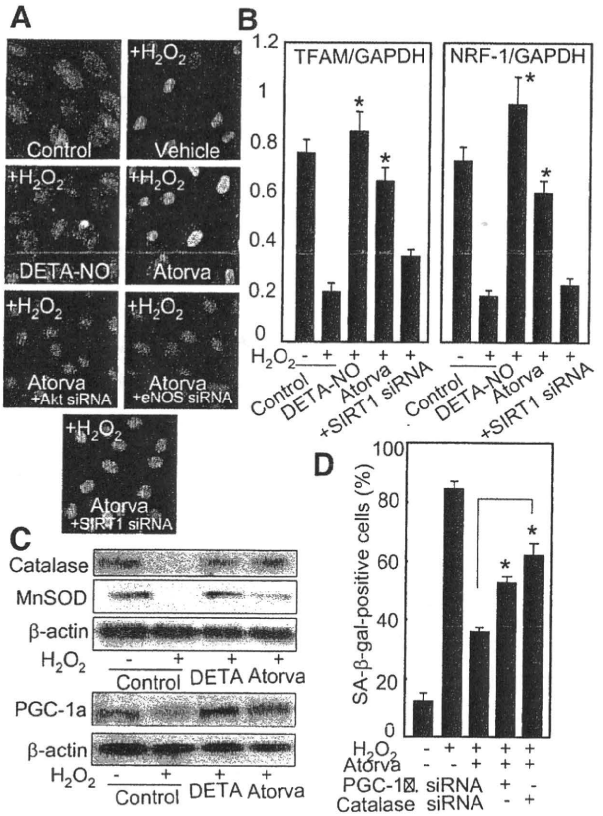

Figure 5. A, MitoTracker Red fluorescence was evaluated in atorvastatin (100 nmol/L)-treated cells at 10 days after addition of H_2O_2. Inhibition of SIRT1, eNOS, and Akt by siRNA abrogated the effect of atorvastatin. B, mRNA levels of TFAM and NRF-1 were quantified by real-time polymerase chain reaction. GAPDH was used as the internal control (*$P<0.05$). C, Expression of PGC-1α, MnSOD, and catalase were assessed by Western blot analysis. D, Knockdown of PGC-1α and catalase reversed the inhibitory effect on senescence of atorvastatin (100 nmol/L, *$P<0.05$, n=3).

scription factor involved in regulating mtDNA transcription) and NRF-1 were quantified by real-time polymerase chain reaction. TFAM and NRF-1 transcripts were increased by treatment with atorvastatin, and SIRT1 inhibition by siRNA completely reversed this (Figure 5B). Concomitantly, the expression of MnSOD and catalase were also increased (Figure 5C). PGC-1α is the principal regulator of mitochondria biogenesis. Therefore, we examined the expression of PGC-1α. As expected, treatment with atorvastatin increased the expression of PGC-1α. To clarify the involvement of PGC-1α and catalase, PGC-1α and catalase siRNA was transfected in atorvastatin-treated cells. Knockdown of PGC-1α and catalase reversed the inhibitory effect of atorvastatin on senescence (Figure 5D). Moreover, to confirm involvement of SIRT1 activation, we treated cells with the SIRT1 direct activator resveratrol. Treatment with resveratrol increased SIRT1, eNOS expression, and eNOS activation (Supplemental Figure IIIA and IIIB). As shown in Supplemental Figure IIIC, activation of SIRT1 by resveratrol inhibited a senescent phenotype, and knockdown of PGC-1α and catalase abrogated it. These results indicated that the molecular mechanism of the antioxidative effect of statins was attributable to increased MnSOD/catalase expression through upregulation of SIRT1 (Supplemental Figure IIID).

Administration of Pitavastatin Inhibits Vascular Endothelial Senescence in STZ-Diabetic Mice

To investigate whether statins have a protective effect against vascular endothelial senescence in vivo, we used STZ-diabetic mice, in which endothelial senescence has been documented.[27] We considered STZ-diabetic mice suitable for investigation of clinical settings. STZ-treated mice with and without pitavastatin administration had elevated plasma glucose associated with decreased plasma insulin level compared with control mice (Supplemental Figure IVA). Body weight, blood pressure, and pulse rate were unaltered in STZ-treated mice with and without pitavastatin (Supplemental Figure IVB). We resected the thoracic aorta of these mice and compared the senescent phenotype with and without pitavastatin administration (Figure 6A and 6B). The number of SA-βgal-stained cells was significantly increased in the

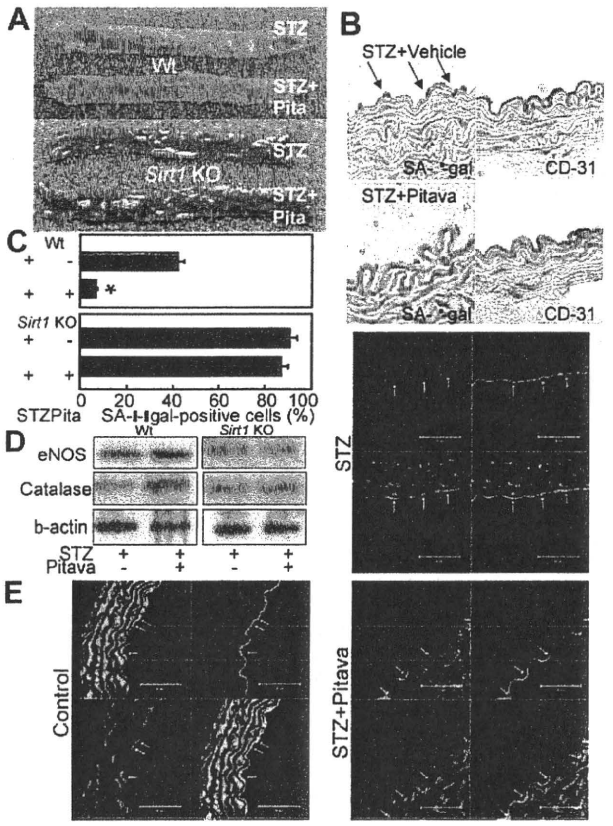

Figure 6. A, SA-βgal staining of thoracic aorta from C57/BL6 wild-type mice or *Sirt1*-heterozygous knockout mice receiving pitavastatin (3 mg/kg per day) at 7 days after a single intraperitoneal injection of STZ (60 mg/kg). B and C, Number of SA-βgal-stained cells in pitavastatin-treated thoracic aorta. SA-βgal-positive cells were mostly located on the luminal surface and stained for CD-31, a marker of vascular endothelial cells. D, The thoracic aortas were lysed, and Western blot was performed. Pitavastatin increased eNOS and catalase expression in the thoracic aorta of wild-type mice, but expression was unaltered in *Sirt1* KO (+/−) mice. E, Immunofluorescent staining for SIRT1 (green), platelet endothelial cell adhesion molecule 1 (red), and TOTO-3 (blue).

thoracic aorta of untreated mice, but it was decreased in the thoracic aorta of pitavastatin-treated mice (Figure 6C). However, in the haploinsufficient *Sirt1* KO (+/−) mice, the number of SA-βgal-stained cells was not completely restored in the thoracic aorta from pitavastatin-treated STZ-diabetic mice (Figure 6C). Cross-sections of aorta stained with SA-βgal showed that positive cells were mostly located on the luminal surface and stained for CD-31, indicating that blue staining originated from vascular endothelial cells and not from the extracellular matrix (Figure 6B). Consistent with in vitro studies, pitavastatin administration increased eNOS and catalase expression in the thoracic aorta of wild-type mice, but we observed unaltered eNOS and catalase expression in the haploinsufficient *Sirt1* KO (+/−) mice (Figure 6D). Immunostaining of sections for SIRT1 showed that SIRT1 expression in aortic endothelial cells was increased by treatment with pitavastatin (Figure 6E).

Discussion

The results of this study demonstrated that statins inhibit oxidative stress-induced endothelial senescence and that, subsequently, upregulation of SIRT1 plays a critical role in prevention of senescence through Akt pathway.

The mechanisms by which statins stimulate the expression and activation of eNOS appear to involve the geranylgeranyl pathway, because mevalonate, GGPP, and FPP reversed the inhibitory effect of statins on senescence. It is well known that inhibition of geranylgeranylation leads to inactivation of Rho kinase. However, pharmacological inhibitors of Rho kinase did not affect endothelial senescence, which indicated that the inhibitory effect of statins on senescence was not mediated by inhibition of Rho kinase. Moreover, treatment with statins increased the phosphorylation of Akt at Ser473. Treatment with Akt siRNA or LY294002, which inhibited phosphorylation of Akt at Ser473, abrogated the eNOS activation and antisenescent property of atorvastatin. These results demonstrate that statins activate the phosphatidylinositol 3-kinase/Akt pathway via isoprenylation, resulting in enhancement of eNOS expression and activation.

The free-radical theory of aging proposes that degenerative senescence is largely the result of the cumulative effect of reactive oxygen species.[28] Previous studies have shown that overexpression of SIRT1 antagonizes cellular senescence through acetylation of p53 with localization of the PML body.[10] In addition, SIRT1 binds to and targets eNOS for deacetylation at lysines 494 and 504 in human endothelial cells.[26] Recently, we reported that SIRT1 overexpression prevented the development of oxidative stress-induced premature senescence in human endothelial cells.[14] Although NO is known to be involved in reducing oxidative stress and the progression of atherosclerosis, the present study suggests that the interaction of SIRT1 with eNOS plays an important role in augmentation of the protective effect of statins against endothelial senescence (Figure 4E).

In this study, we examined the effect of pitavastatin on endothelial senescence, using STZ-diabetic mice as a clinical oxidative condition. Pitavastatin, a lipophilic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is categorized as a strong statin. Pitavastatin was chosen for this in vivo study because it is hardly metabolized by the cytochrome P450 system in the liver. The haploinsufficient *Sirt1* KO (+/−) mice did not show a senescent phenotype of aorta without STZ treatment (Supplemental Figure IVC). In contrast with wild-type mice, the haploinsufficient *Sirt1* KO (+/−) mice showed a senescent phenotype of aorta with STZ treatment, and pitavastatin did not recover it. These findings indicate that the maintenance of SIRT1 expression is important in developing stress tolerance.

It is now apparent that mitochondrial dysfunction is causal in many disease states, and improvement of mitochondria function could be an important therapeutic target. In this study, we observed that treatment with statins increased mitochondria biogenesis in SIRT1-dependent manner. In accordance with our results, it has been shown that overexpression or activation of SIRT1 regulates mitochondrial function and attenuates mitochondrial reactive oxygen species (mtROS) production and cellular H_2O_2 level in human coronary arterial endothelial cells.[29] We observed that expression of MnSOD and catalase were increased. In addition, previous study reported that resveratrol, an activator of

SIRT1, increases mitochondrial content in the vascular endothelium.[30] According to the mitochondrial theory of aging, mitochondria biogenesis reduces the flow of electrons per unit mitochondria; thus, statin-induced mitochondria biogenesis may be attributable to a reduction of oxidative stress in human endothelial cells.

Our results indicated that 100 nmol/L levels of statins are sufficient to exert protective effects against endothelial senescence. Considering that a 1 nmol/L level of statins was hardly able to prevent endothelial senescence under oxidative conditions in this study (data not shown), it becomes apparent that effective concentrations of statins are likely to be slightly higher. The use of statins is relatively safe, with few side effects. However, it should be noted that myopathy is the most common side effect, with symptoms ranging from fatigue, weakness, and pain to rhabdomyolysis.

In summary, we have shown that statins inhibit oxidative stress-induced endothelial senescence and that, subsequently, enhancement of SIRT1 expression through the Akt pathway plays a critical role in the inhibition of a senescent phenotype in human endothelial cells.

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Disclosures

None.

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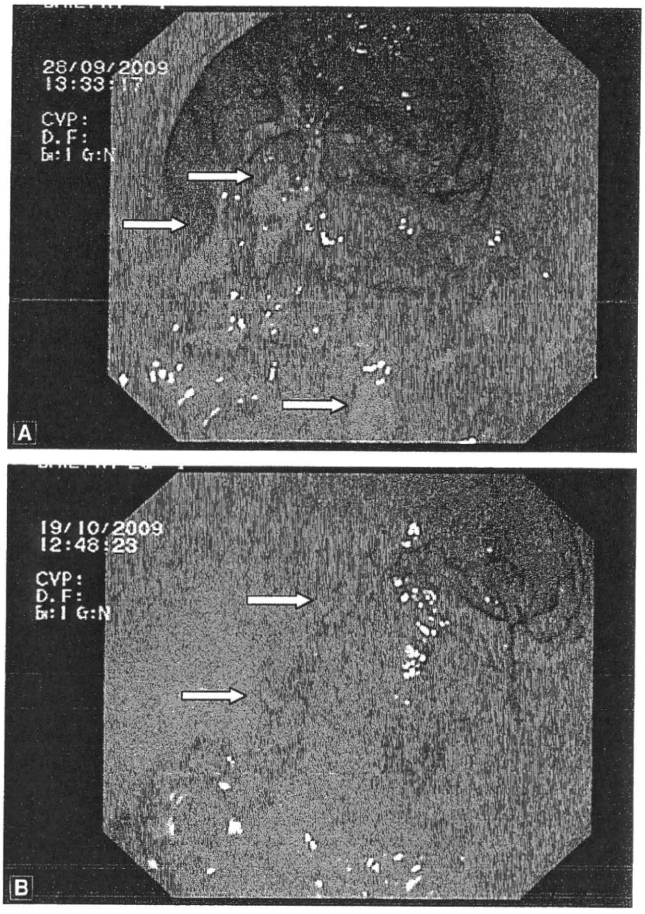

Figure 1. (A) Ulcerations in the left colon (arrows), seen on the first colonoscopy. (B) Colonoscopy after antiviral treatment, ulcerations are in phase of remission (arrows).

DISCUSSION

CMV colitis is a rare cause of diarrhea in older adults; it is more commonly seen in people who are immunosuppressed (with the human immunodeficiency virus or after bone marrow transplant), in whom it is often due to a virus reactivation, or in patients with preexisting inflammatory bowel disease.[4] Nevertheless, although it may be considered an invasive diagnostic test in a frail elderly patient, a sigmoidoscopy with biopsy should be considered as a necessary investigation if culture-negative diarrhea persists. Although some cases of CMV colitis are described in immunocompetent patients, when a diagnosis of CMV colitis is made, screening to exclude the presence of concomitant immunomodulating conditions or inflammatory bowel disease is necessary. In the clinical history of this patient, different coexisting immune-modulating conditions (diabetes mellitus, previous HCV infection, probable essential thrombocythemia) can be identified.

Most cases of CMV infection described in the literature are limited to the left colon, but the infection could theoretically involve all of the digestive tract. In this case, an ulceration was also found in the bulbar duodenum; because the ulcer was bloody, histology was not done, so it was not possible to confirm whether it was a location of CMV infection. Anyway, in a meta-analysis, in which the authors identified 44 cases of CMV bowel infection in immunocompetent patients, the extent of disease was not an independent predictor of survival.[3]

No conclusive statement regarding the need for specific antiviral treatment can be made from the available data in the literature. Although patients with no associated comorbidities seem to have a good rate of spontaneous remission, a trend for higher mortality has been reported in patients aged 55 and older and in patients with diseases affecting immune responses,[3] with a mortality rate of 31.8% in patients aged 55 and older. The patient in the current case belongs to this latter group at high risk of mortality, so it was thought that the antiviral treatment was mandatory. Nevertheless, randomized controlled trials are needed for a more-conclusive answer about antiviral treatment in immunocompetent patients suffering from severe CMV infection.

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EFFECTS OF TESTOSTERONE IN OLDER MEN WITH MILD-TO-MODERATE COGNITIVE IMPAIRMENT

To the Editor: Some population-based studies have found that endogenous testosterone levels are associated with general cognitive function,[1,2] and it has also been reported that testosterone levels are associated with physical and psychological functions, including cognition, in disabled older men,[3] but there have been few studies evaluating the effects of testosterone supplementation in men with cognitive impairment, and the results were inconsistent.[4–7] Thus, additional information is needed for frail or disabled older men with cognitive impairment as the targets of testosterone supplementation. Here, a pilot study to investigate the effect of oral testosterone supplementation for 6 months on

Table 1. Changes in Functional Parameters According to Treatment Group

Functional Parameters	Mean ± Standard Deviation								P-Value
	Testosterone				Control				
	Baseline	3 Months	6 Months	Difference: 0 to 6 Months	Baseline	3 Months	6 Months	Difference: 0 to 6 Months	
Mini-Mental State Examination	20.2 ± 4.5	21.8 ± 4.7	22.6 ± 6.5*	2.4 ± 3.1	21.9 ± 5.3	22.0 ± 4.6	22.0 ± 4.1	0.1 ± 2.7	.03
Hasegawa Dementia Scale, Revised	17.6 ± 5.9	18.2 ± 7.1	20.6 ± 7.3*	3.0 ± 4.3	19.6 ± 5.6	20.1 ± 7.0	18.8 ± 7.7	−0.8 ± 2.3	.02
Barthel Index	91 ± 12	89 ± 17	91 ± 15	0.5 ± 7.1	92 ± 10	91 ± 10	92 ± 7	0.4 ± 7.6	.70
Vitality Index	9.0 ± 0.9	9.3 ± 0.9	7.9 ± 1.3	−1.1 ± 1.0	9.0 ± 1.0	9.4 ± 1.0	9.4 ± 0.9	0.4 ± 1.0	.35

P-values are based on repeated-measures analysis of variance comparing the 6-month change between the groups.
*$P < .05$ compared with baseline.

cognitive function in Japanese older men with mild to moderate cognitive decline is reported.

Eleven men with cognitive impairment, mean age 81 ± 6, receiving long-term care, were assigned to take oral testosterone undecanoate 40 mg daily for 6 months after a breakfast containing 15 to 20 g of fat. The control group of 13 men matched for age and cognitive function were followed without testosterone treatment. Cognitive function was evaluated using the Mini-Mental State Examination (MMSE) and Hasegawa Dementia Scale, Revised (HDS-R) at baseline and at 3 and 6 months. Plasma hormone levels were also measured. The institutional review board approved the study protocol, and all participants or their families gave written informed consent.

At baseline, mean total and free testosterone levels, calculated using the Vermeulen equation,[8] were 14 ± 4 nmol/L and 246 ± 47 pmol/L, respectively. There were no significant differences between the groups in age, length of education, nutritional parameters, functional parameters, or plasma hormone levels. Fasting plasma testosterone levels in the morning did not change significantly during the study, whereas the post-dose levels increased up to 30 ± 8 nmol/L 6 hours after testosterone administration, as reported previously.[9] The changes in functional parameters in each group from baseline to 6 months are shown in Table 1. At 3 months, subjects who received testosterone treatment showed a nonsignificant increase in MMSE and HDS-R scores, whereas at 6 months, cognitive scores were significantly greater than at baseline. In the control group, both cognitive scores remained unchanged. The difference between the groups was significant at 6 months. Prostate-specific antigen and liver function were unchanged, and no adverse effects were observed.

No significant changes were observed in basic activities of daily living (ADL) and ADL-related vitality in either group (Table 1), possibly because these scores were preserved in most subjects at baseline; the Barthel Index and Vitality Index[10] were 91 ± 10 (full score = 100) and 9.0 ± 1.0 (full score = 10), respectively.

This preliminary study needs to be confirmed in a randomized controlled trial with a large sample size. Nevertheless, these results indicate the effects of testosterone treatment on cognitive function in frail elderly men.

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A CASE OF SELECTIVE SEROTONIN REUPTAKE INHIBITOR-INDUCED RAPID EYE MOVEMENT BEHAVIOR DISORDER

To the Editor: Rapid eye movement (REM) sleep behavior disorder (RBD) is often seen in older patients and is characterized by a loss of normal skeletal muscle atonia during REM sleep.[1,2] As a result, the disease manifests as nocturnal motor activity consistent with the enactment of dream content, for example grabbing the bed partner in response to a dream about falling from a cliff. RBD often results in injury to the patient, bed partner, or both.[1,2]

In perhaps up to two-thirds of cases, RBD is associated with neurodegenerative disorders, most notably the alpha-synucleinopathies (Parkinson's disease, Lewy body disease, multiple systems atrophy), often antedating other manifestations of these disorders by many years.[1–4] Other cases seem to be idiopathic, although it has been suggested that various medications, notably selective serotonin reuptake inhibitors (SSRI) and other antidepressants, may commonly induce RBD.[1,4,5] In spite of this assertion, there have been few supporting case reports.[5,6] The authors recently cared for a man who clearly developed RBD as a result of SSRI treatment; the use of the SSRI for posttraumatic stress disorder (PTSD) complicated the clinical picture.

CASE REPORT

An 87-year-old male World War II veteran had been treated for PTSD with associated nightmares but no nocturnal motor activity with bupropion and lorazepam. Past medical history was significant only for essential hypertension. In 1998, after many years of treatment, sertraline was added because of increasing symptoms. Within 6 months of adding sertraline, the patient developed frequent nocturnal motor behavior consistent with the content of his dreams and nightmares, for example punching and choking his wife in the context of a dream about being in a fight. As a result, he and his wife had suffered lacerations and contusions. Other behaviors included running out of his bedroom or running into a window. Upon awakening, he was able to recall portions of the dreams but was unaware of the motor behaviors.

Trials of temazepam, zolpidem, and trazodone were ineffective in improving these behaviors. Ultimately, a diagnosis of RBD was made based on the clinical presentation. Clonazepam 1 mg at bedtime was added, which resulted in a moderate decrease in the frequency of the nocturnal motor activity, from nightly to two or three times per week. After 3 months, sertraline was slowly tapered and discontinued, which resulted in a complete cessation of all nocturnal motor behavior. He remained free of nocturnal motor activity for 5 months, until sertraline was inadvertently restarted after the loss of his wife. Within 1 month of restarting sertraline, the nocturnal motor behavior returned. There has thus far been no evidence of dementia or of parkinsonism.

This patient's clinical presentation was typical of RBD; unfortunately, his and his wife's injuries were also typical. It seems clear that his RBD was SSRI induced; it developed after sertraline was started, did not definitively improve until it was stopped, and recurred after it was inadvertently restarted, and there was no evidence of parkinsonism or dementia over the previous 12 years. Although there are few published cases of SSRI-induced overt RBD, increased electromyography activity during REM sleep has been demonstrated in patients taking SSRIs. (None of the patients were being treated for PTSD.)[7]

The relationship between RBD and PTSD is complex and not fully investigated. There is clinical and polysomnographic evidence of greater motor activity during REM sleep in patients with PTSD,[8] and greater prevalence of RBD was noted in a cohort of patients with PTSD.[9] SSRIs are effective for PTSD-related nightmares[10] but may cause RBD, clonazepam is effective for RBD[1,2,4] but not for PTSD-related nightmares,[10] and RBD is not associated with the typical diurnal symptoms of PTSD. In spite of his long history of PTSD and related nightmares, this patient had never exhibited any significant motor activity during sleep until the SSRI was started.

RBD is relatively common in geriatric practice and should be explored in any patient with nocturnal injuries or motor activity. RBD responds well to treatment, generally with clonazepam. Discontinuation of SSRIs or changing to

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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 ± 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 ± 3.2 vs −2.1 ± 2.2, $P < 0.05$; HDS-R, +2.8 ± 2.8 vs −0.3 ± 4.1, $P < 0.05$; Barthel Index, +3.7 ± 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 impairment[24] 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 μmol/L. The exclusion criteria were history of stroke, extremely low ADL status (Barthel Index[26] <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, MMSE[22] 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, μmol/L	1.8 ± 0.6	4.5 ± 1.3*	5.6 ± 2.9*	3.8 ± 2.8	1.6 ± 0.8	1.8 ± 1.0	1.7 ± 0.8	−0.02 ± 0.4	<0.01
DHEA, nmol/L	7.6 ± 4.7	12.2 ± 4.8*	13.7 ± 7.7*	6.1 ± 8.2	6.6 ± 3.1	7.3 ± 3.7	7.4 ± 4.5	0.9 ± 2.8	0.04
Testosterone, nmol/L	1.4 ± 0.4	2.3 ± 0.7*	2.3 ± 0.8*	0.9 ± 0.8	1.4 ± 0.7	1.4 ± 0.7	1.6 ± 0.8	0.2 ± 0.5	<0.01
Estradiol, pmol/L	88 ± 52	92 ± 48	101 ± 37	13 ± 51	70 ± 26	68 ± 20	67 ± 42	−4.0 ± 38	0.17
Functional parameters									
MMSE	24.0 ± 4.2	24.1 ± 4.6	24.6 ± 4.3	0.6 ± 3.2	23.4 ± 4.4	23.1 ± 5.4	21.3 ± 5.0**	−2.1 ± 2.2	0.04
HDS-R	19.9 ± 5.8	20.5 ± 7.3	22.7 ± 6.3**	2.8 ± 2.8	21.7 ± 5.6	22.1 ± 5.6	21.3 ± 6.4	−0.3 ± 4.1	0.04
Barthel Index	89.6 ± 9.4	92.7 ± 6.5	93.3 ± 6.8	3.7 ± 7.1	89.7 ± 6.4	86.9 ± 7.2	87.0 ± 6.7*	−2.7 ± 4.6	0.04
Vitality Index	9.8 ± 0.6	9.7 ± 0.5	9.7 ± 0.7	−0.1 ± 1.0	9.9 ± 0.3	9.8 ± 0.5	9.7 ± 1.0	−0.3 ± 1.0	0.80
GDS	7.0 ± 4.4	6.2 ± 3.4	6.6 ± 3.7	−0.4 ± 1.7	7.0 ± 4.0	8.3 ± 3.9	7.5 ± 3.5	0.5 ± 3.3	0.60

Values are shown as mean ± 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 ± 2.11, 3.90 (mean ± standard deviation) and 3.42 ± 2.01 μmol/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 ± SD Baseline	3 months	6 months	Change (0–6 months)	*P*
Mobility (55)					
DHEA	46.9 ± 9.2	48.2 ± 6.0	49.2 ± 5.2	2.3 ± 5.4	0.01
Control	47.5 ± 5.4	46.2 ± 5.5	45.0 ± 4.3*	−3.7 ± 3.9	
Self care (45)					
DHEA	42.7 ± 6.1	44.5 ± 1.5	43.1 ± 2.5	0.4 ± 6.9	0.96
Control	41.8 ± 4.2	42.5 ± 3.4	41.2 ± 4.3	0.7 ± 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 ± SD Baseline	3 months	6 months	Change (0–6 months)	*P*
Orientation (10)					
DHEA	8.3 ± 1.9	8.0 ± 2.7	7.5 ± 3.0	−0.1 ± 1.2	0.28
Control	8.3 ± 1.9	8.0 ± 2.8	7.5 ± 2.9	−0.7 ± 1.7	
Verbal memory (9)					
DHEA	5.7 ± 2.1	6.5 ± 2.3	6.7 ± 2.5†	1.0 ± 1.9	0.79
Control	6.5 ± 1.7	7.5 ± 1.8	7.0 ± 1.9	0.5 ± 1.7	
Attention and calculation (5)					
DHEA	2.3 ± 1.9	2.8 ± 2.0	2.7 ± 1.8	0 ± 2.3	0.79
Control	2.0 ± 1.7	1.9 ± 1.2	1.8 ± 1.5	−0.5 ± 1.4	
Visual memory (5)					
DHEA	3.6 ± 0.9	3.6 ± 1.3	3.8 ± 1.2	0.3 ± 1.1	0.91
Control	3.6 ± 1.3	3.9 ± 0.9	3.9 ± 1.0	0.5 ± 1.1	
Language comprehension (9)					
DHEA	8.5 ± 0.8	7.8 ± 2.5	8.7 ± 0.7	0.1 ± 0.3	0.12
Control	8.5 ± 0.8	8.5 ± 0.8	8.4 ± 1.1	−0.1 ± 0.9	
Verbal fluency (5)					
DHEA	2.8 ± 3.3	2.5 ± 2.0	4.3 ± 1.1*	1.5 ± 1.7	0.01
Control	3.2 ± 1.9	3.8 ± 1.6	3.3 ± 1.9	0.1 ± 2.1	
Performance (7)					
DHEA	5.7 ± 0.7	5.5 ± 0.7	4.8 ± 0.4**	−0.8 ± 0.6	0.36
Control	5.6 ± 0.6	5.1 ± 0.6	4.5 ± 0.9**	−1.1 ± 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. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.1$ 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.

Acknowledgments

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ORIGINAL ARTICLE

Association of low testosterone with metabolic syndrome and its components in middle-aged Japanese men

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Epidemiological studies have shown that low testosterone is associated with metabolic syndrome (MetS) in Caucasian men. We investigated whether testosterone level is related to the prevalence of MetS in middle-aged Japanese men. A cross-sectional survey was conducted in 194 men aged 30–64 years (49 ± 9). Blood sampling was performed in the morning after a 12-h fast, and the relationship between plasma hormone and MetS was analyzed. Low total testosterone was associated with MetS according to the Japanese criteria (HRs of 2.02 by quartile of testosterone; 95% CI=1.43–2.87) and the International Diabetes Federation criteria (HRs of 1.68 by quartile of testosterone; 95% CI=1.25–2.25). Age-adjusted regression analyses revealed that testosterone was significantly related to the MetS parameters of obesity (β=−0.365 and −0.343 for waist circumference and body mass index, respectively), hypertension (β=−0.278 and −0.157 for systolic and diastolic blood pressure, respectively), dyslipidemia (β=−0.242 and 0.228 for triglycerides and high-density lipoprotein cholesterol, respectively), insulin resistance (β=−0.253 and −0.333 for fasting plasma glucose and homeostasis model assessment of insulin resistance, respectively) and adiponectin (β=0.216). Inclusion of waist circumference into the model largely weakened the association of testosterone with other metabolic risk factors. In contrast, high estradiol was associated with MetS and its parameters, mostly attributing to the positive correlation between estradiol and obesity. Dehydroepiandrosterone sulfate was not associated with MetS or its parameters. These results suggest that low testosterone is associated with MetS and its parameters in middle-aged Japanese men. The association between estradiol and MetS needs further investigation.
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Keywords: androgen; estrogen; insulin resistance; obesity; sex hormone

INTRODUCTION

There is growing awareness that metabolic syndrome (MetS) is one of the most important threats to public health because of its association with type 2 diabetes mellitus, cardiovascular disease and mortality.[1–3] In men, it is well established that endogenous androgens decline with advancing age,[4] and low testosterone levels have been associated with insulin resistance,[5] type 2 diabetes,[6,7] hypertension[8] and increased cardiovascular and all-cause mortality.[9,10] Moreover, men with low testosterone are likely to have more components of MetS in cross-sectional studies,[11–13] and longitudinal studies show that lower total testosterone predicts higher frequency of MetS.[14,15] These data were mostly from studies with Caucasian men in western countries. Regarding Japanese men, one study showed that testosterone was positively correlated with plasma adiponectin.[16] However, there are no reports showing a relationship between testosterone and MetS or its components in Japanese men.

Recently, we reported that low testosterone is an independent determinant of endothelial dysfunction in middle-aged men[17] and is a predictor of cardiovascular events in men with coronary risk factors,[18] suggesting a link between testosterone and cardiovascular pathology. Given these findings, this study investigated the relationship of endogenous testosterone with MetS in middle-aged Japanese men.

METHODS

Subjects

Enrollment screening included consecutive, apparently healthy male subjects aged 30–64 years who underwent medical examinations at either our department or at two clinics located in Tokyo. After exclusion of subjects who met the exclusion criteria, 194 subjects (104 from our department and 90 from the clinics) were enrolled. Exclusion criteria included history of cardiovascular disease (stroke, coronary heart disease, congestive heart failure and peripheral arterial disease), malignancy or overt endocrine disease or use of steroid hormones, because these conditions may influence plasma sex hormones and/or the components of MetS. Other exclusion criteria were diabetic subjects

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on insulin injection or hypoglycemic agent drugs or with hemoglobin A1c >8%, and subjects on β-blockers[19] or fibrates. History, physical examination and laboratory tests were performed for all subjects. Of the included subjects, 23% (n=44) were taking anti-hypertensive drugs (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers and diuretics), and 22% were taking statins. Each subject gave written, informed consent before study enrollment. The study protocol was approved by the ethics committee of the Graduate School of Medicine at the University of Tokyo.

Assays of metabolic risk factors and plasma hormones

Clinical information was collected at baseline when each patient attended the initial medical examination. Blood sampling and measurement of height, weight, waist circumference and blood pressure were performed in the morning after a 12-h overnight fast. Blood pressure was measured at least twice using an automated, digital electrosphygmomanometer (Omron Healthcare, Kyoto, Japan) on the non-dominant arm in a sitting position, and the average was used for statistical analysis.

Serum total cholesterol and triglyceride were measured enzymatically, and serum high-density lipoprotein (HDL) cholesterol was measured by the heparin-$Ca^{2+}Ni^{2+}$ precipitation method. Low-density lipoprotein cholesterol was determined using the Friedewald formula or the direct, liquid, selective detergent method when triglycerides were >400 mg per 100 ml. Plasma glucose was assayed by the glucose oxidase method, and hemoglobin A1c was measured by high-performance liquid chromatography. Plasma total testosterone, dehydroepiandrosterone sulfate and estradiol were determined using sensitive radioimmunoassays. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin ($\mu IU\,ml^{-1}$)×fasting plasma glucose (mg per 100 ml)/405. Patients with a fasting plasma glucose >140 mg per 100 ml were excluded from the HOMA-IR calculation because of a lack of data reliability. Serum adiponectin was measured using an enzyme-linked immunosorbent assay (Human Adiponectin ELISA kit, Otsuka Pharmaceutical, Tokyo, Japan). These assays were performed by a commercial laboratory (SRL, Tokyo, Japan). The intra-assay coefficients of variation for the measurements were <5%.

Definition of MetS

We applied both the Japanese criteria[20] and the International Diabetes Federation (IDF) criteria for Japanese ethnicity[21] for the diagnosis of MetS. In the Japanese criteria, MetS was diagnosed when waist circumference ⩾85 cm and two or more of the following three components were present: (1) HDL cholesterol <40 mg per 100 ml and/or triglyceride ⩾150 mg per 100 ml; (2) systolic blood pressure ⩾130 mm Hg and/or diastolic blood pressure ⩾85 mm Hg and (3) fasting plasma glucose ⩾110 mg per 100 ml. Subjects taking anti-hypertensive medications were considered hypertensive for statistical purposes.

In the IDF criteria for Japanese ethnicity, MetS was diagnosed when waist circumference ⩾85 cm and two or more of the following four components were present: (1) HDL cholesterol <40 mg per 100 ml; (2) triglyceride ⩾150 mg per 100 ml; (3) systolic blood pressure ⩾130 mm Hg and/or diastolic blood pressure ⩾85 mm Hg and (4) fasting plasma glucose ⩾100 mg per 100 ml. Subjects taking anti-hypertensive medications were considered hypertensive for statistical purposes.

Data analysis

Values are expressed as the mean ± s.d. in the text unless otherwise stated. Pearson's simple correlation coefficients were calculated between plasma hormones and the number of MetS components. Differences between the quartile groups of sex hormones were analyzed using one-factor ANOVA followed by the Newman–Keuls' test. Logistic regression analysis was performed to determine the association of sex hormones with the diagnosis of MetS. Furthermore, multiple regression analysis was performed to determine the association between sex hormones and metabolic risk factors for MetS. A value of $P<0.05$ was considered statistically significant. The data were analyzed using SPSS (Version 17.0, SPSS, Chicago, IL, USA).

RESULTS

Sex hormones and MetS criteria

Characteristics of the study subjects are shown in Table 1. Twenty-three and 32% of the subjects were diagnosed with MetS according to the Japanese criteria and the IDF criteria, respectively. The prevalence is comparable with that reported in middle-aged Japanese men.[22,23]

As plasma total testosterone was negatively correlated with the number of MetS components (Figure 1a), the association of testosterone with MetS was analyzed by quartile of testosterone. As shown in Figure 2a, lower testosterone was associated with a step-wise increase in the number of MetS components. Age-adjusted logistic regression analysis revealed that the hazard ratios for MetS diagnosis by quartile decline of testosterone were 2.02 (95% CI=1.43–2.87) and 1.68 (95% CI=1.25–2.25) according to the Japanese criteria and the IDF criteria, respectively.

Interestingly, plasma estradiol was positively correlated with the number of MetS components (R=0.285, $P<0.001$); therefore, the association with MetS was also analyzed by quartile of estradiol. As shown in Figure 2b, higher estradiol was associated with a step-wise increase in the number of MetS components. Age-adjusted logistic regression analysis revealed that the hazard ratios for MetS diagnosis by quartile increment of estradiol were 1.48 (95% CI=1.06–2.06) and 1.63 (95% CI=1.20–2.21) according to the Japanese criteria and the IDF criteria, respectively. Dehydroepiandrosterone sulfate was not associated with MetS components or diagnosis (data not shown).

Table 1 Characteristics of study subjects (N=194)

Age (years)	49 ± 9	[30–64]
Body mass index ($kg\,m^{-2}$)	25.2 ± 4.0	[17.3–41.9]
Waist circumference (cm)	87 ± 10	[69–125]
Hip circumference (cm)	96 ± 7	[80–125]
Waist/hip ratio	0.94 ± 0.06	[0.78–1.09]
Systolic blood pressure (mm Hg)	126 ± 14	[95–183]
Diastolic blood pressure (mm Hg)	79 ± 11	[50–128]
Triglycerides (mg per 100 ml)	162 ± 135	[32–880]
HDL cholesterol (mg per 100 ml)	54 ± 16	[26–110]
Free fatty acids ($mEq\,l^{-1}$)	0.53 ± 0.28	[0.08–2.08]
LDL cholesterol (mg per 100 ml)	128 ± 29	[54–213]
Fasting plasma glucose (mg per 100 ml)	98 ± 13	[76–158]
Hemoglobin A1c (%)	5.2 ± 0.6	[4.0–8.0]
Insulin ($\mu U\,ml^{-1}$)	6.7 ± 4.0	[1.0–21.2]
HOMA-IR	1.64 ± 1.04	[0.21–5.50]
Total testosterone ($nmol\,l^{-1}$)	19.1 ± 6.2	[4.6–38.2]
DHEA-S ($\mu mol\,l^{-1}$)	5.89 ± 2.37	[1.12–12.0]
Estradiol ($pmol\,l^{-1}$)	92.5 ± 43.7	[18.4–216.6]
Metabolic syndrome (MetS) and its components		
MetS (Japanese criteria), n (%)		44 (23)
MetS (IDF criteria), n (%)		62 (32)
Waist circumference ⩾85 cm, n (%)		110 (56)
High blood pressure, n (%)		89 (46)
HDL cholesterol <40 mg per 100 ml, n (%)		34 (18)
Triglycerides ⩾150 mg per 100 ml, n (%)		79 (41)
Fasting plasma glucose ⩾110 mg per 100 ml, n (%)		23 (12)
Fasting plasma glucose ⩾100 mg per 100 ml, n (%)		73 (38)

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IDF, International Diabetes Federation; LDL, low-density lipoprotein.
Values are expressed as the mean ± s.d. (range). High blood pressure was defined if subjects showed systolic blood pressure ⩾130 mm Hg and/or diastolic blood pressure ⩾85 mm Hg, or were taking antihypertensive medications.

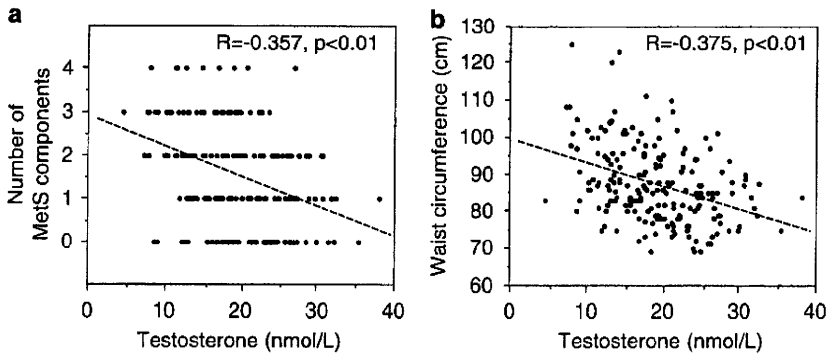

Figure 1 Scattergrams and regression lines (dotted lines) showing the correlation between testosterone and the number of metabolic syndrome (MetS) components (**a**) or waist circumference (**b**).

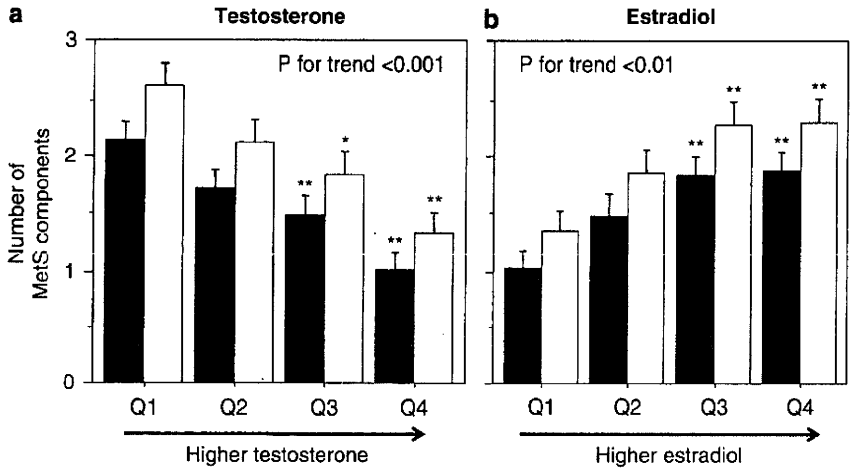

Figure 2 Number of metabolic syndrome (MetS) components according to quartiles of plasma testosterone (**a**) and estradiol (**b**). MetS components were defined according to the Japanese criteria (closed bars) and the IDF criteria for Japanese ethnicity (open bars). Values are expressed as the mean ± s.e.m. $^{*}P<0.05$, $^{**}P<0.01$ *vs.* Q1. Cut offs of the quartiles were 14.1, 18.7 and 23.4 nmol l^{-1} (405, 540 and 674 ng per 100 ml) for testosterone, and 55, 101 and 125 pmol l^{-1} (15.0, 27.5 and 34.0 pg ml^{-1}) for estradiol.

Sex hormones and metabolic risk factors

The associations of plasma sex hormones with each of the metabolic risk factors were analyzed. As shown in Table 2, the unadjusted model shows that testosterone was significantly related to parameters of MetS except for diastolic blood pressure. Testosterone was not related to low-density lipoprotein cholesterol, but this parameter is not included in the definitions of MetS used here. Adjustment for age did not considerably influence the results of the regression analysis, but the association between testosterone and diastolic blood pressure became significant after adjustment for age. In contrast, inclusion of waist circumference into the model weakened the association of testosterone with metabolic risk factors. As a result, systolic blood pressure, triglycerides, fasting plasma glucose and HOMA-IR were significantly related to testosterone. The significant association for diastolic blood pressure, HDL cholesterol, free fatty acids, hemoglobin A1c, insulin and adiponectin were attenuated after adjustment for age and waist circumference. Adjustment for body mass index or waist/hip ratio instead of waist circumference showed similar results (data not shown).

As shown in Table 3, estradiol showed weaker association than testosterone with parameters of MetS, but was significantly related to body mass index, waist circumference, systolic blood pressure, HDL

Table 2 Multiple regression analysis determining the impact of plasma testosterone on metabolic risk factors

	Unadjusted	*Age adjusted*	*Age+waist adjusted*
Body mass index	−0.376*	−0.343*	ND
Waist circumference	−0.378*	−0.365*	ND
Waist/hip ratio	−0.353*	−0.384*	ND
Systolic blood pressure	−0.230**	−0.278*	−0.169***
Diastolic blood pressure	−0.114	−0.157***	−0.098
Triglycerides	−0.247*	−0.242*	−0.182***
HDL cholesterol	0.252*	0.228**	0.065
Free fatty acids	−0.208**	−0.209**	−0.137
LDL cholesterol	−0.054	−0.056	−0.020
Fasting plasma glucose	−0.231**	−0.253**	−0.228**
Hemoglobin A1c	−0.166***	−0.220**	−0.137
Insulin	−0.331*	−0.307*	−0.129
HOMA-IR	−0.349*	−0.333*	−0.159***
Adiponectin	0.222**	0.216**	0.046

Abbreviations: HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; ND, not determined.
Regression coefficients with plasma testosterone as an independent variable and each of risk factors as a dependent variable are shown. Age and/or waist circumference were included in multiple regression models as indicated. $^{*}P<0.001$, $^{**}P<0.01$, $^{***}P<0.05$.

Table 3 Multiple regression analysis determining the impact of plasma estradiol on metabolic risk factors

	Unadjusted	*Age adjusted*	*Age+waist adjusted*
Body mass index	0.279*	0.260*	ND
Waist circumference	0.346*	0.338*	ND
Waist/hip ratio	0.102	0.082	ND
Systolic blood pressure	0.133	0.158**	0.042
Diastolic blood pressure	0.036	0.058	−0.002
Triglycerides	0.105	0.094	−0.012
HDL cholesterol	−0.207***	−0.193***	−0.040
Free fatty acids	0.087	0.091	0.049
LDL cholesterol	−0.056	−0.056	−0.094
Fasting plasma glucose	0.130	0.141	0.095
Hemoglobin A1c	0.040	0.067	−0.030
Insulin	0.240***	0.228***	0.038
HOMA-IR	0.250***	0.243***	0.060
Adiponectin	−0.267*	−0.262*	−0.114

Abbreviations: HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; ND, not determined.
Regression coefficients with plasma estradiol as an independent variable and each of risk factors as a dependent variable are shown. Age and/or waist circumference were included in multiple regression models as indicated. *$P<0.001$, **$P<0.05$, ***$P<0.01$.

cholesterol, insulin, HOMA-IR and adiponectin after adjustment for age. Further adjustment for waist circumference, body mass index or waist/hip ratio (Table 3 and data not shown) eliminated the significant associations between estradiol and these metabolic parameters. Dehydroepiandrosterone sulfate was not significantly related to parameters of MetS in unadjusted or adjusted analyses (data not shown).

DISCUSSION

In this study, cross-sectional analysis of 194 middle-aged Japanese men showed that low testosterone is positively related to MetS, MetS components and additional metabolic risk factors. Adjustment for obesity parameters such as waist circumference, body mass index and waist/hip ratio greatly diminished the association, but low testosterone retained weak associations with some metabolic risk factors including systolic blood pressure, triglycerides, fasting plasma glucose and HOMA-IR. Taken together, results in this statistical model suggest that abdominal obesity is an important contributor to the association between low testosterone and MetS, but additional factors may also impact testosterone. To our knowledge, this is the first report showing the significant association between low testosterone and MetS in Japanese men.

Several mechanisms have been suggested for the causal relationship between low testosterone and abdominal obesity. Activation of the lipoprotein lipase and lipolysis[24] may explain the effect of testosterone on adipose tissue. Many studies including a medium-sized, randomized controlled trial[25] and a meta-analysis[26] showed the inverse effect of testosterone on adiposity. Conversely, it has been reported that men with MetS are prone to hypogonadism.[27] This finding might be due to elevated leptin levels that interfere with gonadotropin-stimulated androgen production[28] and to increased aromatase activity in adipose tissue that leads to higher circulating estradiol and suppression of testosterone production by negative feedback.[29] These findings suggest a bi-directional causal relationship between low testosterone and obesity.

After adjustment for waist circumference, testosterone was weakly but significantly related to some metabolic risk factors including systolic blood pressure, triglycerides, fasting plasma glucose and HOMA-IR, which is consistent with earlier reports.[5,6,8,12] Testosterone is likely to be involved in the pathogenesis of MetS, irrespective of obesity. For example, testosterone increases the hepatic production of apolipoprotein A-1 and consequently increases HDL cholesterol,[30] improves insulin sensitivity and increases muscle strength.[31] There was no significant correlation between age and testosterone ($R=0.114$, $P=0.12$). This result may be because the cohort was limited to middle-aged men (30–64 years old) . However, age was included in the multivariate analyses in this study, because it is well established that testosterone declines with age.[4]

The positive association found between testosterone and adiponectin is in agreement with earlier reports.[16,32,33] However, the direct action of testosterone on adiponectin production/secretion might be different from these findings, because testosterone decreases adiponectin secretion in mice and in adipocytes.[34,35] Accordingly, abdominal obesity may underlie the positive correlation between testosterone and adiponectin in men.

In this study, estradiol was associated positively with MetS and its components, consistent with an earlier report.[12] This relationship may be independent of testosterone because estradiol was not correlated with testosterone by simple regression analysis ($R=-0.019$, $P=0.80$), and the inclusion of both testosterone and estradiol into the multiple regression model as covariates did not influence the association of each other with MetS parameters (data not shown). The relationship between estradiol and MetS might be attributed to increased aromatase activity and subsequent elevation of circulating estradiol in obese subjects.[29] Increased estradiol may subsequently suppress pituitary function,[29] and lead to a further decrease in testosterone. Comprehensive assessment of sex hormone, gonadotropin and components of MetS reveal a causal relationship. Unfortunately, we could not measure gonadotropin because of limited plasma. Further investigation is needed to address the mechanistic and pathophysiological interactions between sex hormones and MetS.

There are some limitations to our study. First, the cross-sectional design does not clarify the causal relationship between sex hormones and MetS. As there may be bi-directional causalities as mentioned above, longitudinal follow-up studies and hormone replacement studies should be performed in Japanese populations. Second, active forms of testosterone such as bioavailable and calculated free testosterone were not measured. A direct assay of bioavailable testosterone or of sex hormone-binding globulin (required for free testosterone calculation) was not available for the study. Third, the potential influence of medications on the measured parameters cannot be denied, although the exclusion of subjects on statins ($n=40$) or anti-hypertensive drugs ($n=44$) did not seriously affect the association of testosterone with waist circumference (statins, $R=-0.304$, $P<0.01$; anti-hypertensives, $R=-0.337$, $P<0.01$) and the number of MetS components (statins, $R=-0.274$, $P<0.01$; anti-hypertensives, $R=-0.278$, $P<0.01$). Fourth, because the sample size ($n=194$) is relatively small, the finding needs to be confirmed in a larger cohort.

In summary, this study suggests that low testosterone is associated with MetS and its parameters in middle-aged Japanese men. We also found a positive but weaker association between estradiol and MetS. These associations were largely attenuated by adjustment for waist circumference. Our results reinforce the need to address the causal relationship and pathophysiological interactions between sex hormones and MetS.

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