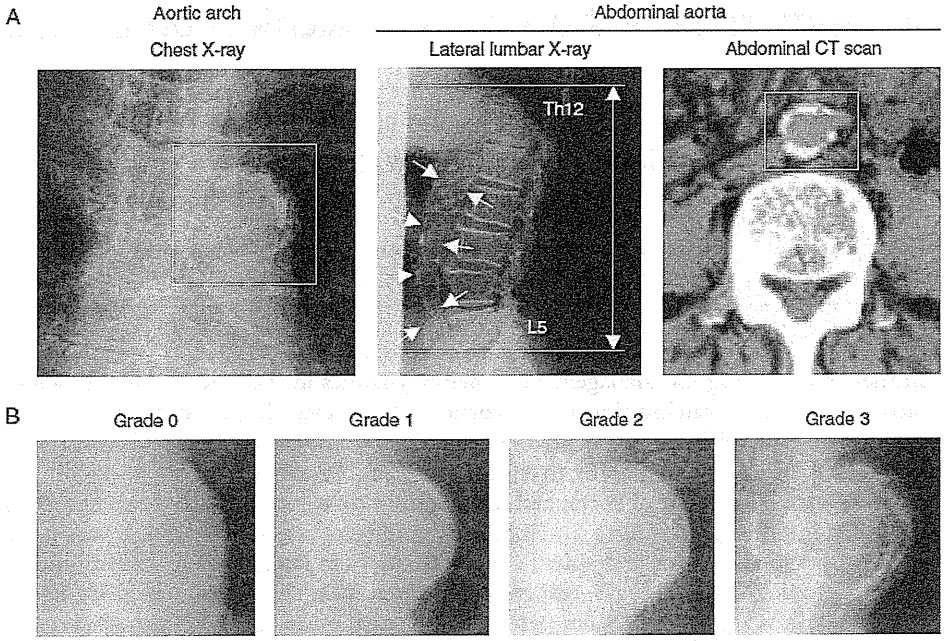

Fig. 1. Clinical assessment of calcification in aortic arch and abdominal aorta.

(A) Aortic calcification was assessed using three independent non-invasive examinations. The extent of aortic arch calcification (AAC) in a simple postero-anterior chest X-ray was divided into four grades. Calcification level in the abdominal aorta was assessed by quantitative measurements using two examinations, lateral radiograph of the lumbar spine (sum length; mm) and plain abdominal CT scan (%ACI). (B) Representative chest X-rays for each AAC grade are shown.

tram-track[13]. In contrast, intimal calcification is characteristically identified as a spotty and patchy radiopaque finding; however, it is difficult to distinguish these calcified changes in the arterial wall solely by radiographic techniques without a pathological approach. Recently, high-tech non-invasive examinations, such as electron beam-computer tomography (EB-CT) and multi-detector row CT (MD-CT), have been shown to be the gold standard for evaluating coronary artery calcification (CAC), with the power of quantifying its severity and progression[14]. Several reports have demonstrated that the extent of CAC as assessed by these examinations is a good predictor of coronary events[15, 16]; however, these examinations can not be easily or commonly performed.

In this study, we evaluated the extent of AAC by reviewing a chest X-ray, and compared it with abdominal aortic calcification. In addition, the AAC grade was evaluated in comparison with traditional atherosclerotic risk factors.

Methods

Study Population

First, the accuracy of two independent examinations to assess the extent of abdominal aortic calcification was confirmed in 27 patients who underwent both examinations described below. Second, AAC grade was determined by reviewing a simple chest X-ray in 239 consecutive asymptomatic outpatients (male/female=115/124, mean age 61.9±10.8 years) and was compared with the extent of abdominal aortic calcification. The Medical Ethics Committee of The University of Tokyo approved this study. Informed consent was obtained from all patients before the study.

Assessment of Aortic Arch Calcification

The extent of AAC was assessed in a routine postero-anterior chest X-ray. As shown in **Fig. 1**, AAC extent was divided into four grades according to the categorization proposed in a previous report[17]. Briefly, we scored the area of calcification as four grades: grade 0, no visible calcification; grade 1, small spots of calcification or a single thin area of calcification of the aortic knob; grade 2, one or more areas of thick calcification; grade 3, circular calcification of the aortic knob.

Quantitative Assessment of Abdominal Aortic Calcification

To quantify the extent of abdominal aortic calci-

fication, two independent examinations were carried out (**Fig. 1**). First, calcification visualized on a lateral radiograph of the lumbar spine was assessed. Briefly, we measured the sum length of the calcified lesions in the anterior and posterior walls of the abdominal aorta from thoracic vertebra no. 12 to lumbar vertebra no. 5. Second, an index of lower abdominal aortic calcification using a plain CT scan was also measured, as previously described[18]. Using the TES-100 image software program, the percentage of calcified area against the whole vascular area was calculated from images of four consecutive slices just above the bifurcation of the common iliac arteries. The index (abdominal aortic calcification index: %ACI) was presented as an average value.

Atherosclerotic Risk Factors

Hypertension was defined as a systolic blood pressure (BP) of more than 140 mmHg, diastolic BP of more than 90 mmHg, and/or use of anti-hypertensive drugs. To measure serum low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride, a blood sample was obtained after overnight fasting. Dyslipidemia was defined as an LDL-C level of more than 140 mg/dL, HDL-C level of less than 40 mg/dL, triglyceride level of more than 150 mg/dL and/or use of lipid-lowering drugs. Diabetes mellitus was defined as a fasting glucose level of more than 126 mg/dL, post-prandial glucose level of more than 200 g/dL, and/or use of anti-diabetic drugs. In addition, renal dysfunction was defined as an estimated glomerular filtration rate (eGFR) level of less than 60 mL/min/1.73 m^2.

Measurement of Carotid Artery IMT

Ultrasound measurements of intima-media thickness (IMT) of the common carotid artery were performed by longitudinal scanning. IMT of the carotid artery was measured on high-resolution, 2-dimensional ultrasound images obtained with an SSA-270A ultrasound machine (Toshiba) with a 7.5-MHz linear-array transducer. This procedure was performed by an examiner who was unaware of the subjects' clinical background, as previously described[19].

Statistical Analysis

Analysis of variance (ANOVA) and paired Student's *t*-test were used for parametric procedures. The Mann-Whitney *U* test and Wilcoxon tests were alternatively used as nonparametric tests. When data were normally distributed, the two groups were compared by unpaired *t*-test; otherwise, the Mann-Whitney *U* test was used. Statistical comparisons among more than three groups were performed by the Kruskal-Wallis test. Data in the text, tables, and figures are expressed as the mean ± standard deviation (SD). A value of $p<0.05$ was considered significant.

Results

Positive Correlation of Calcification Extent Between Aortic Arch and Abdominal Aorta

First, the extent of calcification in the aortic arch and abdominal aorta was assessed by three independent non-invasive examinations, postero-anterior chest X-ray (grading), lateral radiograph of the lumbar spine (sum length; mm) and plain abdominal CT scan (%ACI) (**Fig. 1**). The positive correlation between the sum length and %ACI showed consistent accuracy in the quantitative assessment of abdominal aortic calcification ($r=0.671$, $p<0.01$) (data not shown); thereafter, we evaluated AAC grade by reviewing the chest X-rays of 239 patients. Baseline characteristics of the population are shown in **Table 1**. There was no difference between male and female groups in atherosclerotic risk factors (hypertension, diabetes, dyslipidemia and renal dysfunction), except for smoking. Regarding medication, there was no difference between male and female groups, except calcium channel blockers. AAC grade was 0, 1, 2, and 3 in 46%, 22%, 29% and 4% of the population, respectively (**Fig. 2A**). The accuracy and reproducibility of this technique for grading were 82%, 79%, 75% and 88% in grade 0, 1, 2 and 3, respectively. Regarding sex differences, the distribution of AAC grade was similar (**Fig. 2B**). Elderly patients (over 65 years) had a significantly higher AAC grade than those go anser than 65 years, and the tendency was more marked in patients over 75 years (**Fig. 2C**).

In 27 patients who underwent all examinations, the correlation between AAC grade and the extent of abdominal aortic calcification was investigated. Background characteristics of these patients showed hypertension (40.7%), dyslipidemia (29.6%), diabetes (7.4%), and renal dysfunction (25.9%). The sum length of abdominal aortic calcification was positively associated with AAC grade (30.3 ± 15.5, 58.9 ± 14.2, 75.3 ± 21.2, and 145.0 ± 14.2 in AAC grade 0, 1, 2, and 3, respectively) (**Fig. 3A**). Similarly, comparison of AAC grade with %ACI also showed a positive correlation (2.5 ± 0.8%, 7.4 ± 1.7%, 15.6 ± 2.7%, and 21.2 ± 4.3% in AAC grade 0, 1, 2, and 3, respectively) (**Fig. 3B**).

Association of Aortic Calcification with Pulse Pressure and Carotid IMT

AAC grade was not associated with systolic or diastolic BP (systolic BP; 130 ± 18, 135 ± 24, 134 ± 17,

Table 1. Baseline characteristics of patients

	All	Male	Female	*p* value
Number	239	115	124	
Age (y)	61.9±10.8	60.6±11.6	63.2±10.0	n.s.
Risk factors				
Hypertension	135 (56.5%)	72 (62.6%)	63 (50.8%)	n.s.
Diabetes	70 (29.3%)	38 (33.0%)	32 (25.8%)	n.s.
Dyslipidemia	162 (67.8%)	82 (71.3%)	80 (64.5%)	n.s.
Renal dysfunction	62 (25.9%)	31 (27.0%)	31 (25.0%)	n.s.
Smoking	83 (34.7%)	72 (62.6%)	11 (8.9%)	<0.01
BMI (kg/m^2)	24.2±4.3	24.9±4.4	23.7±4.2	n.s.
Blood pressure (mmHg)				
Systolic BP	132.5±18.9	133.2±18.0	131.9±19.8	n.s.
Diastolic BP	76.2±12.2	77.9±12.8	74.7±11.4	n.s.
Pulse Pressure	56.3±14.7	55.3±14.0	57.2±15.3	n.s.
Medication				
Anti-hypertensive drugs				
ACEI/ARB	27 (11.3%)	16 (13.9%)	11 (8.9%)	n.s.
CCB	95 (39.7%)	53 (46.1%)	42 (33.9%)	0.01
α/β blockers	25 (10.5%)	14 (12.2%)	11 (8.9%)	n.s.
Anti-diabetic drugs				
Sulfonyl urea	23 (9.6%)	12 (10.4%)	11 (8.9%)	n.s.
Biganide	1 (0.4%)	1 (0.9%)	0 (0%)	n.s.
αGIs	5 (2.1%)	1 (0.9%)	4 (3.2%)	n.s.
Insulin	7 (2.9%)	2 (1.7%)	5 (4.0%)	n.s.
Lipid-lowering drugs				
Statins	43 (18.0%)	16 (13.9%)	27 (21.8%)	n.s.
Others	10 (4.2%)	5 (4.3%)	5 (4.0%)	n.s.

Abbreviations; BMI, body mass index; BP, blood pressure; ACEI/ARB, angiotension-converting enzyme inhibitors and/or angiotension II receptor blockers; CCB, calcium channel blockers; αGIs, αglucosidase inhibitors; statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors; *p* value, probability value; n.s., not significant

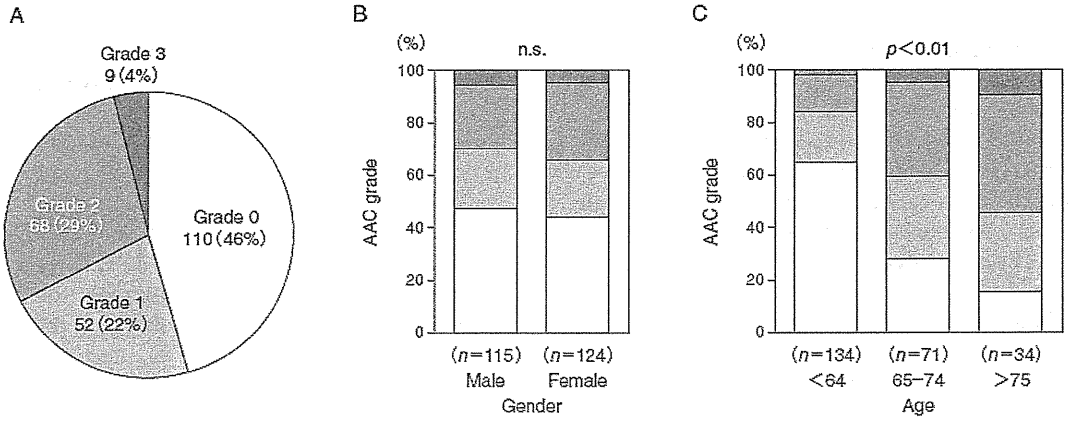

Fig. 2. Distribution of AAC grade in chest X-ray.

(A) Distribution of AAC grade determined by reviewing chest X-rays in all subjects. (B) There was no significant sex difference in the distribution of AAC grade. (C) Elderly patients (over 65 years) had a significantly higher AAC grade than those less than 65 years, and the tendency was more marked in patients over 75 years ($p<0.01$).

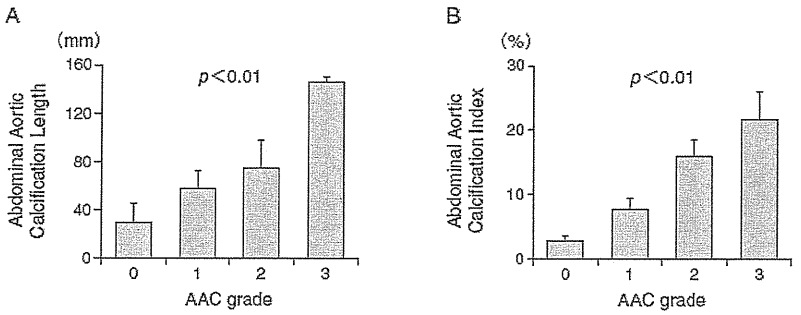

Fig. 3. Comparison of AAC grade with extent abdominal aortic calcification.

AAC grade was positively correlated with the extent of abdominal aortic calcification as assessed by two independent quantitative examinations, sum length (A) and %ACI (B).

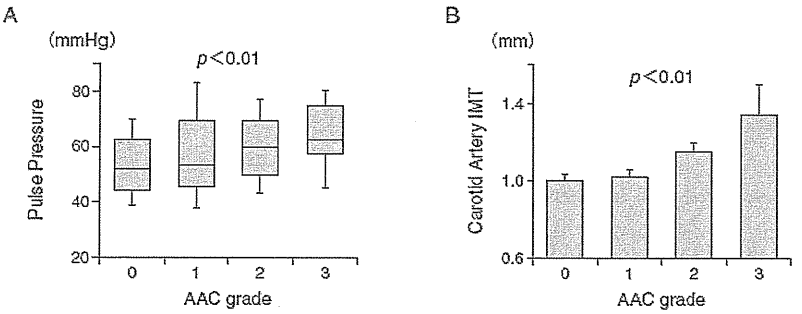

Fig. 4. Positive correlation of AAC with pulse pressure and IMT.

(A) AAC grade was positively correlated with pulse pressure. (B) Intima-media thickness (IMT) of the carotid artery was measured by ultrasound. Patients with a higher AAC grade had greater IMT than those with no or trivial AAC.

and 136±14 mmHg, diastolic BP; 77±13, 78±13, 74±10, and 172±10 mmHg in AAC grade 0, 1, 2, and 3, respectively). AAC grade was positively correlated with pulse pressure (53±12, 58±19, 60±14, and 65±14 mmHg, in AAC grade 0, 1, 2, and 3, respectively) (**Fig. 4A**). Significantly greater IMT, a marker of atherosclerosis, was found in patients with higher AAC grade (**Fig. 4B**).

Correlation of Aortic Calcification with Risk Factor Clustering

AAC grade was compared between the presence and absence of traditional risk factors (**Fig. 5A**). AAC grade in patients with diabetes was significantly higher than in those without diabetes. In addition, a similar significance was found in patients with renal dysfunction (eGFR level of less than 60 mL/min/1.73 m^2). There was no significant difference in grade according to the presence or absence of hypertension and dyslipidemia. The clustering of these risk factors (up to 4 factors) was significantly associated with increasing AAC grade (**Fig. 5B**).

Discussion

The present study demonstrated the value of assessing AAC grade using a simple chest X-ray examination to allow semi-quantitative evaluation for atherosclerotic risk stratification. Because the progression of arterial calcification is very slow, it is easier to detect slight and time-related alterations of calcium deposition in the arterial wall using high-tech non-invasive imaging, such as EB-CT and MD-CT. In contrast, the detection of macroscopic arterial calcification by plain radiographs is relative crude[20, 21]. In addition, the extent of arterial calcification and its significance are generally easily disregarded in routine clinical work; however, this simple detection of arterial calcification is reproducible, inexpensive, and readily available for large populations, as compared to such high-tech imaging. In the present study, AAC grade was positively correlated with the extent of abdominal aortic calcifi-

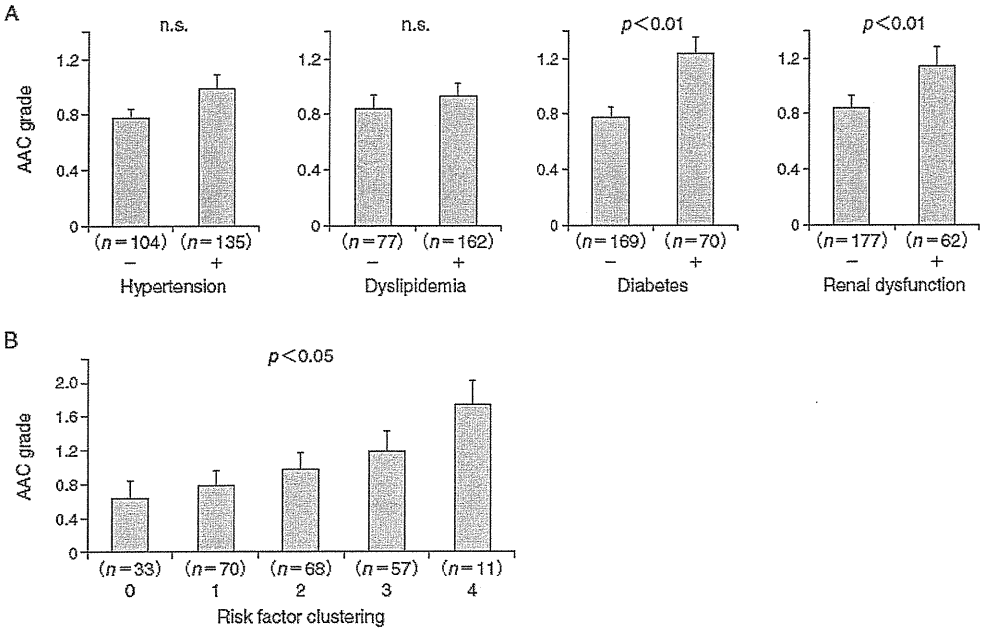

Fig. 5. Positive correlation between AAC grade and accumulation of atherosclerotic risk factors.

(A) AAC grade was compared between the presence and absence of traditional risk factors; hypertension, dyslipidemia, diabetes and renal dysfunction. A significantly higher grade of AAC was found in patients with diabetes or renal dysfunction, but not in those with hypertension or dyslipidemia. (B) The number of accumulated risk factors was positively associated with AAC grade (p for trend <0.05).

cation in independent examinations, suggesting that AAC grade as assessed by a simple chest X-ray may reflect the degree of calcification in the whole aorta. Thus far, few reports have fully evaluated the correlation of macroscopic calcification between the aortic arch and the abdominal aorta using independent examinations; therefore, our observations support the view that this simple examination, which is easy to follow up routinely, is indispensable for atherosclerotic risk evaluation, and may consequently help provide more information to predict the risk of CV events.

The atherosclerotic vasculature has many features, such as 'atheroma' and 'sclerosis'[4]. The condition leads to functional changes (such as arterial stiffening) and localized morphological changes (such as arterial wall thickening)[1, 4]. Arterial calcification can be seen at two different anatomical sites in the vessel wall; medial calcification (known as Mönckeberg's sclerosis) and atherosclerotic intimal calcification[22]; however, it is difficult to distinguish these two calcified lesions clearly using only a plain radiographic approach, including simple chest X-ray, without a pathological approach. This difficulty is marked in the elderly, because there are likely to be mixed calcified lesions in their arteries. In fact, if spotty intimal calcification exists with massive medial calcification visible as linear tram-tracks radiographically, it will probably not be possible to distinguish them.

Several reports have shown a positive correlation of abdominal aortic calcification with CV events, using lateral lumbar X-ray[23]. Several huge clinical studies, including the Framingham Heart Study, have demonstrated that the extent of abdominal aortic calcification was a good predictor of CV events and congestive heart failure over a 20-year follow-up period (relative risk 1.9 and 2.2, respectively)[23-25]; however, this examination is not very common in clinical routine work, because it is basically used to assess bone mineralization in patients with suspected osteoporosis. In the present study, we confirmed the good correlation between AAC grade and the extent of abdominal aortic calcification. This result suggests that grading AAC detectable on a chest X-ray can be strongly recommended to evaluate the stratification of risk factors.

On the other hand, there are relatively few reports regarding the predictive value of AAC for CV events using a simple chest X-ray, although many studies have previously shown a positive correlation of abdominal aortic calcification with CV events. One report demonstrated that more CV events occurred in patients

with AAC than in those without AAC[26]; however, the evaluation method in at report was dependent upon the presence or absence of AAC only using chest X-rays, without considering the extent of AAC; therefore, our evaluation method may be favorable to assess the prognostic value of AAC grade for the new onset of CV events. It is necessary to further evaluate whether this assessment of AAC grade has incremental predictive value, beyond traditional coronary risk factors, for the new onset of CV events.

IMT, a prognostic indicator, is commonly used to assess the progression of atherosclerosis[27]. Although few reports have compared IMT with aortic calcification, a good correlation between both factors was found in the present study. Indeed, carotid arterial wall thickening associated with plaques is frequently seen in patients with advanced atherosclerosis, and its magnitude is positively correlated with CV events[28]; therefore, similar to IMT, the risk of CV events is probably higher in patients with a higher AAC grade on chest X-rays.

In the present study, we compared AAC grade with traditional risk factors; diabetes, hypertension and dyslipidemia. First, among these factors, we found a significant association of diabetes with AAC grade among coronary risk factors. In general, diabetes is thought to be a strong risk factor for the progression of not only atherosclerosis, but also arteriosclerosis. It has been reported that high glucose-induced expression of the osteopontin gene and advanced glycation end products accelerated mineralization in microvascular pericytes in a culture model[29, 30]. In addition, our observations showed that patients with insulin treatment had a higher AAC grade than those without insulin in simple correlation analysis (data not shown), suggesting that the status of diabetic patients who need insulin treatment is probably more serious.

Systolic hypertension, in conjunction with a decline in diastolic blood pressure, results in a decrease in end-organ perfusion, including coronary flow[31]. In addition, arterial stiffness augments an increase in myocardial afterload, finally leading to left ventricular hypertrophy[3]. In this study, patients with a higher AAC grade had significantly higher pulse pressure, consistent with previous reports[2]. The Framingham Heart Study demonstrated that the risk of coronary artery diseases was negatively correlated with diastolic BP at any level of systolic BP[32], suggesting that pulse pressure is a good predictor of coronary events; therefore, in our population, the new onset of CV events may frequently occur in patients with high AAC grade. Further investigation of the new incidence of CV events during long-term follow up in this population is necessary.

Many reports have shown the relationship of abnormal serum lipid levels (such as elevated cholesterol and TG and reduced HDL-C) to aortic calcification, suggesting that dyslipidemia promotes calcium deposition in the arterial wall[33]; however, most these studies evaluated abdominal aortic calcification, but not AAC. In addition, the relationship between lipids and aortic calcification is likely to remain controversial, because several studies have indicated that no correlation is present between abdominal aortic calcification and serum lipid components[34]. In this study, there was no association between AAC grade and the presence of dyslipidemia, although our cohort included 67.8% patients with dyslipidemia, including statin users (18%). One possible reason may be the small size iof this cohort, suggesting that higher-powered studies are required to clearly define the relationship between lipids and AAC. Several retrospective studies have demonstrated that statins inhibit the progression of coronary artery calcification as a pleiotropic effect beyond their cholesterol-lowering effects[35, 36]. In addition, statins have also been shown to possess inhibitory effects on vascular smooth muscle cell calcification *in vitro*[37]. Further analysis to evaluate the longitudinal change of AAC grade during a long-term follow-up period is necessary.

Renal dysfunction (so-called chronic kidney disease, CKD) has been shown to be associated with atherosclerotic diseases[38]. Many reports have shown that CKD patients have arterial calcification due to mineral disorders, including hyperphosphatemia. Similar to diabetes in this study, the evidence that patients with a decline in eGFR had an increased AAC grade was consistent with previous reports.

Recently, the accumulation of atherosclerotic risk factors has been shown to act in a pro-atherogenic fashion, like metabolic syndrome. In the present study, the accumulation of these three factors was significantly associated with the AAC grade. This result suggests that the clustering of multiple risk factors, which provoke metabolic disorder, such as oxidative stress and assressive adipocytokines, may augment calcium deposition in the aortic wall. Thus far, there is no established therapy to prevent arterial calcification, and therefore, at present, the most appropriate therapeutic strategy may be to manage modifiable risk factors strictly.

The principal limitation of the present study is that our assessment using AAC grades was relatively crude. This approach is not a quantitative method, and it is possible that the true calcium deposition in the aortic wall was underestimated. Clinically, CT

scan is frequently used to quantify abdominal aortic calcification as a percentage of the cross-sectional area of the aorta[39]. In AAC, it has been thought to be difficult quantify the calcified level using plain chest CT scan. In the present study, the present population did not all undergo a chest CT scan. To make this evaluation of AAC grade more valid, further investigation to compare AAC grade by assessing chest X-ray and chest CT scan is necessary. In addition, the population in the present study was small; therefore, a further large-scale prospective study is necessary.

In conclusion, our cross-sectional study demonstrated that AAC grade was associated with the extent of abdominal aortic calcification, and the accumulation of traditional atherosclerotic risk factors was positively correlated with the AAC grade. Our results emphasize that the magnitude of aortic calcification is worthy of greater attention in routine clinical work. Our data strongly suggest that risk stratification by a simplified approach to assess aortic calcification may provide supportive information for the primary preventive management of atherosclerotic disease.

Acknowledgements

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Association of plasma sex hormone levels with functional decline in elderly men and women

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Aim: We aimed to determine whether plasma sex hormone levels are associated with activities of daily living (ADL), cognition, depression and vitality in elderly individuals with functional decline.

Methods: Two hundred and eight consecutive persons 70 years or older (108 men and 100 women; mean ± standard deviation, 81 ± 7 years) with a chronic stable condition, receiving long-term care at a long-term care facilities located in Nagano Prefecture, Japan, were enrolled. Plasma total testosterone, free testosterone (only in men), dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S) and estradiol levels were determined in the morning after an overnight fast. Comprehensive geriatric assessment was performed including basic ADL by Barthel Index, instrumental ADL, cognitive function by Hasegawa Dementia Scale – Revised, mood by Geriatric Depression Scale and ADL-related vitality by Vitality Index.

Results: Simple regression analysis showed that, in men, plasma total and free testosterone levels were associated with basic ADL ($R = 0.292$ and $R = 0.282$), instrumental ADL ($R = 0.261$ and $R = 0.408$), cognitive function ($R = 0.393$ and $R = 0.553$) and vitality ($R = 0.246$ and $R = 0.396$), while DHEA(-S) was associated with cognitive function, and estradiol with cognitive function as well as vitality. In women, the only significant correlation was between DHEA(-S) and basic ADL. Adjustment for age and nutritional markers did not influence the associations of plasma sex hormone levels with functional scores except for that of free testosterone with Barthel Index.

Conclusion: These results suggest that sex hormones have sex-specific associations with physical and neuropsychiatric functions in elderly individuals, and that endogenous testosterone is related to global function in elderly men.

Keywords: activities of daily living (ADL), comprehensive geriatric assessment, dehydroepiandrosterone sulfate, estradiol, testosterone.

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Introduction

In addition to the abrupt reduction in estrogen production in women during the menopause, both men and women experience an age-associated decrease in the levels of androgens.[1–3] Physical and neuropsychiatric

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function also declines with age; however, the association of sex hormones with functional decline is not fully understood. One nursing home study found that a higher total testosterone (T) level was associated with better activities of daily living (ADL) performance such as transferring and eating among frail elderly men, while estrone and dehydroepiandrosterone (DHEA) levels were inversely related to ADL in women.[4] Although several observational studies examining the relationship between endogenous androgen and cognitive function in elderly men have also been published,[5-8] most surveys have investigated only a few aspects of functions rather than the whole spectrum and have been carried out based on community samples of white people in Western countries. In addition, many studies are restricted to one sex and few have focused on frail or disabled elderly individuals.

Thus, additional data are needed to elucidate the relationship between plasma hormone levels and functional status in elderly individuals with functional decline to better understand the application of hormone replacement therapy to bring about the most beneficial effects. In our preliminary study in a small sample of frail elderly men, a higher plasma T level was associated with higher functional scores.[9] To extend this pilot study, we included a larger sample of elderly men and women with functional decline, and evaluated whether sex hormone levels, including DHEA sulfate (DHEA-S) and estradiol, are associated with functioning on the basis of comprehensive geriatric assessment.

Methods

Study design and participants

In this cross-sectional observational study, 208 consecutive persons aged 70 years or older (108 men aged 70–95 years and 100 women aged 70–93 years; mean ± standard deviation, 82 ± 7 and 81 ± 6 years, respectively) who attended health service facilities for the elderly (facilities that provide 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 either for facility admission or day-care service. The principal exclusion criteria were malnutrition (serum albumin, <3.5 mg/dL), extremely low ADL status (Barthel Index,[10] <50), malignancy, acute inflammation (fever, white blood cell count of >10 000/mL, or other signs of infection within 4 weeks before enrollment), severe anemia (blood hemoglobin, <10.0 g/dL) and overt endocrine diseases because these diseases may affect both plasma sex hormone levels and functions. The following information was collected from medical history charts or by interviewer-administered questionnaire; past medical history, present diagnosis of any disease, medication and nutritional intake. Comorbid conditions included in the current analysis were hypertension, chronic heart disease (angina, myocardial infarction, congestive heart failure, arrhythmia), stroke, osteoarthropathy (arthritis, rheumatism, osteoporosis, history of fractures) and diabetes mellitus. We also obtained data on anti-androgenic treatment or intake of glucocorticoids, opiates or hormone supplements which could affect plasma hormone levels, but no subject was taking any of these. The institutional review board of Kikyogahara Hospital 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). Free-T, DHEA-S and DHEA were assayed using sensitive radioimmunoassays. Total-T and estradiol were assayed using chemiluminescence 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%.

Functional and anthropometric measurements

Trained nurses and physical therapists visited the participants at the health service facilities and performed comprehensive geriatric assessments. Basic ADL was assessed by Barthel Index,[10] instrumental ADL (IADL) by Lawton and Brody's IADL,[11] cognitive function by Hasegawa Dementia Scale – Revised (HDS-R, 30-point scale),[12] mood by Geriatric Depression Scale (GDS, 15 items)[13] and ADL-related vitality by Vitality Index (10-point scale).[14] In the current study, three items (food preparation, household tasks and laundering) were removed from the original version of Lawton and Brody's IADL scale to assess men; thus, IADL scale ranged 0–5 points in men and 0–8 in women. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Statistical analysis

Data were analyzed using SPSS statistical software (version 11.0). Data were compared between men and women using the Student's *t*-test for continuous variables and χ^2-tests for categorical variables. Pearson's simple correlation coefficients were determined by plasma sex hormone levels, age and functional

measures. Standardized regression coefficients from multivariate linear regression analysis of functional measurements in relation to age, nutritional markers and plasma hormone levels were determined. An unpaired Student's *t*-test was used for the differences in hormone levels and functional status according to associated diseases. $P < 0.05$ was considered statistically significant.

Results

The characteristics of the study subjects are presented in Table 1. Sex differences were found in the levels of hemoglobin and total cholesterol, and also in the percentage of subjects with heart disease and stroke. On average, subjects showed mild-to-moderate functional decline, and scores of Barthel Index, HDS-R and Vitality Index were higher in women than in men. Plasma level of total-T in male cohorts was lower than that reported in healthy elderly men,[15] but comparable to those in frail elderly men.[4] All plasma hormone levels were significantly higher in men than in women.

In simple regression analysis, age was negatively correlated with most of the functional scores except for instrumental ADL and GDS in men and GDS in women (data not shown). Because an age-associated decline of plasma sex hormone levels[1–3] and an influence of nutritional status on hormone levels[16,17] have been reported, we analyzed the correlations between hormone levels, age and BMI (Table 2). However, only free-T in men was significantly associated with age, and only total-T in women was correlated with BMI. Because DHEA, testosterone and estradiol have precursor-metabolite relationships in the steroid-hormone biosynthesis cascade, we evaluated the correlations between each of the plasma hormone levels (Table 2). Some, but not all, plasma sex hormone levels showed significant correlations in both sexes.

Table 1 Distribution of variables in study subjects

	Men	Women
No. of subjects	108	100
Age, years	82 ± 7 (70–95)	81 ± 6 (70–93)
Nutritional parameters		
Body mass index, kg/m^2	21.8 ± 3.3 (15.1–29.0)	22.9 ± 3.8 (16.0–33.6)
Hemoglobin, g/dL	13.7 ± 1.7 (10.4–18.7)	12.8 ± 1.3 (10.0–15.6)**
Albumin, g/dL	4.2 ± 0.3 (3.5–4.9)	4.2 ± 0.3 (3.5–4.9)
Total cholesterol, mg/dL	181 ± 32 (119–273)	205 ± 33 (126–288)**
Chronic diseases		
Hypertension, *n* (%)	31 (28.7)	36 (36.0)
Heart disease, *n* (%)	9 (8.3)	19 (19.0)*
Stroke, No. (%)	35 (32.4)	19 (19.0)*
Osteoarthropathy, *n* (%)	23 (21.3)	31 (31.0)
Diabetes mellitus, *n* (%)	10 (9.3)	14 (14.0)
Functional parameters		
Barthel Index	84 ± 17 (50–100)	93 ± 9 (60–100)**
Instrumental ADL[†]	2.6 ± 2.0 (0–5)	5.9 ± 2.3 (0–8)
HDS-R	19 ± 7 (2–30)	23 ± 6 (5–30)**
Vitality Index	9.2 ± 1.1 (5–10)	9.7 ± 0.6 (6–10)**
GDS	5.6 ± 3.2 (0–13)	5.4 ± 3.0 (0–13)
Hormones		
Total testosterone, nmol/L	14.8 ± 5.8 (2.5–30.5)	1.3 ± 0.6 (0.2–2.9)**
Free testosterone, pmol/L	22.2 ± 8.7 (3.1–43.4)	
DHEA-S, μmol/L	1.75 ± 1.18 (0.26–5.47)	1.34 ± 0.54 (0.38–2.70)**
DHEA, nmol/L	7.63 ± 3.82 (2.43–25.7)	4.86 ± 2.08 (1.04–11.1)**
Estradiol, pmol/L	109.4 ± 48.1 (14.7–228.0)	59.5 ± 38.9 (14.7–206.7)**

Probability values of chronic diseases were compared between men and women by χ^2-test; *$P < 0.05$. Age, nutritional parameters, functional parameters (except for instrumental ADL) and hormone measurements were compared between men and women by Student's *t*-test; **$P < 0.001$. Values except those for chronic diseases are shown as mean ± standard deviation (range). [†]Lawton and Brody's instrumental ADL scale ranges 0–5 points in men and 0–8 in women, respectively. ADL, activities of daily living; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; GDS, Geriatric Depression Scale; HDS-R, Hasegawa Dementia Scale – Revised.

Table 2 Correlation between plasma sex hormone levels, age and body mass index

	Age	BMI	Total-T	Free-T	DHEA-S	DHEA	Estradiol
Men							
Age	–	0.035	−0.121	−0310**	−0.254	−0.111	−0.047
BMI		–	0.006	0.026	−0.177	−0.151	−0.055
Total-T			–	0.672***	0.043	0.075	0.476***
Free-T				–	0.468***	0.392**	0.414***
DHEA-S					–	0.382**	0.342*
DHEA						–	0.084
Estradiol							–
Women							
Age	–	−0.187	0.079		−0.062	0.017	−0.028
BMI		–	0.320*		0.121	−0.070	0.040
Total-T			–		0.202*	0.355**	0.162
DHEA-S					–	0.561***	0.131
DHEA						–	0.097
Estradiol							–

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. All data are presented as Pearson correlation coefficients. BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; Free-T, free testosterone; Total-T, total testosterone.

We also assessed whether plasma hormone levels were different between individuals with or without chronic diseases including hypertension, heart disease, cerebrovascular disease, osteoarthropathy and diabetes mellitus, using a Student's *t*-test, but there were no significant differences in hormone levels according to these conditions (data not shown). On the other hand, a significant difference was observed in Barthel Index scores between subjects with and without cerebrovascular disease in men (77 ± 16 vs 86 ± 16, $P < 0.01$).

The associations between plasma hormone levels and functional scores were evaluated. As shown in Table 3, in men, plasma total-T and free-T levels were positively correlated with functional scores except for GDS. DHEA(-S) and estradiol were positively correlated with cognitive function, and DHEA and estradiol were associated with Vitality Index as well. In contrast, in women, a significant correlation was observed only between DHEA(-S) and Barthel Index.

Multiple regression analysis revealed that the associations between sex hormones and functions were independent of age and BMI except that the associations between free-T and Barthel Index in addition to DHEA and vitality were not significant after adjustment. The statistical results were similar when serum albumin or total cholesterol was entered into the regression model instead of BMI (data not shown).

Because all measured sex hormones were associated with HDS-R in men, we entered free-T, DHEA-S and estradiol into the regression model as covariates in addition to age and BMI (Table 4). Free-T remained a significant determinant of HDS-R, while DHEA-S and estradiol did not hold a significant association with HDS-R. When a stepwise model (forward selection) was used to test for the determinants for HDS-R with the same five covariates, the *P*-value for the regression was minimized when only free-T was chosen as a variable ($R^2 = 0.227$, overall *P*-value for the regression <0.05).

Discussion

The present study demonstrated that men with higher plasma T levels had better ADL, cognitive function and vitality. Also, a higher estradiol level was related to better cognitive function as well as vitality, and a higher DHEA(-S) level was related to better cognitive function. In women, DHEA(-S) level was related to higher basic ADL, but T and estradiol levels showed no correlation with functional scores. The positive associations between sex hormones and functional scores were independent of age and nutritional status, suggesting that plasma sex hormone levels, especially that of testosterone in men, are independently related to functional status in elderly individuals.

Concerning cognitive function, our findings are consistent with the results of the previous observational studies examining the relationship between endogenous androgen and cognitive function in elderly men.[5,7,8] Several interventional studies have shown an improvement in spatial cognition and working memory after treatment with T, suggesting that T might have a beneficial effect on cognitive function.[18–21] Also, DHEA(-S), the most abundant circulating steroid in both sexes and the biosynthetic precursor of T, has been shown to have neurotrophic and neuronal remodeling activity.[22,23]

Table 3 Linear regression model of hormone levels on functional scores unadjusted and adjusted for age, and age and body mass index

	Total-T	Free-T	DHEA-S	DHEA	Estradiol
Men					
Unadjusted					
Barthel Index	0.292**	0.282**	0.094	−0.058	0.110
Instrumental ADL	0.261*	0.408**	0.239	0.140	0.129
HDS-R	0.393***	0.553***	0.390*	0.393**	0.266*
Vitality Index	0.246*	0.396***	0.210	0.297*	0.291*
GDS	−0.103	−0.097	−0.181	−0.027	−0.060
Adjusted for age					
Barthel Index	0.250**	0.183	0.044	−0.077	0.107
Instrumental ADL	0.255*	0.402***	0.216	0.137	0.124
HDS-R	0.366***	0.488***	0.317*	0.361**	0.243*
Vitality Index	0.218*	0.348***	0.160	0.176	0.288*
GDS	−0.068	−0.065	−0.146	−0.024	−0.005
Adjusted for age and BMI					
Barthel Index	0.281**	0.112	0.101	0.109	0.114
Instrumental ADL	0.229*	0.414**	0.314*	0.400*	0.053
HDS-R	0.340**	0.443**	0.329*	0.480**	0.236*
Vitality Index	0.285*	0.321*	0.140	0.227	0.292*
GDS	−0.067	−0.015	−0.013	0.002	0.079
Women					
Unadjusted					
Barthel Index	0.085		0.280*	0.293*	−0.068
Instrumental ADL	−0.050		0.071	0.171	0.071
HDS-R	−0.051		0.080	−0.034	0.121
Vitality Index	−0.076		0.167	0.091	0.043
GDS	0.004		−0.087	−0.014	0.052
Adjusted for age					
Barthel Index	0.120		0.225*	0.288*	−0.068
Instrumental ADL	−0.003		0.041	0.166	0.052
HDS-R	−0.028		0.038	−0.037	0.120
Vitality Index	−0.060		0.140	0.089	0.043
GDS	−0.008		−0.066	−0.012	0.052
Adjusted for age and BMI					
Barthel Index	0.142		0.269*	0.221*	0.035
Instrumental ADL	−0.067		0.092	0.178	0.046
HDS-R	−0.110		0.045	−0.051	0.106
Vitality Index	−0.103		0.137	0.043	0.104
GDS	0.063		−0.020	0.038	0.056

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Data are presented as standardized regression coefficients. ADL, activities of daily living; BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; Free-T, free testosterone; GDS, Geriatric Depression Scale – 15 items; HDS-R, Hasegawa Dementia Scale-Revised; Total-T, total testosterone.

In addition, our recent study showed that a low plasma T level is related to endothelial dysfunction in middle-aged men,[24] suggesting a mechanistic link between T and cerebrovascular function.

With respect to mood, although some large scale epidemiological studies[25,26] failed to show a clear correlation between T and depression in middle-aged men, another study has shown that low T levels are associated with depression in healthy elderly men.[27] The reason is unknown but it might be due simply to the cohort difference between community-dwelling healthy men and frail elderly men, or to the low reliability of GDS in demented people.[14] In the current study, in men, estradiol was also associated with cognitive function and vitality. However, multiple regression analysis with both free-T and estradiol as covariates suggested that estradiol is merely a marker as a metabolite of androgens and does not exert a direct action on neuropsychiatric

Table 4 Multiple regression analysis on cognitive function with free-T, DHEA-S and estradiol as covariates in addition to age and BMI in men

	HDS-R β	*P*
Age	−0.346	0.087
BMI	0.091	0.649
Free-T	0.466	0.030
DHEA-S	0.011	0.964
Estradiol	−0.213	0.321

β, standardized regression coefficient; BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate; Free-T, free testosterone; HDS-R, Hasegawa Dementia Scale – Revised.

function in men consistent with the results of cross-sectional studies.[5,7,8]

Because T has anabolic effects on muscle and may improve cognition, our findings on the association of T with ADL are not surprising. While several observational studies have demonstrated the correlation of endogenous testosterone with muscle mass and strength[28–31] and physical performance[4,32] in older men, interventional surveys have provided mixed findings[33–38] and the studies using healthy men have found only increased muscle mass and strength but not improved physical function.[35–38] In addition, results of studies investigated the correlation between endogenous testosterone and fall risks are inconsistent.[30,32] Future interventional studies enrolling frail and/or disabled elderly men might clarify the causal relationship between testosterone and frailty. Although the correlation between sex hormones and physical function or ADL in women is contradictory across studies,[39–43] our findings are consistent with one report showing that the plasma level of DHEA(-S) is related to basic ADL in middle-aged to elderly women.[39]

The explanations for the sex difference in the correlation between hormones and function could be due to sex differences in hormone secretion and metabolism.[41,44,45] In fact, plasma estradiol level in women was approximately half of that in men, and distributed in a narrow range (52% cases fell into a range of 14.7–53.1 pmol/L), providing a possible explanation for no association of estrogen levels with functioning in women. Measurement of active forms of estrogens such as free or bioavailable estradiol, although the assays are not available in Japan, might show some significant correlations with functional levels; however, most of the previous studies investigating the relationship between endogenous estrogen levels and physical performance or cognitive function in postmenopausal women, including one study that measured bioavailable estradiol levels, found negative results.[46–50] Accordingly, in the ranges of circulating endogenous hormone levels, estradiol may not be related to functional levels in older women. On the other hand, information on the sex-specific distribution of steroid hormone receptors is limited. Recently, Bezdickova *et al.* reported that nuclear androgen receptor staining was observed in the mammillary body, precentral gyrus and hippocampus in the human male brain but not in the female brain.[51] The sex difference in the correlation between hormones and functions should be further determined based on the ligand–receptor relationship.

The limitations of our study should be acknowledged. First, we cannot exclude an influence of the associated diseases or the comorbid condition on our results, although no significant differences were observed in hormone levels or functional status in subjects with or without chronic diseases, except that the Barthel Index was significantly lower in male subjects with cerebrovascular disease. Second, only free-T was measured as the active form of T by radioimmunoassays instead of bioavailable or calculated free-T, because sex hormone-binding globulin and direct assays of bioavailable T were not available. Finally, it should be recognized that our results were obtained from a cross-sectional study and do not provide direct evidence of a causal relationship; therefore, it is possible that high sex hormone levels were the result of enhanced physical or mental health.

In summary, our cross-sectional survey revealed that sex hormones have sex-specific relationships with physical and neuropsychiatric function in elderly individuals. In men, endogenous androgen is independently associated with ADL, cognitive function and vitality. Although it has been reported that testosterone or DHEA supplementation in healthy elderly men did not affect physical or cognitive function,[37,38] our findings suggest that elderly men with functional decline could be a better target for androgen replacement to improve physical and cognitive function.

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ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Age-related changes in plasma androgen levels and their association with cardiovascular risk factors in male Japanese office workers

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Aim: To assess the age-related change in plasma androgen levels in healthy middle-aged men and whether any clinical parameters are associated with the hormonal change.

Methods: The study was comprised of male Japanese office-workers aged 40–64 years, who had undergone an annual health check-up in 2002 and 2007 (96 and 76 men, respectively). Body mass index and blood pressure were measured, and serum concentration of lipids, glucose and uric acid in addition to plasma total testosterone, free testosterone and dehydroepiandrosterone sulfate (DHEA-S) levels were determined in the morning after an overnight fast. The 5-year hormonal changes and their associations with clinical parameters were analyzed in 33 men who repeated the examination at both check-ups. The cross-sectional associations of hormonal levels with clinical parameters were also investigated.

Results: Age was negatively associated with free testosterone ($r = -0.399$, $P < 0.001$ in 2002; $r = -0.458$, $P < 0.001$ in 2007) and DHEA-S ($r = -0.233$, $P = 0.02$ in 2002; $r = -0.336$, $P < 0.01$ in 2007) but not with total testosterone, while the 5-year changes of free testosterone and DHEA-S levels were not significant and showed no associations with major cardiovascular risk factors. Cross-sectionally, after adjustment for age, linear regression analysis showed a positive association between free testosterone and blood hemoglobin and a negative association between total testosterone and serum uric acid.

Conclusion: In Japanese middle-aged men, 5-year androgen decline is too subtle to detect, and endogenous androgen levels seem to have relatively weak association with cardiovascular risk profiles. **Geriatr Gerontol Int 2010; 10: 32–39.**

Keywords: aging male, dehydroepiandrosterone sulfate, hypogonadism, testosterone.

Introduction

The concept of age-related androgen deficiency in men, namely late-onset hypogonadism (LOH),[1] has opened public awareness of the significance of men's health. LOH is not a rare condition in aging men, although often left undiagnosed and untreated. Recently, not only

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endocrinologists but also geriatricians and general physicians are required to be more vigilant in diagnosing and treating symptomatic hypogonadism as the front line of health-care delivery. Based on current guidelines,[1] assessing plasma testosterone levels are recommended when an adult man exhibits signs of hypogonadism such as feelings of low energy, decreased libido, decreased muscle strength, and also as part of normal medical screening in men starting at age 40–50 years to establish a baseline. The application of androgen replacement therapy (ART) should be discussed for symptomatic patients with low testosterone level. However, reliable standard values of androgen levels in healthy men are essential to determine the application of ART.

Age-associated decline in plasma androgen concentration has been confirmed by a large series of cross-sectional studies. Reference range of serum total testosterone (TT) and free testosterone (FT) in Japanese male adults has also been established.[2] Several longitudinal studies have also found that TT and FT concentrations fall by 0.8% and 2% per year, respectively, in middle-aged men, contributing to an increasing prevalence of LOH with advancing age.[3–5] These surveys, however, have been performed based on samples of Caucasian men in the Western countries; therefore, additional data are needed to elucidate the longitudinal change in Japanese middle-aged men, especially whose testosterone levels are declining gradually.

In addition to the age-associated decline in plasma androgen levels, large inter- subject variations are seen at all ages. Although the mechanisms of this variability have not been completely elucidated, several physiological factors or chronic diseases such as obesity,[6–8] diabetes mellitus,[9–13] hypertension[14–17] and dyslipidemia[12,14,18] appear to play a role. In the present study, we aimed first to assess the age-related change in plasma androgen levels in healthy Japanese middle-aged men, and second to elucidate whether any clinical parameters measured in health check-ups are associated with the hormonal change both in cross-sectional and longitudinal surveys.

Methods

Study design and participants

This survey was conducted as a part of annual health check-ups of office workers at a company located in Tokyo, Japan, in 2002 and 2007. A total of 139 men aged 40–64 years who had undergone the health check-up (96 men in 2002 [mean ± standard deviation age = 52.7 ± 5.9] and 76 men in 2007 [51.5 ± 6.8 years]) were enrolled. Among the 96 men who attended in 2007, 33 men repeated the check-up in 2007 (Fig. 1). Those under testosterone or dehydroepiandrosterone (DHEA) replacement, those being treated with androgen deprivation therapy and those with malignancies including prostate cancer were excluded. The study protocol was approved by the ethics committee of the Graduate School of Medicine, University of Tokyo. Each subject gave a written informed consent for the enrollment in this study.

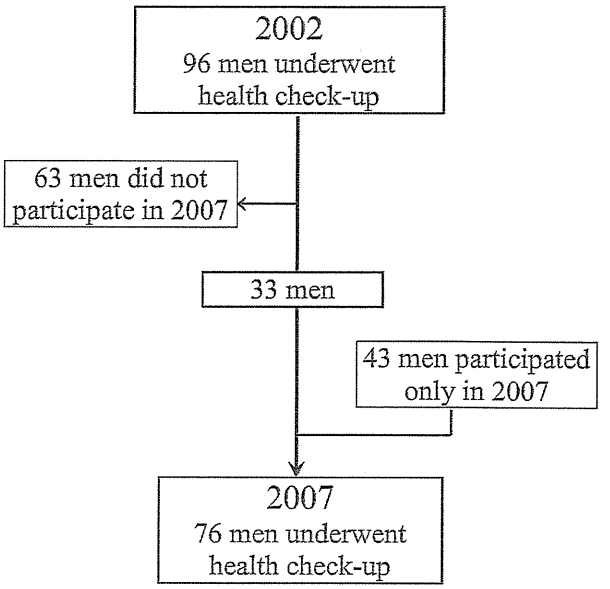

Figure 1 Participant flow diagram.

Annual health check-ups

A history was taken, physical examination including brachial blood pressure in addition to laboratory tests were performed in all subjects. Height and weight were measured with participants wearing light clothing without shoes, and body mass index (BMI) was calculated as weight in kg/m^2. Waist circumference was measured only in 2007. Blood sampling was performed in the morning (10.00–12.00 hours) of the health check-up after an overnight fast, to measure plasma hormones in addition to blood cell counts and blood chemical parameters.

Laboratory assays

Blood cell counts and blood chemical parameters were assayed by a commercial laboratory (Byotaiseiri Laboratory, Tokyo, Japan). Plasma TT and DHEA sulfate (DHEA-S) concentrations were determined using sensitive radioimmunoassay (RIA) in 2002 and chemiluminescence immunoassays (CLIA) method in 2007 by commercial laboratories (SBS [Tokyo, Japan] and SRL [Tokyo, Japan], respectively). In order to compare the measurements in both years, the following conversion formulas provided by SRL were applied: y (CLIA) =

1.03 × (RIA) + 14, the coefficient of determination (R^2) was 0.96 for TT and y (CLIA) = 0.10 × (RIA) + 2.23, R^2 was 0.97 for DHEA-S. Plasma FT concentrations were measured using the RIA method in both years, with the same measurement reagent (Mitsubishi Kagaku Iatron, Tokyo, Japan), although in different laboratories; SBS in 2002 and SRL in 2007. Hence, the following conversion formula was applied to adjust the slight difference between the two laboratories: y(SRL) = 0.95 (SBS) + 0.94, with an R^2 of 0.98. The intra-assay coefficients of variation for these measurements were less than 5%.

Statistical analysis

Data were analyzed using SPSS statistical software (ver. 11.0). The values are expressed as the means ± standard deviation in the text. Pearson's correlation coefficients were used to assess the relationship between plasma androgen levels and age. A paired Student's *t*-test was used to assess the 5-year change in hormone levels and clinical and biochemical parameters. Differences in the characteristics of the subjects of two health check-up year groups were compared with an unpaired Student's *t*-test. Standardized regression coefficients from multivariate linear regression analysis of potential cardiovascular disease risk profiles in relation to age and plasma androgen levels were determined. $P < 0.05$ was considered statistically significant.

Results

The subject profiles of 139 men who underwent annual health check-ups in 2002 and in 2007 are summarized in Table 1. Of 2002 and 2007 subjects, 27% and 29% were obese (BMI > 25) and 34% and 38% had hypertension, respectively. None of the subjects had a TT level below 8 nmol/L (the range may benefit from testosterone treatment[1]), and only 4.2% and 5.3% of subjects in each year (aged 47–58 years in 2002 and 44–59 years in 2007) had TT levels between 8 and 12 nmol/L (the range might require hormone therapy after ruling out other causes[1]).

In cross-sectional analysis, using the linear regression model, age was negatively associated with plasma concentration of FT and DHEA-S but not with TT (Fig. 2). The estimated 5-year decline of plasma hormone levels, calculated with the linear regression equations from the samples in 2002 and 2007, were −1.27 and −1.29 pg/mL (−4.41 and −4.50 pmol/L) for FT, and −16.1 and −23.4 μg/dL (−0.44 and −0.64 μmol/L) for DHEA-S, respectively. However, the 5-year changes of FT and DHEA-S levels were not significant in the longitudinal survey of 33 men, which was started when the average age of the subjects was 49 years (Table 2). Moreover, regression analysis showed no significant associations between each hormonal change and neither major cardiovascular risk factors at baseline nor their 5-year changes (data not shown).

Cross-sectionally, after adjustment for age, linear regression analysis demonstrated a positive association between FT and hemoglobin and a negative association between TT and uric acid, while androgen levels did not show statistical interactions with other health parameters (Table 3). Multiple regression analysis revealed that the negative association between TT and uric acid level was independent of age, BMI, estimated glomerular filtration rate and blood hemoglobin ($\beta = -0.257$, $P < 0.01$ in 2002; $\beta = -0.226$, $P < 0.05$ in 2007). The

Table 1 Characteristics of subjects in 2002 and 2007

Characteristics	Subjects in 2002	Subjects in 2007	*P*-value
n	96	76	
Age, years	52.7 ± 5.9	51.5 ± 6.8	0.234
Systolic blood pressure, mmHg	127 ± 16	124 ± 13	0.189
Diastolic blood pressure, mmHg	79 ± 10	80 ± 12	0.430
Body mass index, kg/m^2	23.6 ± 2.7	24.0 ± 3.0	0.301
Total cholesterol, nmol/L	5.34 ± 0.75	5.67 ± 0.73	0.005
HDL cholesterol, nmol/L	1.42 ± 0.31	1.58 ± 0.39	0.003
Triglyceride, nmol/L	1.56 ± 1.03	1.54 ± 1.08	0.924
Fasting glucose, nmol/L	5.77 ± 1.33	5.44 ± 0.89	0.060
Plasma hormone levels			
Total testosterone, nmol/L	22.0 ± 5.1	20.8 ± 5.6	0.167
Free testosterone, pmol/L	46.8 ± 12.8	55.9 ± 13.5	<0.001
DHEA-S, μmol/L	5.5 ± 2.2	6.2 ± 2.6	0.052

Data are shown as mean ± standard deviation. *P*-values represent differences between measurements of 2002 and 2007 with unpaired Student's *t*-test. DHEA-S, dehydroepiandrosterone sulfate; HDL, high-density lipoprotein.

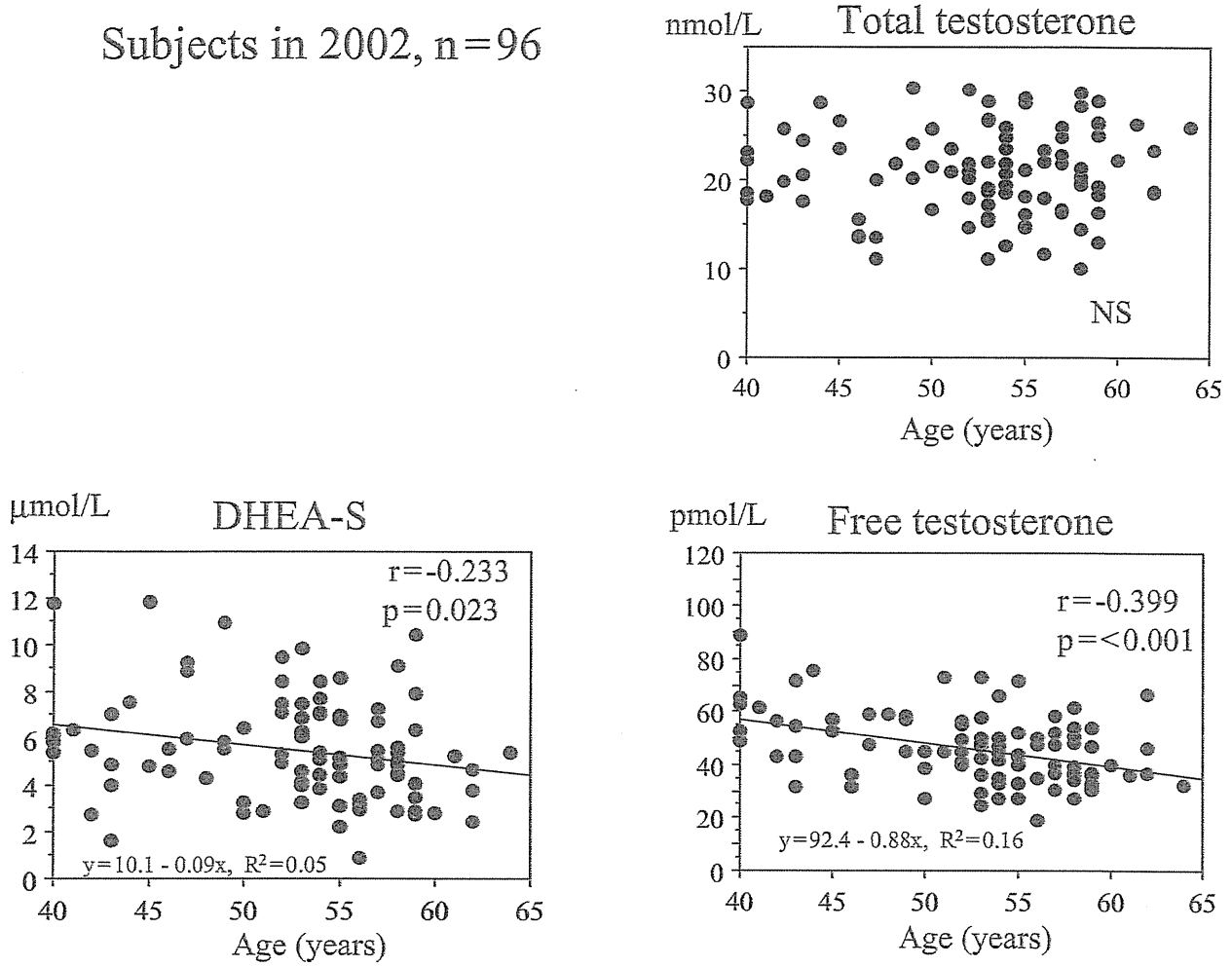

Figure 2 Scatter plots illustrate the correlations between age and plasma androgen concentrations. DHEA-S, dehydroepiandrosterone sulfate; NS, not significant.

statistical result was similar after waist circumference was entered as a covariate into the multiple regression model instead of BMI in 2007 ($\beta = -0.234$, $P < 0.05$). With respect to the association between FT and hemoglobin, there was a significant correlation after adjusting for age and BMI in the subjects in 2007 ($\beta = 0.288$, $P < 0.01$); however, statistical significance disappeared in the subjects in 2002 ($\beta = 0.140$, $P = 0.176$).

Discussion

The present study demonstrated that 5-year androgen decline from age 40–50 years among Japanese middle-aged men is very small with large individual variations. In addition, in these healthy men, endogenous androgen levels were not significantly associated with cardiovascular risk profiles neither cross-sectionally nor longitudinally, except that TT was inversely correlated with serum uric acid in both 2002 and 2007 subjects.

Despite the results from previous longitudinal studies that TT, FT and DHEA-S concentrations decline with aging in men;[3–5,19,20] the 5-year changes in these hormonal levels were not significant in the present study. The estimated annual decline of FT level, calculated with the linear regression equations from our cross-sectional samples (Fig. 2), was approximately −2.5% per year. However, the number of samples needed in the longitudinal study was calculated to be approximately 91 in power analysis, and the sample size was not enough to enable statistical judgment due to large standard deviation. Further, although not significantly, the mean FT level increased after 5 years compared to baseline; this could be attributed to the relatively short follow-up period or to too small number of subjects to detect the subtle hormonal decline with considerably large between-subject variations.

In the current study, as shown in Table 3, no clear associations were observed between plasma androgen