Distribution of Dehydroepiandrosterone Sulfate and Relationships Between its Level and Serum Lipid Levels in a Rural Japanese Population

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Dehydroepiandrosterone sulfate (DHEAS) is a major secretory product of the adrenal glands. DHEAS is inversely associated with death from cardiovascular disease in males, but not in females. This cross-sectional study examined the relationships between serum DHEAS levels and atherosclerosis in free living subjects in Japan. We measured the serum DHEAS levels of 990 apparently healthy subjects aged 35-81 years old in a rural area in Japan; 431 males and 559 females. The levels were determined by the radioimmunoassay method. The frequency distribution was skewed to a lower value in both sexes. Both unadjusted and age-adjusted mean DHEAS levels were statistically higher in males than in females. A marked linear decline of levels with age was observed in both sexes. DHEAS levels were positively correlated with high density lipoprotein-cholesterol (HDL-C), and negatively correlated with low density lipoprotein-cholesterol (LDL-C) even after adjustment for age in both sexes. The mean atherogenic index (AI) was significantly inversely correlated with the rise of tertiles of the DHEAS level, both before and after adjustment for age, Total cholesterol (TC), HDL-C and Triglyceride (TG). These results suggest high levels of serum DHEAS may have an inhibitory effect on the development of atherosclerosis and have an important role in its etiology and prevention.

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MATERIALS AND METHODS

The subjects for this study were 1789 males and females aged 35-81 years, who participated in these mass medical examinations conducted since 1983 in accordance with the health and medical service law for aged in Japan, during the 2 months from June through July in 1996, in a rural town, north of Nagoya. The distribution of the total population in this distinct and the subjects by sex and age are shown in Table 1. The participation rate of subjects to the total population was 68 percent for males and 72 percent for females. Of these persons, 510 were excluded from the present analysis, 415 (23.2 percent) because of missing data on serum levels of DHEA, and 232 (13.0 percent) because of missing data on any serum levels of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDLC). Also 152 (8.5 percent) were excluded because of missing data on age, height or weight. These exclusions left 431 males and 559 females (55.3 percent of 1789 subjects) for analysis. Medical history, habitual food intake, smoking habits, alcohol consumption and physical activity were determined by a self-administered, structured questionnaire.

Height and body weight were measured with the participants wearing light clothing without shoes. Body mass index was calculated as weight (kg)/height(m)^2.

Blood samples were obtained before noon after an overnight fast from the antecubical vein and separated immediately after collection.

Serum DHEAS levels were measured by radioimmunoassay method. The serum levels of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDLC) were determined by enzymatic methods. High-density lipoprotein cholesterol (HDL-C) was measured by the phosphate-manganese chloride precipitation method. The serum levels of low density lipoprotein cholesterol (LDLC) were calculated according to the formula of Friedewald et al. The atherogenic index (AI) was calculated by (TC-HDL-C)/HDL-C. Statistical analyses were performed with SAS statistical package.

The distribution of DHEAS levels was highly skewed, and a logarithmic (ln) transformation was performed. TG values also were log transformed. Pearson correlation coefficient were used to examine the association between the DHEAS levels and serum lipid levels. Partial correlation analysis was used to adjust the DHEAS levels relationship for potentially confounding effects of age and BMI. We computed AI by tertiles for DHEAS (<33%, 33-66%, >66 %), using analysis of covariance to remove the effects of age, TC, TG and HDLC.

To test for a linear trend, we used multiple linear regression analysis models with AI as the dependent variable and DHEAS, age, TC, HDLC and TG as the independent variable. All statistical tests were two-tailed, with statistical significance defined as p<0.05.

RESULTS

The descriptive characteristics of the subjects in this study are shown in Table 2. There were no significant differences in mean age between males and females, 48.6±11.4 and 49.7±10.3, respectively. The mean serum total cholesterol concentration of 190.8±32.5 mg/dl in this study was similar to the result of the National Survey of Circulatory Disorders. DHEA levels were statistically significant higher in males than in females. Serum levels of TC, HDLC, LDLC were statistically significantly higher in females than in males, whereas the serum TG level and AI were significantly higher in males.

The relative frequency distribution of DHEAS is shown in Fig 1. The DHEAS frequency distribution was highly skewed toward the low level in both sexes. The DHEAS distribution differed between sexes. The peak frequency distribution of DHEAS levels in females shifted to a lower level compared with that in males. In the lowest class of DHEAS (DHEAS<500 ng/dl), the relative frequency of females (15.2%) was about 4 times higher than that of males (4.4%).

The arithmetic mean DHEAS levels by sex and age are shown in Tables 3 and Fig.2. DHEAS was distributed widely in 718-3950 ng/dl for males and in 431-2156 ng/dl for females. The mean DHEAS levels were significantly higher in males than in females for any age group. The age-adjusted means levels of DHEAS in males (1698 ng/dl) was significantly higher than in females (1065 ng/dl). The mean DHEAS levels and age were negatively correlated in males and females (p=0.000 and p=0.000, respectively).

Selected percentiles for serum DHEAS levels in males and females are shown in Table 4. The quartiles, 10 percentile, 50

Table 1. Number of total population and subjects by sex and age category.

| Sex   | Age category (y) | Total population | Subjects |
|-------|------------------|------------------|----------|
| Males | ≤39              | 273              | 153      |
|       | 40-49            | 295              | 215      |
|       | 50-59            | 223              | 152      |
|       | 60-69            | 161              | 125      |
|       | ≥70              | 114              | 80       |
| Total |                  | 1066             | 725      |
| Women | ≤39              | 272              | 163      |
|       | 40-49            | 453              | 362      |
|       | 50-59            | 313              | 281      |
|       | 60-69            | 305              | 195      |
|       | ≥70              | 120              | 53       |
| Total |                  | 1463             | 1054     |
Table 2. Serum DHEAS levels and serum lipids levels by sex, for 431 male and 559 females aged 35-81 years.

|                      | Males (Mean±SD) | Females (Mean±SD) | p value |
|----------------------|-----------------|-------------------|---------|
| Age (years)          | 48.8±10.9       | 49.7±10.3         | N.S.    |
| Total cholesterol (mg/dl) | 184.6±28.8     | 196.9±31.1        | 0.000   |
| Triglyceride (mg/dl)  | 134.5±92.1      | 102.1±70.6        | 0.000   |
| HDL cholesterol (mg/dl) | 52.4±12.8       | 60.2±15.1         | 0.000   |
| LDL cholesterol (mg/dl) | 440.6±98.0     | 459.9±117.1       | 0.001   |
| Atherogenic index     | 2.7±1.2         | 2.5±1.0           | 0.043   |
| Dehydroepiandrosterone sulfate | 1699.5±970.2 | 1050.8±685.1 | 0.000   |

N.S.: Not significant

Figure 1. Distribution of serum DHEAS levels by sex.

Table 3. Mean serum DHEAS levels (ng/dl) by sex and age, for 431 males and 559 females aged 35-81 years.

| Sex and Age category | Number of subjects | Males DHEAS Mean ± (SD) (ng/dl) | Females DHEAS Mean ± (SD) (ng/dl) | P value |
|----------------------|--------------------|---------------------------------|----------------------------------|---------|
| ≤39                  | 95                 | 2374.0 (982.3)                 | 98                               | 1423.0 (624.9) | 0.000 |
| 40-49                | 155                | 1746.0 (933.0)                 | 200                              | 1175.8 (787.6) | 0.000 |
| 50-59                | 101                | 1495.0 (679.3)                 | 156                              | 881.7 (589.9)  | 0.000 |
| 60-69                | 60                 | 1091.6 (683.9)                 | 85                               | 723.8 (354.2)  | 0.000 |
| ≥70                  | 20                 | 991.0 (594.2)                  | 20                               | 684.9 (410.8)  | 0.000 |
| P for trend          |                    | 0.000                           |        | 0.000               |        |
| Age-adjusted         | 1689               | 1065                            |        | 0.000               |        |

Significance for difference between males and females.

‡Arithmetic mean
percentile of DHEAS levels were higher in males than in females.

Crude and age-adjusted correlations coefficients between logarithmic DHEAS levels and TC, TG, HDLC, and LDLC in both sexes are shown in Table 5. The crude DHEAS level was significantly inversely correlated with age and LDLC, and positively correlated with HDLC in both sexes. Even after adjustment for age, DHEAS levels were significantly positively correlated with HDLC, and significantly negatively correlated with LDLC in both sexes.
Sex-specific unadjusted and adjusted comparison of the mean AI by tertiles of DHEAS level are shown in Table 6. There was a significantly linear increase in AI for increasing tertile of DHEAS level both before and after adjustment for age, TC, HDLC and TG in both sexes.

**DISCUSSION**

We measured DHEAS in the apparently healthy population of a rural area in Japan using a mass screening program. Some epidemiological studies have revealed that lower DHEAS levels is a significant and independent risk factor for cardiovascular disease. However, few data are available on normal range and distribution of the DHEAS level in healthy adults in both Japan and Western countries. Frequency distribution of DHEAS in this study was a skewed pattern to a lower value, which is similar to other reports. Accordingly, we concluded that there was no difference in the DHEAS frequency distribution between Japanese and foreign adults. The mean DHEAS level in this study was similar to previous study in Japan 9, 12, but was lower than in North America or Europe 4, 18, 19. However, men of Japanese ancestry residing in Honolulu showed higher published levels than Japanese men residing in Japan 20. Wang observed that serum DHEAS levels among British women were higher than among Japanese women 21. We thought these differences in DHEAS levels in different geographic locations were caused by environmental rather than racial or ethnic differences.

We observed a marked linear decrease in DHEAS level with advancing age the same as previous studies in Japan and Western countries 3, 4, 18, 19. Metabolic studies have suggested that the decrease in DHEAS levels with age is due to decreased production of DHEAS rather than increased clearance, and is consistent with reduced adrenal activity in older men, and with enzymatic alternations with aging 22.

DHEAS has been inversely associated with cardiovascular mortality in men, but not in women. Since most of the studies on the relationships between serum DHEAS levels and cardiovascular risk factors or atherosclerotic were restricted to males, little data are available on the relation between DHEAS and lipids in women except for a preliminary report suggesting that higher DHEAS levels are associated with a less atherogenic lipoprotein profile 20.

Recent research has examined the relationships between serum DHEAS levels and cardiovascular risk factors and atherosclerosis in both males and females. Firstly, in both sexes, we found that serum DHEAS levels had a positive association with TC, but the association was weak and not statistically significant, likewise our previous report used 90 males 11. Several studies 23, 24, 25 have shown an inverse association between serum DHEAS levels and TC. The reasons for these differences in the association between serum DHEAS levels and TC in current report and previous studies is unknown, but could to be due to the smaller sample size and lower power in some earlier reports. However, the present study showed that serum DHEAS levels had a significantly positive association with HDLC and a negative association with LDLC after adjustment for age, TC and TG in both sexes. This is in agreement with several previous studies that serum DHEAS levels were positively correlated with HDLC 23) and inversely with LDLC 24). In a number of previous studies, estrogen level was positively related to the HDLC levels and inversely related to the LDLC levels 26. Serum DHEAS levels can be converted into many other steroids including more potent androgens and estrogens 27. This suggests that serum DHEAS levels may indirectly affect HDLC or LDLC levels through DHEAS-estrogen associations. In a case series, patients fed a pharmacologic dose of DHEAS had marked reductions in angina symptoms 28. However, in case-control studies on the association between serum DHEAS levels and myocardial infarction used postdiagnostic sera, the association was found to be inconsistent, showing inverse associations 29, no association 30 or positive associations 31. One possible limitation of retrospective studies is that myocardial infarction patients may alter serum DHEAS levels after occurrence.

In a cross-sectional angiographic study, men with stenosis of ≥50 percent had significantly lower DHEAS levels than men without stenosis 6. Many studies have been reported in

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**Table 6. Comparison of unadjusted and adjusted atherogenic index by tertiles of DHEAS level for 431 male and 559 females aged 35-81 years.**

| Tertiles  | Males | | Females | |
|----------|-------|-----|---------|-----|
|          | Unadjusted | Adjusted‡ | Unadjusted | Adjusted‡ |
| Lowest   | 3.01  | 2.98 | 2.71  | 2.68 |
| Middle   | 2.78  | 2.82 | 2.50  | 2.42 |
| Highest  | 2.56  | 2.66 | 2.37  | 2.31 |
| P value  | 0.035 | 0.026| 0.042 | 0.032|

‡: Adjusted for age, BMI, TC, TG, and HDLC
Western countries on the negative association with atherosclerosis \(4,5,29\). Some prospective studies \(6-8\) based on population studies have shown an inverse correlation between serum DHEAS levels and death from ischemic heart disease in adult men. The most compelling evidence relating the DHEAS level to coronary heart disease comes from a prospective epidemiological study of 242 men that found a threefold higher risk of coronary heart disease death over 12 years of follow-up among men with low compared to higher DHEAS levels \(5\). Hautanen et al \(8\) found, in their nested case-control study of 103 men with myocardial infarction in the Helsinki Heart Study, that cases had significantly higher levels. However, no studies have also reported the relation between serum DHEAS levels and mortality from ischemic heart disease among Japanese adult population. We have observed that a significant inverse relation was observed between serum DHEAS and Al in our previous study used 90 males employees \(10\). In this cross-sectional study used more larger samples, we also found the same finding as previous study, not only in males, but also in females. Accordingly, this finding suggests that the serum DHEAS acts protective against atherosclerosis in both sexes.

Several mechanisms have been suggested for the protective action of DHEAS against atherosclerosis. In this study, the serum DHEAS level had a significant positive association with the HDLC level which is known as a anti-atherogenic factor, and inversely association with the LDLC level, which is known as an atherogenic factor. This suggests DHEAS may indirectly inhibit the development of atherosclerosis through an increase in HDLC and a decrease in LDLC. Several studies suggest DHEAS is directly associated with the inhibition of the development of atherosclerosis. At the cellular level, DHEAS has been shown to interfere with atherogenesis by affecting adherence of platelets and macrophages, the release of chemotactants and growth factors, the proliferation of cellular elements or uptake of cholesterol in the atheroma \(20\). In addition, DHEAS has been demonstrated to interfere with the generation of superoxide radicals by phorbol ester-stimulated human granulocytes.

It may be possible that DHEAS also protects against atherosclerosis by reducing local free radical generation \(26\).

Furthermore, DHEAS is a potent non-competitive inhibitor of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose cycle, which is necessary for extra-mitochondrial production of reduced nicotinamide adenine dinucleotide phosphate (NADPH), a co-enzyme important in the synthesis of fatty acid, cholesterol and thromboplastin \(30\). Animal studies \(31,30\) have shown that rabbits fed high-cholesterol diets had a significant reduction in atherosclerosis plaque size in the aorta when they also received DHEAS. Fatty infiltration of the heart and liver was also markedly reduced. This study, which observed the reduction of atherosclerosis in rabbits fed DHEAS and cholesterol, not only suggested that DHEAS inhibits the development of atherosclerosis following endothelial injury, but also is particularly strong finding that DHEAS has a direct inhibitory effect on atherosclerosis. However, the mechanism for a protective effect of DHEAS against atherosclerosis is difficult to explain only a direct inhibitory effect. Accordingly, it seems reasonable to suppose that both direct and indirect inhibitory effect with DHEAS itself play a role in the development of atherosclerosis, although it seems to be differences in the strength of inhibitory effect.

In this study, we observed that serum DHEAS levels had a significantly inversely association with age and a rise of Al in both sexes. The present findings suggest DHEAS may have an important role in the etiology and prevention of atherosclerosis in both sexes, although longitudinal follow-up studies are need to utilize the DHEAS level as an index of atherosclerosis.

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