Physiological Levels of Estradiol Correlate with Lipid / Lipoprotein Profiles in Healthy Men

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Abstract

In this cross-sectional study, the relationships of the physiological levels of estradiol (E2) and dehydroepiandrosterone-sulfate (DHEA-S) to the lipid and lipoprotein profiles were investigated in 212 apparently healthy men aged from 18 to 59 years old. These subjects were divided into tertiles according to the levels of E2 and DHEA-S, respectively. We performed one-way analysis of variance and analysis of co-variance controlling for age, body mass index, percent body fat, waist to hip ratio, maximal oxygen uptake, alcohol and cigarette consumptions as confounding factors.

A lower E2 level was associated with higher concentrations of total cholesterol (TC) and low density lipoprotein cholesterol. A lower DHEA-S level was associated with a lower concentration of high density lipoprotein cholesterol (HDL) and a higher ratio of TC / HDL. These correlations remained significant after adjusting for the above confounding factors. Neither free testosterone nor sex hormone-binding globulin was associated with the lipids and lipoproteins. These results suggest that higher levels of E2 and DHEA-S, at least in physiological concentrations, are related to the favorable lipid and lipoprotein levels in men.

Key words: Estradiol, Dehydroepiandrosterone-sulfate, Lipid metabolism, Men

Introduction

Sex hormones have been established as risk factors for coronary heart disease (CHD) and the influences of sex hormones to lipid and lipoprotein metabolism have been demonstrated^{1, 2)}. However, results in men were inconclusive. In previous studies, a higher level of estradiol (E2) and/or a higher ratio of E2 / testosterone (T) were found in men with myocardial infarction³⁾, diabetes mellitus⁴⁾, hypercholesterolemia⁵⁾, hypertriglyceridemia⁶⁾, hypertension⁷⁾ and obesity⁸⁾ compared with control subjects. The administration of high doses of estrogen to men with prostatic carcinoma increases the high density lipoprotein cholesterol (HDL) level and triglycerides (TG) and decreases the low density lipoprotein cholesterol (LDL) level⁹⁾, while another clinical study demonstrated that selective E2 deficiency in healthy men induced decreases in HDL and apolipoprotein (apo) A1¹⁰⁾. The administration of high dose T to normal men did not significantly suppress HDL. However, when the normal conversion of T to E2 was blocked, the HDL levels decreased significantly¹¹⁾. From these studies physiological levels of both E2 as well as T are thus assumed to be necessary to maintain the HDL level in healthy men. However, the role of estrogen on the lipid metabolism in healthy men has not been as

well investigated as T in a population-based study.

Dehydroepiandrosterone (DHEA) and its sulfate ester, dehydroepiandrosterone-sulfate (DHEA-S) decrease with aging¹²⁾. Weak androgenic activity and other physiological actions such as anti-obesity and anti-atherosclerotic effects as well as anti-carcinogenic action¹³⁻¹⁵⁾ have been recognized. Low DHEA-S level can predict the development of angiographically diagnosed coronary atherosclerosis¹⁶⁾ and mortality in CHD¹⁷⁾ in men. Regarding the relationship between DHEA-S and lipid metabolism, however, studies with conflicting results or absence of correlation were reported¹⁶⁻²¹⁾.

In the present study, we thus evaluated the relationships of the concentrations of E2 and DHEA-S to the lipid and lipoprotein levels in apparently healthy Japanese men from a wide range of ages and with different degrees of adiposity.

Subjects and Methods

1. Subjects

The subjects in this study were apparently healthy Japanese men (n=212) between 18-59 years old who worked at a food company located in the southwestern part of Japan. Any subjects taking medications that might affect their lipid or glucose metabolism and subjects with a fasting blood glucose in excess of 120 mg/dl were excluded.

2. Anthropometric parameters

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Table 1 Characteristics of the study subjects (n=212)

Variables		Mean	S.D.	Min.	Max.
Age (yrs)		39.1	13.8	18	59
BMI (kg/m ²)		22.4	2.7	16.7	35.9
%Fat (%)		18.3	6.4	6.0	39.0
WHR		.84	.06	.69	.99
VO ₂ max (ml/kg/min)		36.6	5.5	23.7	51.3
Cigarette smoking	no 112 (52.8%)				
	yes 100 (47.2%) number/day	21.5	10.3	1	60
Alcohol drinking	no 62 (29.2%)				
	yes 150 (70.8%) unit/week	6.8	6.6	0.25	31.5
Blood parameters					
TC (mg/dl)		200.6	36.4	114.0	291.0
LDLC (mg/dl)		124.5	32.7	38.0	224.2
HDLC (mg/dl)		53.4	11.2	32.9	81.1
TC/HDLC		3.91	1.07	2.15	7.45
TG (mg/dl) §		97.7	48.9	50.0	656.0
Free T (pg/ml)		23.1	7.5	6.3	44.8
E2(pg/ml)		26.0	12.9	10.0	60.0
DHEA-S (ng/ml)		2108.3	892.4	320.0	5880.0
SHBG (nmol/l)		38.5	18.3	7.0	97.0

Abbreviations: See text. § : Log-transformed for statistical testing.

light clothing in an upright position were measured by the same researcher. Adiposity was evaluated by the body mass index (BMI), the percent body fat (%Fat), and the ratio of the waist to hip circumference (WHR). The BMI was calculated as the weight (kg) divided by the height (m) squared. The %Fat was calculated from the subscapular and triceps skinfold thickness measured by a Harpenden caliper using Brozek's formula²²⁾ after estimating the body density according to Nagamine's method²³⁾. The circumferences of the waist and hip were measured at the level of the umbilicus and at the level of the greater trochanter, respectively. The WHR was used as an index of abdominal fat distribution.

3. Physical fitness and habitual behavior

Physical fitness was evaluated by the maximal oxygen uptake (VO₂max) predicted indirectly using an electric cycle ergometer (ML1400 ; Fukuda Denshi, Japan). The usual daily consumption of cigarettes and the usual weekly consumption of alcohol were recorded by questionnaire. We transformed the alcohol consumption expressed as "units" (1 unit = 1 Go (180ml) of Japanese sake).

4. Blood parameters

Blood samples were drawn from the antecubital vein after a 12 hour fast to determine the following parameters: total cholesterol (TC), HDLC, TG, E2, free T, sex hormone-binding globulin (SHBG), and DHEA-S. The TC, HDLC and TG were analyzed by the enzymatic method. The LDLC was calculated using Friedwald's method²⁴⁾. The mean ratio of TC / HDLC was used as coronary risk factor criteria. E2, free T and DHEA-S were measured by a radio-immunoassay technique using commercial kits (DPC, Los Angeles, USA). SHBG was measured by an immunoradiometric assay technique using SHBG [125 I] assay kit (Farmous Daiagnostica, Oulunsalo, Finland). Intra- and inter-assay coefficients of variation were 1.1% and 0.8% for TC; 2.1% and 1.8% for HDLC; 1.2% and 0.8% for TG; 4.2% and 8.5% for E2; 1.3% and 7.1% for free T; 3.2% and 6.3% for SHBG; 4.1% and 8.6% for DHEA-S, respectively.

5. Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the lipid and lipoprotein variables for each group. Analysis of co-variance (ANCOVA) was used to adjust the effects of several confounding factors. The log-transformed values were used because TG was not normally distributed. A probability level of 0.05 was considered to denote statistical significance.

Results

The subjects in the present study showed a wide range in age, adiposity, physical fitness level, blood lipids and sex hormone levels (Table 1). As shown in Table 2, the level of E2 was inversely associated with the alcohol and cigarette consumption and the concentration of TC and LDLC. The level of DHEA-S correlated inversely with TC/HDLC. The age-related decreases of E2 and DHEA-S were observed.

As demonstrated in Table 3, the levels of TC, LDLC, TC/HDLC, and TG correlated positively with age and adiposity, and correlated inversely with VO₂max. The level HDLC correlated inversely with adiposity and positively with VO₂max. There was a positive correlation between alcohol consumption

Table 2 Pearson's correlation coefficients of E2 and DHEA-S

	E2	DHEA-S
Age	-.164	-.365
BMI	n.s.	n.s.
%Fat	n.s.	n.s.
WHR	n.s.	n.s.
VO ₂ max	n.s.	n.s.
Alcohol	-.145	n.s.
Cigarettes	-.154	n.s.
TC	-.189	n.s.
LDLC	-.196	n.s.
HDLC	n.s.	n.s.
TC/HDLC	n.s.	-.178
TG §	n.s.	n.s.

Above listed coefficients are significant at the 5% level.

§ : Log-transformed for statistical testing

See text for the explanation of abbreviations.

n.s.: not significant.

and the level of TC and TG. Cigarette smoking was positively associated with TG and TC/HDL, and inversely associated with HDL.

Table 4 shows the mean values of the serum lipids and sex hormones classified by the tertiles of E2. The range of E2 concentration was from 10 pg/ml to 18 pg/ml (mean: 12.7 pg/ml) in the low group, from 19 pg/ml to 31 pg/ml (mean: 24.1 pg/ml) in the middle group, and from 32 to 60 pg/ml (mean: 41.3 pg/ml) in the high group. By ANOVA, TC and LDLC were significantly higher in the low E2 group and the concentrations of free T and DHEA-S were higher in the high E2 group (Table 4). By ANCOVA, a significant difference for LDLC remained after adjusting for age, %Fat, WHR, VO₂max, cigarettes, and alcohol. Furthermore, when free T and DHEA-S were added as covariates to adjust the intercorrelation between the sex hormones, such significant difference still existed (Table 4, Figure 1).

Table 5 shows mean values of the serum lipids and sex hormones classified by the tertiles of DHEA-S. The range of DHEA-S concentration was from 320 to 1640 ng/ml (mean: 1231 ng/ml) in the low group, from 1650 to 2370 ng/ml (mean: 2010 ng/ml) in the middle group, and from 2380 to 5880 ng/ml (mean: 3098 ng/ml) in the high group. HDL was slightly higher in the high DHEA-S group, while TC / HDL was significantly lower. The concentration of free T was higher and

SHBG was lower in the high DHEA-S group. After adjusting for age, %Fat, WHR, VO₂max, cigarettes, alcohol, free T and SHBG, the trends of HDL and TC / HDL were still significant among the tertiles of DHEA-S (Table 5, Figure 2).

Discussion

The major finding of the present study was that the physiological E2 level correlated inversely with the TC and LDLC concentrations. Generally, the E2 level is lower and has less variability in men than in women. The role of estrogen on the lipid and lipoprotein metabolism in men, particularly in healthy men, has attracted little interest so far. In earlier studies, hyperestrogenemia was reported in men with myocardial infarction³ and a positive correlation was found between E2 and apo B in healthy men and patients with recent myocardial infarction⁹. In these patients, the range of E2 was significantly higher than that in normal men. Contrary to these studies, Dai et al.²⁰ showed that E2 was negatively but weakly correlated with LDLC in healthy men aged from 35 to 57 years old. Stefanic et al.²⁵ found an inverse relationship between E2 and LDLC after adjusting for the influences of smoking, alcohol intake, and adiposity in healthy but sedentary men aged from 33 to 55 years old. In the present study, we confirmed that estrogen plays an important role in lipid metabolism in healthy men with a wide range of age regardless of its physiological concentration. Although the mechanism has not been clarified, it is speculated that estrogen increases LDL receptor activity in liver and induces enhancement of lipid metabolism by altering the ratio of muscle to adipose lipoprotein lipase activity in animal study²⁶.

Another important finding of this study was that the DHEA-S level correlated positively with HDL and negatively with TC / HDL independent of any potential confounding factors. This result also confirmed the observations of the previous cross-sectional population studies of men^{18, 19}. Lower DHEA-S level compared with healthy control and positive correlation between HDL3-C and DHEA-S were found in young men after myocardial infarction²⁷. Furthermore, a negative dose-response relation between DHEA-S and angiographically defined coronary atherosclerosis¹⁶ and inverse associations between the baseline DHEA-S level and subsequent mortality of any cause and cardiovascular disease in a prospective study of older men¹⁷ have

Table 3 Pearson's correlation coefficients of TC, HDL, TC/HDL and TG

	TC	LDL	HDL	TC/HDL	TG §
Age	.503	.415	n.s.	.385	.370
BMI	.305	.309	-.218	.385	.271
%Fat	.215	.246	-.218	.300	.225
WHR	.501	.468	-.174	.487	.407
VO ₂ max	-.425	-.392	.192	-.427	-.380
Alcohol	.192	n.s.	n.s.	n.s.	.326
Cigarettes	n.s.	n.s.	-.145	.186	.161

Above listed coefficients are significant at the 5% level.

§ : Log-transformed for statistical testing

See text for the explanation of abbreviations.

Table 4 Means of variables shown by the tertiles of E2 concentration

Tertile of E2	low	middle	high	P value
Variables				ANOVA(ANCOVA)
TC (mg/dl)	206.4	205.2	190.2	0.012 (0.067)
LDL (mg/dl)	130.1	128.7	114.9	0.009 (0.024)
HDL (mg/dl)	54.6	51.3	54.1	n.s.
TC/HDL	3.9	4.2	3.7	0.0017 (0.067)
TG (mg/dl)	95.5	107.2	91.2	0.105 (0.136)
Free T (pg/ml)	21.3	23.5	24.4	0.039
DHEA-S (ng/ml)	2091.0	1915.7	2313.2	0.029
SHBG (nmol/l)	39.5	39.9	36.2	n.s.

See text for the explanation of abbreviations.

*p<0.05 by Fisher's PLSD.

n.s.: not significant.

Table 5 Means of variables shown by the tertiles of DHEA-S concentration

Tertile of DHEA-S	low	middle	high	P value
Variables				ANOVA(ANCOVA)
HDL (mg/dl)	51.7	52.5	55.9	0.062 (0.052)
TC/HDL	4.10	3.99	3.59	0.006 (0.040)
TG (mg/dl)	104.7	97.7	89.1	0.081 (0.265)
E2(pg/ml)	25.1	25.4	27.4	n.s.
Free T (pg/ml)	21.8	21.8	25.6	0.002
SHBG (nmol/l)	43.2	41.0	31.3	0.0002

See text for the explanation of abbreviations.

*p<0.05 by Fisher's PLSD.

n.s.: not significant.

Tertiles of E2

Figure 1

Means LDLC shown by the tertiles of estradiol concentration after adjusting for age, BMI, %fat, WHR, VO₂max, smoking, alcohol, free T and DHEA-S by ANCOVA. **p*<0.05. See text for the explanation of abbreviations.

been demonstrated. On the other hand, confounding results have been also found in clinical studies. Thus, a decrease in the TC and LDLC level in young normal men using supraphysiological doses of DHEA-S has been reported²⁸, while no changes in the lipid level were observed after the same dose of DHEA in young obese men²⁹ or after a physiological dose in middle-aged and elderly men³⁰. Considering these results, DHEA-S may possibly have a beneficial effect on the lipid metabolism at least in normal men, however, further studies are still needed.

One of the reasons for the above conflicting results may be due to the differences in physiological and supraphysiological sex hormone levels. In the present study, we focused our attention on the physiological effects in healthy men. Another reason for such conflicting results may be the concomitant relationships among the variables. Most previous studies controlled the level of adiposity, while a few studies simultaneously considered the influences of body fat distribution, alcohol and cigarette consumption, and physical fitness level which may affect the levels of lipid and lipoprotein as well as the level of sex hormones.

We took account of the possible confounding factors to clarify the relationships between sex hormones and lipids. 1) Since the ages of the subjects in this study had a wide range and significant associations with both the lipids and sex hormones, the effect of aging should thus be controlled. 2) We took account of not only total body fatness but regional fat distribution, because it has been shown to be an important determinant of dyslipidemia and CHD³¹. 3) Relatively low physical activity and/or physical fitness levels have been reported to be independent risk factors for CHD³². Some reports have demonstrated that the fitness level evaluated by the VO₂max^{32, 33, 34} was one of the predictors of the lipid parameters. In the present study, the VO₂max was significantly associated with free T as well as the lipid variables. 4) Several epidemiological studies have

Tertiles of DHEA-S

Figure 2

Means of HDLC and the ratio of TC/HDLC shown by the tertiles of DHEA-S concentration after adjusting for age, BMI, %fat, WHR, VO₂max, smoking, alcohol, free T and SHBG by ANCOVA. **p*<0.05. See text for the explanation of abbreviations.

demonstrated that light to moderate alcohol consumption is associated with a higher level of HDLC³⁵, and lower concentrations of TG³⁶ and LDLC³⁷. On the other hand, heavy drinking appeared to increase the risk of stroke and sudden death in prospective studies³⁶. In the present study, the alcohol consumption was positively associated with TC and TG, while inversely associated with E2. It seems that alcohol stimulates protein synthesis with SHBG and the level of free T and E2 would fall³⁸. 5) Regarding the relationship between cigarette smoking and E2 in men, inconsistent results have been demonstrated. Some previous studies have reported an increased E2 level in male smokers^{39, 40} or a positive correlation between cigarette smoking and E2⁴¹, but others did not find any such correlations⁴². 6) There were intercorrelations among sex hormones and SHBG (see Table 4, 5). We thus took these

variables into account as the confounding factors.

In conclusion, the present study demonstrated that the higher levels of serum E2 and DHEA-S within the physiological ranges in men partially relate to the maintenance of favorable

lipid and lipoprotein levels. However, because of the limitations of the cross-sectional design of this study, both the mechanism and the causality remain to be clarified.

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