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Dehydroepiandrosterone Supplementation Improves Endothelial Function and Insulin Sensitivity in Men

HIROAKI KAWANO, HIROFUMI YASUE, AKIRA KITAGAWA, NOBUTAKA HIRAI, TOSHIAKI YOSHIDA, HIROFUMI SOEJIMA, SHINZO MIYAMOTO, MASAHIRO NAKANO, AND HISAO OGAWA

Departments of Cardiovascular Medicine (H.K., N.H., T.Y., H.S., S.M., H.O.) and Pharmacy (A.K.), Kumamoto University School of Medicine, Kumamoto Aging Research Institute (H.Y.), Kumamoto 860-8556, Japan; and Department of Clinical Pharmacy Graduate School of Pharmaceutical Sciences, University of Shizuoka (M.N.), Shizuoka 422-8529, Japan

The dehydroepiandrosterone (DHEA) concentration decreases with age. There is evidence that DHEA has a protective effect against age-related disorders, including cardiovascular disease. Accordingly, we examined the effect of DHEA supplementation (25 mg/d) on endothelial function, insulin sensitivity, and fibrinolytic activity in 24 men with hypercholesterolemia (mean age, 54 ± 1 yr). All subjects were enrolled in a randomized, double-blind study. Flow-mediated dilation of brachial artery after transient occlusion, which was expressed as the percent change from the baseline value of the diameter, increased significantly with DHEA supplementation [DHEA: baseline, 3.9 ± 0.5%; 4 wk, 6.9 ± 0.7%; 8 wk, 7.9 ± 0.6%; 12 wk, 8.4 ± 0.7% ($P < 0.01$ *vs.* baseline for all, by ANOVA); placebo: 4.1 ± 0.6%, 4.5 ± 0.5%, 3.9 ± 0.5%, and 4.4 ± 0.6% ($P < 0.01$ for all, by ANOVA)]. There was a significant concurrent reduction in the plasma levels of plasminogen activator inhibitor type 1 during DHEA supplementation [DHEA: 9.1 ± 2.2, 6.4 ± 2.3, 5.5 ± 2.8, and 5.1 ± 2.0 IU/ml ($P < 0.01$ *vs.* baseline, by ANOVA); placebo: 9.0 ± 2.1, 10.4 ± 2.2, 9.5 ± 2.2, and 9.6 ± 2.1 IU/ml ($P < 0.01$, by ANOVA)]. DHEA supplementation also decreased steady state plasma glucose [DHEA: baseline, 178.9 ± 12.2; 12 wk, 132.0 ± 12.8 mg/dl ($P < 0.01$, by ANOVA); placebo: 181.0 ± 13.8 and 179.6 ± 12.4 mg/dl ($P < 0.01$, by ANOVA)]. In contrast, steady state plasma insulin did not change during the study in either group. The low dose DHEA supplementation improves vascular endothelial function and insulin sensitivity and decreases the plasminogen activator inhibitor type 1 concentration. These beneficial changes have the potential to attenuate the development of age-related disorders such as cardiovascular disease. (*J Clin Endocrinol Metab* 88: 3190–3195, 2003)

ALTHOUGH DEHYDROEPIANDROSTERONE (DHEA) and its sulfate compound (DHEAS) are the most abundant steroids produced by the adrenal (1), their biological significance remains to be fully established. The secretion of DHEA(S) peaks by the second decade and then declines steadily at an average of approximately 10% each decade (1). Studies have shown an inverse relationship between plasma levels of DHEA(S) and coronary artery disease (2) and as the prevalence of coronary artery disease increases with age (3). It has been suggested that higher levels of DHEA(S) may protect against the development of atherosclerosis (4–6).

Endothelium-dependent vasodilation becomes impaired with old age (7, 8). Endothelial dysfunction has been reported to contribute to the pathogenesis of atherosclerosis and cardiovascular disease (9). There is now increasing evidence that changes in endothelial function precede the development of insulin resistance (10). It is also well established that aging is associated with a decrease in insulin sensitivity (11) and a deterioration in glucose tolerance even in the absence of fasting hyperglycemia (12). Several studies have shown a close relationship between these changes in insulin function and age-related diseases, such as cardiovascular disease (12, 13). Other endothelial factors, such as plasminogen activator inhibitor type 1 (PAI-1), have also been implicated in this disease process. This peptide produced by the vascular endothelium is a first-acting inhibitor of plasminogen activation. Endothelial dysfunction increases the PAI-1 concentration, resulting in suppression of fibrinolysis and exacerbation of coronary artery disease (14). On the basis of these associations and the apparent cardioprotective potential of DHEA, we investigated the effect of dietary supplementation of DHEA on insulin sensitivity and endothelial function, including plasma PAI-1 concentration, in middle-aged men with hypercholesterolemia.

Abbreviations: DHEA, Dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; PAI-1, plasminogen activator inhibitor type 1; SSPG, steady state plasma glucose; SSPI, steady state plasma insulin.

Subjects and Methods

Study subjects

The study group comprised 24 men with hypercholesterolemia (mean age, 54 ± 1 yr). Hypercholesterolemia was defined as more than 220 mg/dl. All subjects were asymtomatic, normotensive, nondiabetic, and nonsmokers. Written, informed consent was obtained from all subjects before the study was commenced. The present study was approved by the ethics committee at our institution. All medications were stopped at least 10 d before the study. We tested glucose tolerance in all subjects using a 75-g oral glucose tolerance test according to the criteria of the American Diabetes Association before the study (15).

Study design

The study was a randomized, double-blind, placebo-controlled study of 12-wk duration to evaluate the effects of DHEA supplementation. After selection and consent, the clinical data of each subject were provided to two pharmacologists (A.K. and M.N.), who had sole responsibility for randomization and blinding procedures using random envelopes in the study.

Vascular studies and blood samples were collected at baseline and at 4-wk intervals, and insulin sensitivity was determined at baseline and at the end of the study. Twelve men were randomized to receive DHEA supplementation at a dose of 25 mg/d (mean age, 54 ± 1 yr), whereas the remaining 12 individuals received placebo, consisting of lactose (mean age, 53 ± 1 yr). All investigations were carried out in the morning at 0700 h after the men had fasted for the previous 12 h.

The aim of the present study was to estimate the effect of secretory levels of DHEA in young adults after DHEA supplementation on endothelial function and insulin sensitivity in middle-aged men. According to the results reported by Baulieu *et al.* (16), 25 and 50 mg/d doses were the most effective. Furthermore, the administration of 50 mg/d DHEA over 1 yr had no harmful consequences (16). In accordance with this previous report, we performed a preliminary study before the present study. The oral supplementation of DHEA (25 mg/d) to middle-aged men for 1 wk increased DHEAS levels to young adult values. Thus, we chose the dose of 25 mg/d DHEA.

Endothelial function was assessed by measuring the increase in the diameter of the brachial artery during reactive hyperemia after transient occlusion of the forearm. This vasodilation is caused mainly by endothelium-derived nitric oxide or related substances (17). Flow-mediated endothelium-dependent vasodilation and nitroglycerin-induced vasodilation were measured using ultrasonography according to a procedure that has been validated in our earlier studies and also by other investigators (8, 18–21). Ultrasonography was performed in a quiet, temperature-controlled room (22–23 C) by two experienced investigators who were blinded to the treatment group of the participants. The brachial arteries were scanned in the antecubital fossa region in a longitudinal fashion. The diameter of the left brachial artery above the bifurcation in the non-dominant arm was measured from B-mode ultrasound images, using a 7.5-MHz linear array transducer (SONOS 2500, Philips, Amsterdam, The Netherlands). Optimal brachial artery images were obtained between 1 and 5 cm above the antecubital crease. When a satisfactory transducer position was located, the surface of the skin was marked, and the arm was restrained in the same position throughout the course of the measurements. In addition, to ensure that the same point of the brachial artery was measured at all four time points in the study, a record was made for each subject of the machine settings and the exact distance from the antecubital crease to the measured point on the skin surface.

The subjects lay quietly for 10 min before the first scan. After baseline measurements of the diameter and flow velocity in the brachial artery, a blood pressure cuff placed around the forearm (between 5 and 17 cm under the antecubital crease) was inflated to a pressure of 250–300 mm Hg. After 5 min, the cuff was released. Measurements of brachial artery diameter and flow velocity were continuously performed between cuff inflation and after cuff deflation. Thereafter, the subjects lay quietly for 15 min. After confirming that vessel diameter and flow velocity had returned to baseline levels, 0.3 mg sublingual nitroglycerin was administered, and measurements were repeated 3–4 min later.

The ultrasound images were recorded on a super-VHS videocassette recorder (BR-S601M, Victor, Tokyo, Japan). The measurements were made using ultrasonic calipers at a fixed distance from an anatomical marker. These measurements were taken from the anterior to the posterior m line (*i.e.* the interface between media and adventitia) at the end of diastole, defined by the R wave on a continuously recorded electrocardiogram. Vessel diameter during four cardiac cycles was analyzed for each scan, and the measurements were averaged. The responses of the vessel diameters to the reactive hyperemia and nitroglycerin were expressed as the percent increase from the baseline diameter. Volumetric blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by heart rate and vessel cross-sectional area. The percent increase in brachial blood flow observed immediately after cuff deflation was calculated as the maximum flow recorded within the first 15 sec after cuff deflation divided by baseline flow.

The inter- and intraobserver variabilities for the repeated measurements of resting vessel diameter were 0.05 ± 0.02 and 0.02 ± 0.02 mm, respectively. When these measurements were performed at the same time on 2 separate d in 20 volunteers, the average intrasubject test-retest difference during reactive hyperemia was 0.05 ± 0.04 mm (19–21).

Insulin sensitivity was evaluated before and after 12 wk of DHEA supplementation by the steady state plasma glucose (SSPG) method using octreotide acetate (Sandostatin, Novartis, Basel, Switzerland). Sandostatin inhibits the endogenous secretion of insulin, glucagons, and GH. The validity of this method has been confirmed in our previous studies and by other studies (20–22). After an overnight fast, human insulin (Novolin R-40, 7.5 mU/kg) and Sandostatin (10 μg) were injected as a initial bolus, followed by constant infusion for 2 h of a solution containing glucose (6 mg/kg·min), insulin (0.77 mU/kg·min), Sandostatin (120 μg/2 h), and KCl (0.5 U/kg·min). Blood samples were collected 2 h after the start of the infusion to determine SSPG and steady state plasma insulin (SSPI) concentrations. Plasma glucose levels were confirmed to increase and reach the steady state between 1.5 and 2 h after the start of the infusion (20–22).

The levels of DHEAS, testosterone, and insulin were measured using specific immunoradiometric assays (20, 21, 23). The plasma concentrations of PAI-1 were measured by a commercial chromogenic single-point, poly-D-lysine-stimulated assay kit (Biopool, Inc., Umea, Sweden) (24). Inter- and intraassay coefficients of variation in the PAI-1 assay were 11.4% and 9.4%, respectively (24).

Statistical analysis

The clinical characteristics of the two groups were compared using χ^2 analysis while temporal. Changes in the variables were assessed by two-way ANOVA with repeated measures, followed by *post hoc* testing with Scheffé's test. Statistical significance was defined as $P < 0.05$.

Results

All subjects completed the study without any adverse side-effects. Neither physical examination, sense of well-being, nor libido differed in the DHEA and placebo groups throughout the study.

The data from the vascular and biochemical analyses are summarized in Table 1. At baseline, heart rate, lipid profile, mean arterial pressure, nitroglycerin-induced (endothelium-independent) vasodilation, and fasting plasma levels of glucose, insulin, DHEAS, and testosterone were similar in the two groups. DHEA supplementation resulted in a significant increase in plasma DHEAS concentration, whereas all other parameters remained unchanged in both groups during the study.

Flow-mediated dilation of the brachial artery was similar in the two groups at baseline (Fig. 1). DHEA supplementation did not elicit any change in basal arterial diameter, blood flow, or the percent increase in blood flow during reactive hyperemia in either group (Table 1). Flow-mediated vasodilation increased significantly in the DHEA group during the study compared with the placebo group, as shown in Fig. 1 [DHEA group: baseline, 3.9 ± 0.5; 4 wk, 6.9 ± 0.7%; 8 wk, 7.9 ± 0.6%; 12 wk, 8.4 ± 0.7% ($P < 0.01$ *vs.* baseline); placebo group: baseline, 4.1 ± 0.6%; 4 wk, 4.5 ± 0.5%; 8 wk, 3.9 ± 0.5%; 12 wk, 4.4 ± 0.6%]. This difference in flow-mediated vasodilation between the two groups analyzed by two-way ANOVA was significant ($P < 0.01$).

Figure 2 shows that the plasma PAI-1 concentration was similar in the two groups at baseline. The levels decreased significantly with DHEA supplementation, but remained unchanged in the placebo group [DHEA group: baseline, 9.1 ± 2.2; 4 wk, 6.4 ± 2.3; 8 wk, 5.5 ± 2.8; 12 wk, 5.1 ± 2.0 IU/ml ($P < 0.01$ *vs.* baseline); placebo group: baseline, 9.0 ± 2.1; 4 wk, 10.4 ± 2.2; 8 wk, 9.5 ± 2.2; 12 wk, 9.6 ± 2.1 IU/ml]. This difference in PAI-1 level in the two groups was also significant when analyzed by two-way ANOVA.

Baseline SSPG and SSPI were similar in the two groups (Fig. 3). Twelve weeks of DHEA supplementation decreased SSPG levels in the DHEA group (baseline, 178.9 ± 12.2; 12

TABLE 1. Hemodynamic and biochemistry data

	Placebo group (n = 12)				DHEA group (n = 12)			
	Baseline	4-wk	8-wk	12-wk	Baseline	4-wk	8-wk	12-wk
Heart rate (bpm)	61.7 ± 2.2	63.2 ± 3.5	63.4 ± 2.5	65.2 ± 3.6	62.5 ± 2.7	62.2 ± 3.7	63.4 ± 3.8	63.6 ± 2.7
Mean arterial pressure (mm Hg)	95.5 ± 2.8	92.4 ± 2.7	96.6 ± 1.9	94.2 ± 3.7	94.6 ± 2.4	93.3 ± 4.1	93.7 ± 2.5	91.9 ± 3.8
Baseline diameter (mm)	3.6 ± 0.16	3.7 ± 0.28	3.9 ± 0.28	3.8 ± 0.39	3.7 ± 0.19	3.9 ± 0.21	3.8 ± 0.26	3.9 ± 0.34
Baseline blood flow (ml/min)	277 ± 31.1	268 ± 39.8	272 ± 38.8	277 ± 39.7	283 ± 39.9	280 ± 38.9	264 ± 35.8	267 ± 38.8
Increase in flow during RH (%)	254 ± 13.6	269 ± 14.1	264 ± 17.7	267 ± 19.8	275 ± 17.1	269 ± 16.4	265 ± 16.1	264 ± 16.4
Nitroglycerin-induced dilation (%)	14.8 ± 2.8	14.5 ± 3.4	15.1 ± 2.7	15.2 ± 2.3	14.9 ± 2.3	15.1 ± 2.5	15.2 ± 3.0	14.7 ± 2.4
Total cholesterol (mg/dl)	233 ± 15	231 ± 13	232 ± 15	231 ± 14	234 ± 15	233 ± 14	231 ± 14	232 ± 15
LDL-cholesterol (mg/dl)	152 ± 11	151 ± 13	152 ± 15	151 ± 15	152 ± 14	152 ± 13	152 ± 13	159 ± 16
HDL-cholesterol (mg/dl)	53 ± 9	53 ± 8	54 ± 7	56 ± 8	52 ± 7	53 ± 8	52 ± 8	53 ± 9
Triglycerides (mg/dl)	136 ± 18	137 ± 17	139 ± 18	131 ± 17	137 ± 16	136 ± 18	135 ± 19	139 ± 17
FBS (mg/dl)	92 ± 1.8	93 ± 1.9	92 ± 1.7	93 ± 1.8	93 ± 1.6	92 ± 1.8	94 ± 1.9	93 ± 1.7
Fasting Insulin (μIU/ml)	7.8 ± 0.5	7.9 ± 0.4	7.7 ± 0.6	7.8 ± 0.7	7.6 ± 0.8	7.8 ± 0.7	7.7 ± 0.8	7.8 ± 0.8
Testosterone (ng/dl)	456.4 ± 15.5	460.2 ± 17.4	456.5 ± 16.6	461.1 ± 17.1	452.2 ± 16.1	462.4 ± 15.9	460.3 ± 16.9	457.6 ± 17.2
DHEAS (μg/dl)	111.3 ± 25.5	121.1 ± 24.6	119.2 ± 19.9	115.8 ± 18.8	117.4 ± 27.1	487.6 ± 29.2[a]	490.3 ± 26.3[a]	488.7 ± 28.8[a]

Data are expressed as means ± SE. FBS, Fasting blood glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RH, reactive hyperemia.

[a] $P < 0.01$ *vs.* baseline.

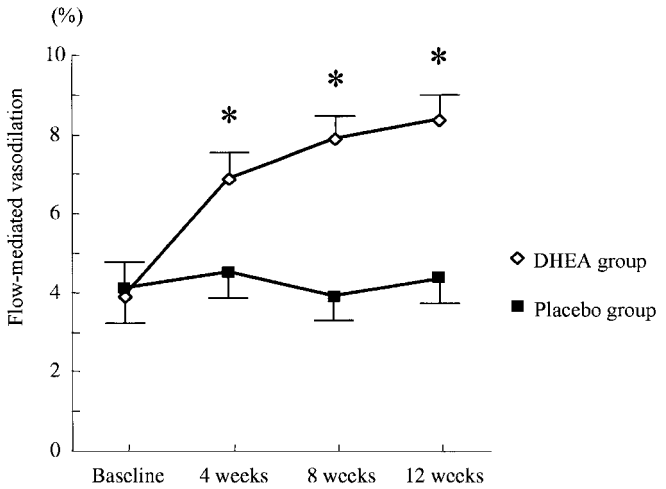

FIG. 1. Effects of DHEA supplementation on flow-mediated, endothelium-dependent dilation of the brachial artery. *, $P < 0.01$ *vs.* baseline. Data are expressed as the mean ± SE.

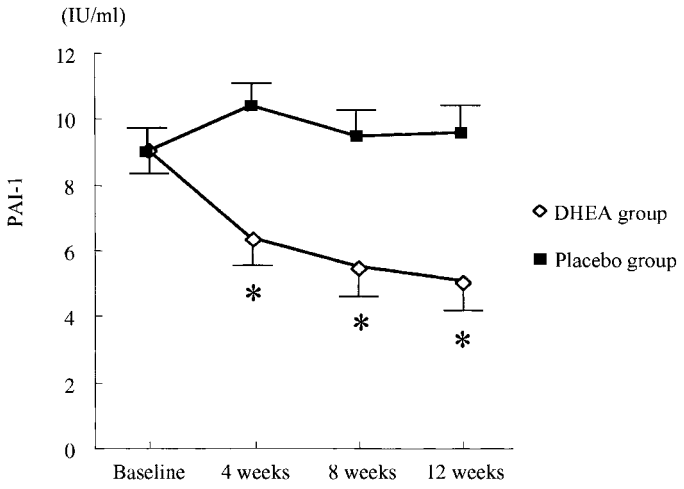

FIG. 2. Effects of DHEA supplementation on plasma PAI-1 concentration. *, $P < 0.01$ *vs.* baseline. Data are expressed as the mean ± SE.

wk, 132.0 ± 12.8 mg/dl; $P < 0.01$), whereas values in the placebo group remained unaltered (baseline, 181.0 ± 13.8; 12 wk, 179.6 ± 12.4 mg/dl). The change in SSPG in the two groups analyzed by two-way ANOVA was significant ($P < 0.01$). In contrast, SSPI did not change during the study in either group (DHEA group: baseline, 28.0 ± 4.1; 12 wk, 29.8 ± 4.6; placebo group: baseline, 29.4 ± 4.6; 12 wk, 28.6 ± 4.2 μIU/ml).

Discussion

It is well established that the plasma DHEA(S) concentration usually far exceeds the level of other sex steroid hormones and decreases with age (1). DHEA(S) has been demonstrated to have an antiatherosclerotic effect in animal models (4–6), and there are some reports that DHEA(S) may have a protective effect against age-related illnesses in humans (16), although the mechanism(s) of these actions remains unknown. Possible explanations for this antiatherogenic effect of DHEA(S) include prevention of platelet aggregation (25) and uptake of cholesterol (4, 5) and a decrease in the proliferation of vascular smooth muscle cells (26).

Our study showed that supplementation of DHEA improved flow-mediated dilation of the brachial artery, a function dependent on endothelium-derived nitric oxide (17). As the endothelial function of the brachial artery and that of the coronary arteries are closely related (18, 19), the modulation of brachial artery endothelium-dependent dilation we observed implies that similar changes may have occurred in the

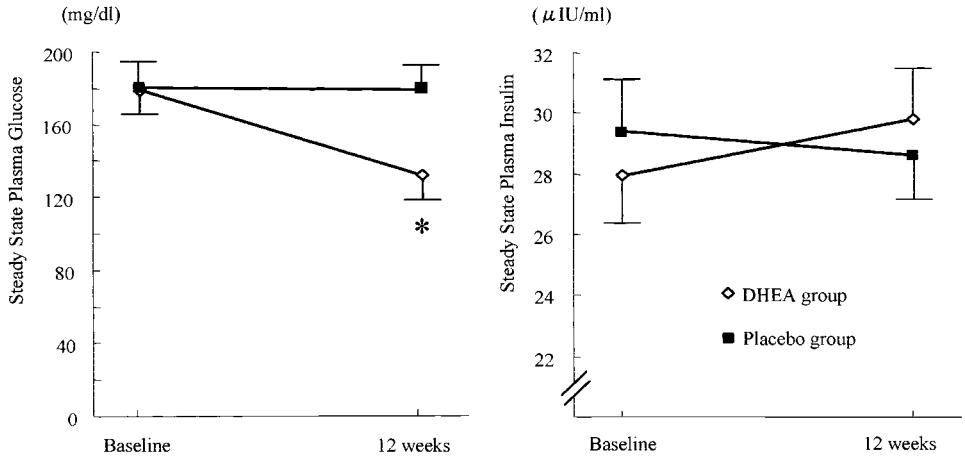

FIG. 3. Effects of DHEA supplementation on insulin sensitivity. *, $P < 0.01$ *vs.* baseline. Data are expressed as the mean ± SE.

coronary arteries. Impairment of endothelial-dependent coronary artery dilation is known to be closely associated with both future cardiovascular events and prognosis (9, 27). DHEA administration has been demonstrated to increase blood flow in uterine and ophthalmic arteries (28, 29). It appears likely that the administration of DHEA is associated with an improvement in systemic arterial blood flow. These effects of DHEA supplementation at least in part may be caused by amelioration of endothelial dysfunction. We anticipate that the improvement in endothelial function after DHEA supplementation may have the potential to minimize the development of cardiovascular disease.

Despite this apparent cardioprotective action of DHEA, little is known about the direct effect of the hormone on the vasculature. Although the adrenal steroid DHEA has no known cellular nuclear receptors, other steroid hormones have specific intracellular receptors that function as ligand-dependent gene transcription factors (30, 31). In contrast to this classical pathway of steroid hormone action, there are also rapid, plasma membrane-dependent, nongenomic effects of steroids in various tissues that lead to several important physiological responses (31). Recently, DHEA receptors have been shown to be expressed on endothelial cell plasma membranes and are coupled to endothelial nitric oxide synthase (32). Thus, DHEA has the potential to stimulate nitric oxide generation. In addition, DHEA may be converted *in vivo* to estradiol (33). This hormone also increases nitric oxide synthase activity and nitric oxide production by either genomic or nongenomic mechanisms within the vascular endothelium (31). Therefore, it is possible that estradiol converted from DHEA may also have contributed to the improvement in endothelial function we observed in this study.

Our finding of a decrease in plasma PAI-1 after DHEA supplementation is consistent with a previous report, which showed that DHEA administration reduced PAI-1 in men (34). Suppression of fibrinolysis by PAI-1 is known to have an important pathogenic role in coronary artery disease, especially acute coronary syndromes (14, 24). PAI-1 is produced by the vascular endothelium and is an important regulatory factor in fibrinolysis. Endothelial dysfunction increases PAI-1 concentrations (14), a change that is especially marked in subjects with insulin resistance independent of whether they have normal glucose tolerance or type 2 diabetes. Interventions that improve insulin sensitivity, such as weight loss, are invariably accompanied by a reduction in plasma PAI-1 concentration (14). In our study the glucose tolerance of all subjects at baseline was normal, as assessed by an oral glucose tolerance test, although it must be recognized that insulin resistance may be present even in patients with a normal oral glucose tolerance test (20). Insulin sensitivity, assessed by SSPG, remained unchanged in the placebo group, whereas we observed a significant improvement in this parameter after DHEA supplementation. From our data it appears that DHEA supplementation may be associated with a lower steady state plasma glucose level without any significant change in circulating insulin levels. These results are consistent with other studies that found DHEA and its sulfate to have a role in the regulation of insulin sensitivity (35, 36) and therefore to have the potential to attenuate age-related increases in insulin resistance.

As the endothelium modulates vascular tone and regulates blood flow to insulin-sensitive tissues such as skeletal muscle (37), it would be anticipated that endothelial dysfunction may lead to impaired blood flow to these tissues that, in turn, results in insulin resistance (10, 20, 21). Other investigators, however, have reported that insulin resistance may occur even in the absence of reduced blood flow to skeletal muscles (38). In addition to improving endothelial function, it is possible that DHEA has a postinsulin receptor effect that also has an important role in determining insulin sensitivity (39). Taken together, our results indicate that long-term DHEA supplementation may assist in maintaining optimal insulin sensitivity. We contend that it is also possible, but as yet unproven, that the combined effect of improved insulin sensitivity and amelioration of endothelial dysfunction after DHEA supplementation may have a role in decreasing PAI-1 concentration.

When interpreting the results of the present study it is important to recognize that similar studies have reported conflicting findings on the effects of DHEA on insulin sensitivity. Casson *et al.* (40) reported that DHEA (50 mg/d) supplementation had beneficial effects on insulin sensitivity in middle-aged postmenopausal women, whereas Morales *et al.* (41) found that this dosage of DHEA had no effect on insulin sensitivity in either men or women. Several other

investigations have also reported that insulin sensitivity is unaltered by DHEA supplementation (42–44). The precise reason for the different findings between these other studies and the present investigation is unclear. Our study had the limitation that it examined the effects of DHEA on impaired endothelial function in hypercholesterolemic males, and to confirm our findings it is necessary to carry out a similar study in men without hypercholesterolemia. Not withstanding this limitation, it is possible that the different results of the studies may be a consequence of the markedly higher dosage of DHEA (*i.e.* 1600 mg/d) administered in the earlier studies (42–44). Another possibility is variability in the age of the study subjects. In contrast with two of the other studies that included subjects less than 37 yr of age (43, 44), our study involved middle-aged men in whom a small dose of DHEA caused DHEAS levels to increase to values seen in young adults. As testosterone levels did not change during the study, our results appear to reflect the physiological actions of DHEA, and it is therefore possible that supplementation of the compound may alter insulin sensitivity if administered to a group of individuals with preexisting insulin resistance.

In conclusion, this double-blind, placebo-controlled study in men with mild hypercholesterolemia demonstrated that low dose DHEA supplementation improved both vascular endothelial function and insulin sensitivity and decreased the PAI-1 concentration. The combined effect of these beneficial changes would be expected to minimize the progression of age-related disorders such as cardiovascular disease.

Acknowledgments

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Address all correspondence and requests for reprints to: Hiroaki Kawano, M.D., Ph.D., Department of Cardiovascular Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto City 860-8556, Japan. E-mail: koumei@gpo.kumamoto-u.ac.jp.

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