

# Reduction of Atherosclerosis by Administration of Dehydroepiandrosterone

## A Study in the Hypercholesterolemic New Zealand White Rabbit with Aortic Intimal Injury

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### Abstract

Dehydroepiandrosterone (DHEA) is an endogenous steroid that blocks carcinogenesis, retards aging, and exerts antiproliferative properties. *In vitro*, it is a potent inhibitor of glucose-6-phosphate dehydrogenase, the first committed step of the pentose phosphate pathway. In man, serum levels of DHEA and its sulfate peak in early adulthood and drop markedly with age. Epidemiologic evidence indicates that low levels of DHEA or its sulfate conjugate are linked to an increased risk of developing cancer or of death from cardiovascular disease. Like cancer, atherosclerosis is a proliferative process characterized by both initiation and promotion phases. This similarity provided a framework in which to study the antiatherogenic effects of DHEA.

Rabbits were randomly assigned to four groups. Two groups of rabbits received aortic endothelial injury by balloon catheter and were fed a 2% cholesterol diet for 12 wk. DHEA, 0.5%, was incorporated into the diet of one group receiving the 2% cholesterol diet and endothelial injury and also into the diet of one of the control groups. Animals were killed after 12 wk and aortas, hearts, and livers were studied. Plasma samples were analyzed for total cholesterol, VLDL, LDL, HDL, triglycerides, DHEA, and DHEA-sulfate levels.

The atherogenic insult resulted in severe atherosclerosis in animals not treated with DHEA. In those receiving DHEA there was an almost 50% reduction in plaque size ( $P = 0.006$ ), inversely related to the serum level of DHEA attained. Fatty infiltration of the heart and liver were also markedly reduced. These beneficial actions were not attributable to differences in body weight gain, food intake, total plasma cholesterol or distribution of cholesterol among the VLDL, LDL, or HDL fractions.

The results show that high levels of plasma DHEA inhibit the development of atherosclerosis and they provide an important experimental link to the epidemiologic studies correlating low DHEA-sulfate plasma levels with an enhanced risk of cardiovascular mortality.

### Introduction

Atherogenesis, like carcinogenesis, is now viewed as a proliferative disorder with identifiable initiating and promoting events

Presented in part at the American Federation for Clinical Research National Meeting, San Diego, CA, May 1987, and published in abstract form (*Clin. Res.* 35:281a). Address reprint requests to Dr. Weisman, Division of Cardiology, Carnegie 568, the Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21205.

Received for publication 20 November 1987 and in revised form 7 March 1988.

J. Clin. Invest.

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0021-9738/88/08/0712/09 \$2.00

Volume 82, August 1988, 712-720

preceding the proliferative stage (1, 2). Both diseases may be characterized by monoclonal cellular proliferation and in both diseases cellular proliferation is stimulated by growth factors (3-5). Just as chemical carcinogens cause cellular proliferation, these agents can increase the frequency and size of spontaneous atherosclerotic lesions (6, 7). Because of these similarities between the pathogenesis of cancer and atherosclerosis, we considered the possibility that compounds that interfere with carcinogenesis might have similar inhibitory effects on atherosclerosis.

Dehydroepiandrosterone (DHEA)<sup>1</sup> is an endogenous steroid with a wide variety of restraining effects on cell proliferation and differentiation and carcinogenesis in animals and their cells. DHEA has been shown to reduce the incidence of a variety of both chemically induced and spontaneous tumors in mice (8). Furthermore, DHEA affects both initiation and promotion stages of carcinogenesis (9). Circulating levels of DHEA and its sulfate, dehydroepiandrosterone sulfate (DHEAS), decline profoundly with age as the incidences of atherosclerosis and cancer rise (10-13). This suggests that higher levels of DHEA or DHEAS may be protective against these proliferative disorders. Low plasma levels of these steroids in humans have been associated with the presence of cancer and may be related to increased risk of developing some types of cancer (14, 15). Furthermore, low plasma levels of DHEAS are predictive of increased risk of cardiovascular mortality in men (10), and are associated with some known risk factors for cardiovascular disease including hypertension (16), and elevated serum lipids (17-19). DHEA has been reported to lower serum cholesterol in animals and to cause subjective improvement of intermittent claudication in humans (20, 21).

Other properties of DHEA include inhibition of fat synthesis, amelioration of diabetes in genetically diabetic mice and inhibition of cell proliferation and transformation (8). DHEA also blocks the differentiation of 3T3-L1 fibroblasts to adipocytes (22). DHEA is a specific and potent inhibitor of glucose-6-phosphate dehydrogenase (23, 24) and it has been suggested that this inhibition is central to some of the actions of DHEA (8, 22, 25). Given the possible effects of DHEA on these proliferative and biosynthetic processes, we studied the effects of DHEA on atherosclerosis in cholesterol fed New Zealand White rabbits.

### Methods

2- to 3-kg male, New Zealand White rabbits were obtained from Bunyville Inc, Littlestown, PA. They were individually caged and housed

1. *Abbreviations used in this paper:* DHEA, dehydroepiandrosterone; DHEAS dehydroepiandrosterone sulfate; CONTROL, group of animals fed regular chow; DHEA only, group of animals fed chow supplemented with 0.5% DHEA; CHOL/INJ, group of animals fed chow supplemented with 2% cholesterol and subjected to an aortic intimal injury; DHEA/CHOL/INJ, group of animals fed chow supplemented with 2% cholesterol and 0.5% DHEA and subjected to an aortic intimal injury.

in temperature and humidity controlled rooms. Light-dark cycles were 12 h each. After several days of acclimation the animals were sequentially assigned to four feeding groups: standard rabbit chow (Ralston-Purina Co., St. Louis, MO), standard rabbit chow containing 0.5% DHEA, standard rabbit chow containing 2% cholesterol, and standard rabbit chow containing both 2% cholesterol and 0.5% DHEA. DHEA was obtained from Diosynth, Oss, Holland. The diets were prepared and certified by Bioserv, Inc., Frenchtown, NJ. Diets were administered for 12 wk, after the animals had adjusted to their new environment.

Body weights were determined at weekly intervals. Food bins, which allowed continuous ad lib. feeding, were refilled three times per week and average daily food intake calculated from the total food consumed per week. Food spillage was similar in each group.

**Surgery.** After 1 wk on the assigned diet, animals in the two groups receiving the 2% cholesterol diet underwent aortic intimal injury; animals in the two normal cholesterol groups had sham operations. The surgery was performed as described by Baumgartner (26). Briefly, the animals were anesthetized with sodium thiamylal (80–100 mg/kg), intubated with a 3.5- or 4.0-mm interior diameter pediatric endotracheal tube, and maintained on an animal respirator (Harvard Apparatus Co., S. Natick, MA). Additional anesthesia was given as required. Aortic intimal injury was accomplished by the insertion of a 5 French balloon-tipped catheter through the iliac artery. The catheter was inserted a premeasured distance sufficient to reach the aortic arch. The balloon was then inflated with 1 cm<sup>3</sup> of air and withdrawn and advanced three times to cover the span of the thoracic and abdominal aorta. The catheter was then removed and the vessel ligated. Immediately postoperatively all animals received 1.2 million U of procaine penicillin G intramuscularly. Sham operated animals were subjected to the same procedure except that the catheter was not inserted into the vessel. Operative mortality was less than 2.5%.

The assignment to the various diet or diet and surgery groups resulted in four analyzed groups: CONTROL (normal diet, no DHEA, *n* = 6); DHEA only (DHEA supplementation without cholesterol, *n* = 6); CHOL/INJ (cholesterol supplementation and aortic intimal injury, *n* = 11); and DHEA/CHOL/INJ (cholesterol supplementation and aortic intimal injury with DHEA added to the diet, *n* = 11).

**Histopathology.** All animals were sacrificed by sodium pentobarbital overdose. Animals were weighed at time of sacrifice and the aortas were removed and placed in Hepes-buffered saline solution. The adherent adventitia was removed and the aortas opened lengthwise, pinned, and placed in formalin for 24 h. The aortas were stained for lipid with Sudan IV and photographed. The aortas were then sliced transversely at five predetermined sites (Fig. 1) and 5-mm sections obtained. The tissue blocks were embedded in paraffin and two 5- $\mu$ m sections prepared; one section was stained with hematoxylin-eosin; the other with Verhoeff-Van Gieson stain for connective and elastic tissue.

Hearts and livers were also removed, weighed after blotting, and then fixed in formalin. After fixation the hearts were sliced transversely parallel to the atrioventricular groove in 4-mm sections from apex to base. These sections were then processed as described above. Sections from liver were also collected.

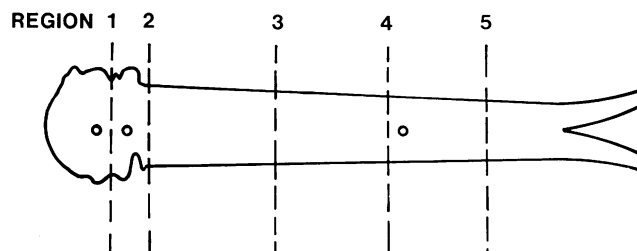


Figure 1. Schematic representation of aortic regions sampled for histologic and topographic analysis.

The extent of aortic disease was assessed by two measures. First, color slides of the whole aortas were projected and traced at magnification of 5. The entire aortic surface area and the fraction with Sudan positive staining were digitized using a Videoplan image analysis computer (Carl Zeiss, Thornwood, NY). The topographic extent of gross disease of the entire aorta was graded as mild (< 40% of total aortic area exhibiting Sudan staining), moderate (40–90% of aorta with Sudan staining) or severe (> 90% staining with Sudan). As a second measure of the extent of disease, histologic atherosclerotic plaque size was determined in five regions (Fig. 1). The slides of the aortic cross-sections were projected at a magnification of 7 and the plaque cross-sectional area and total cross-sectional aortic wall area traced and digitized with the Videoplan computer. The percentage of plaque cross-sectional area to total cross-sectional aortic wall area was used as the index of regional plaque size.

**Lipid analysis.** Blood samples were obtained by ear bleeds before initiation of the assigned diet and just before sacrifice. To control for possible diurnal differences in measured blood values within the same rabbit and between rabbits, all bleeds were performed at approximately the same time of day (0800 to 1000 hours). In some animals an additional sample was collected ~ 2 wk before sacrifice. In these cases the values were not found to be significantly different from the terminal values and the average of the two determinations was used for data analysis. Plasma cholesterol, VLDL, HDL, and LDL were determined using standard techniques (30) in the Johns Hopkins Lipid Research Clinic. Samples were collected into glass tubes containing disodium EDTA (final concentration of 1.5 mg/ml). Plasma was prepared by centrifugation for 15 min at 1,500 *g* at 4°C. The plasma was stored at 4°C until analysis. Briefly, plasma fractions of density < 1.006 g/ml and > 1.006 g/ml were prepared by ultracentrifugation for 18 h at 105,000 *g* (10°C). The top fraction containing VLDL (and chylomicrons if present) and the bottom fraction containing LDL and HDL were recovered and reconstituted to the original volume with isotonic saline and stored at 4°C. The HDL fraction was prepared by heparin/MnCl<sub>2</sub> precipitation after establishing the conditions appropriate for rabbit plasma. These conditions were established by titration of rabbit plasma with heparin and MnCl<sub>2</sub>. The completeness of precipitation of apolipoprotein B containing lipoproteins was established by agarose gel electrophoresis of the heparin-MnCl<sub>2</sub> supernatants.

Cholesterol in the unfractionated and fractionated samples was determined using an enzymatic cholesterol procedure (Boehringer Mannheim Diagnostics, Indianapolis, IN). The cholesterol content of the lipoprotein fractions was calculated as follows: [VLDL-cholesterol] = [Total cholesterol] – [*d* > 1.006 g/ml cholesterol]; [LDL-cholesterol] = [*d* > 1.006 g/ml cholesterol] – [HDL-cholesterol].

Plasma triglycerides were determined by an enzymatic assay (Abbott Laboratories, North Chicago, IL). Triglyceride blanks were performed using a similar reagent without lipase (Abbott Laboratories). Triglyceride standards (20.8 and 52 mg/dl) were obtained from Seragen Diagnostics, Indianapolis, IN.

**DHEA and DHEAS analysis.** DHEA and DHEAS plasma levels were determined in duplicate using commercial radioimmunoassays (Diagnostic Products Corporation, Los Angeles, CA) and the average of the two determinations used for analysis. In some animals an additional sample was collected ~ 2 wk before killing. In these cases the values obtained at both times were averaged for use in the analysis presented in this report.

**Statistical methods.** The study was viewed as a two-way analysis of variance with a two-level intervention factor (high cholesterol diet with aortic intimal injury or control) and a two level treatment factor (DHEA supplementation or no supplementation) (27). This resulted in four analyzed groups. A repeated measures design using multivariate profile analysis was utilized in the analysis of the aortic histologic data with location of the aortic section (from the five designated regions) as the repeated measure (28). A similar repeated measures design with time as the repeated measure was used for data acquired at the beginning and end of the study. All hypotheses were tested by analysis of the main effects and interaction effects. Multiple regression analysis using

a multivariate general linear model was used to assess the independent effects of plasma DHEA, DHEAS, and cholesterol on plaque size (27). Comparisons between individual groups were made using Student's unpaired *t* test corrected for multiple comparisons (29). Frequency data were analyzed using either chi-square analysis, or where appropriate, Fisher's exact test (29). A two-tailed *P* value < 0.05 was considered significant. The results are reported as the mean and the 95% confidence interval of the mean for values within individual groups and mean ± the overall standard error for between-group comparisons.

## Results

**Survival.** Overall survival in this set of experiments was good. There was one operative death. There were no deaths in either the CONTROL or the DHEA/CHOL/INJ groups. One rabbit in the DHEA only group and two in the CHOL/INJ group developed clinically significant *Pasteurella* infections and were killed by barbiturate overdose before completion of the experiment. There were no other deaths. Final number of rabbits analyzed in each group were CONTROL = 6, DHEA only = 5, CHOL/INJ = 9 and DHEA/CHOL/INJ = 11.

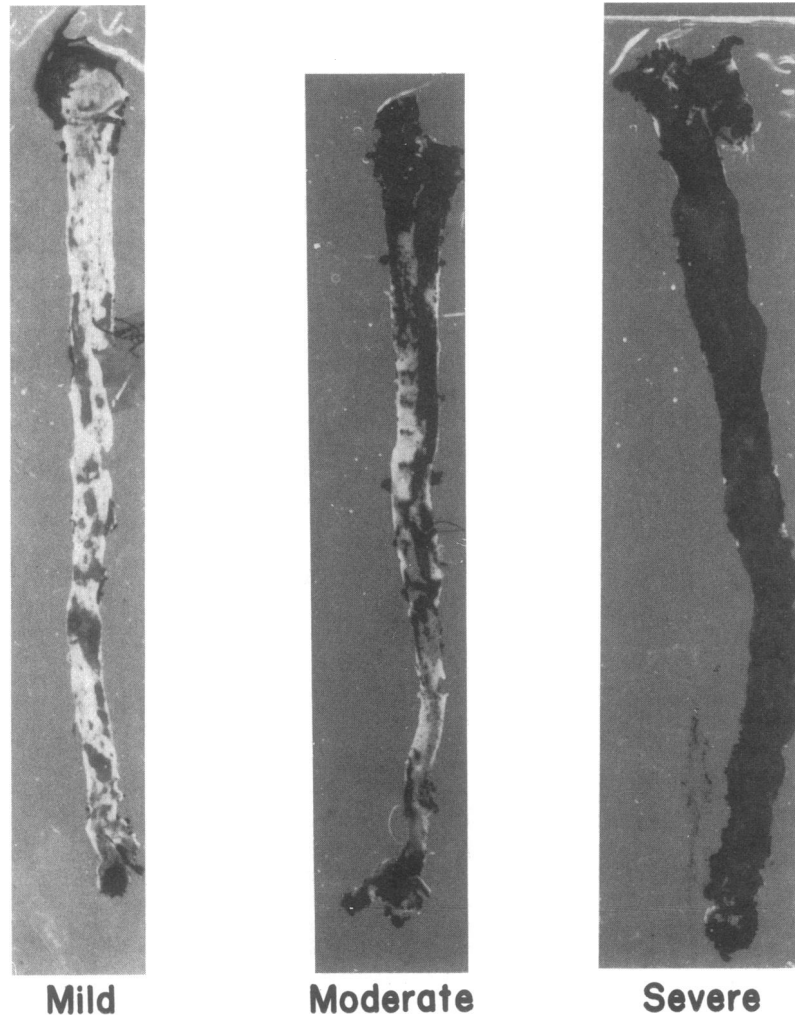
**DHEA and DHEAS levels.** The overall mean plasma level of DHEA before treatment was 582 (353–811) pg/ml. Dietary supplementation with 0.5% DHEA resulted in a significant increase in the plasma DHEA level to 3,481 (1995–4967) pg/ml. Plasma DHEAS was not detectable by the assay used

before dietary treatment with DHEA. This finding is in marked contrast to human plasma, which normally contains 25–50 times higher levels of DHEAS than DHEA (11–13). After 12 wk of DHEA dietary supplementation, plasma DHEAS concentration was 81 (13–149) pg/ml, which is still significantly less than the plasma DHEA levels prior to treatment.

### Severity of atherosclerosis

**Gross morphologic findings.** The percentage of the total aortic surface area covered by plaque was 62 (36–88) in the DHEA/CHOL/INJ group compared with 85 (70–100) in the CHOL/INJ group (*P* = 0.105). When the topographic extent of disease was classified as mild (< 40%), moderate (40–90%), or severe (> 90%), the 9 aortas from rabbits in the CHOL/INJ group had moderate (*n* = 4) or severe (*n* = 5) disease, whereas the 11 aortas from rabbits in the DHEA/CHOL/INJ group were distributed over all three grades with four (36%) of the aortas classified as having mild disease, three with moderate and four with severe disease (Fig. 2). This difference in the distribution of topographic extent of atherosclerosis did not reach statistical significance (*P* = 0.094). All aortas from the control group and the DHEA group showed no evidence of atherosclerosis.

**Histopathology of the atherosclerotic lesions.** Histologic examination of the aortic sections that were stained with hema-



**Figure 2.** Sudan IV stained aortas demonstrating the grading system. After cleaning and fixation the aortas were stained with Sudan IV and were graded with respect to extent of atherosclerosis (mild, < 40% surface involvement; moderate, 40–90% surface involvement; severe, > 90% surface involvement). All of the aortas from the control and DHEA only groups were without evidence of disease. Mild disease was seen only in the DHEA/CHOL/INJ group (4/11), whereas moderate (CHOL/INJ, 4/9; DHEA/CHOL/INJ, 3/11) and severe disease (CHOL/INJ, 5/9; DHEA/CHOL/INJ, 4/11) were seen in both groups.

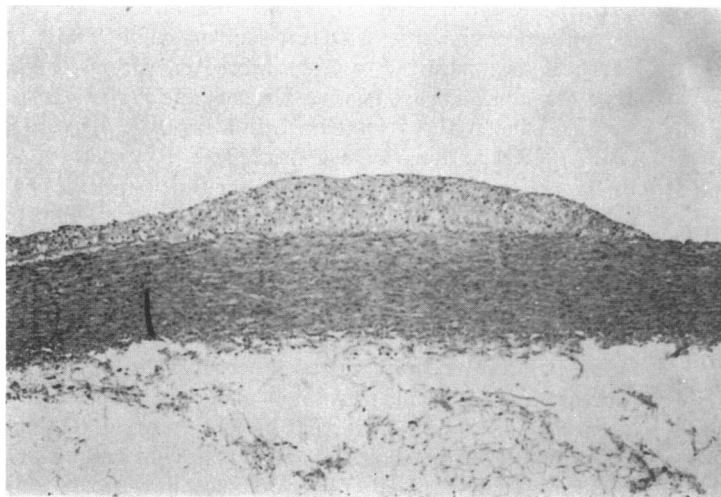
toxylin-eosin showed the characteristic intimal thickening with foam cell infiltration typical of this model. The extent of intimal thickening was greater in the CHOL/INJ aortas compared to aortas from the DHEA/CHOL/INJ group (see below). In addition, some Verhoeff-Van Gieson stained sections showed early collagen deposition and formation of a fibrous cap in the aortas of both groups. Many sections from the CHOL/INJ aortas showed destruction of the internal elastic membrane with foam cells in the media. This finding was uncommon in the DHEA/CHOL/INJ group. There was otherwise no significant difference in the histologic appearance of the aortic sections from the two groups.

**Plaque size.** To evaluate the effect of DHEA treatment upon the atherosclerotic process quantitatively, plaque size, defined as the percent of cross-sectional wall area involved with atherosclerosis, was determined from Verhoeff-Van Gieson stained sections in the five regions indicated in Fig. 1. Fig. 3 shows typical results for the DHEA/CHOL/INJ group and the CHOL/INJ group. DHEA/CHOL/INJ treated rabbits showed an overall 48% reduction in atherosclerotic cross-sectional plaque size compared to CHOL/INJ treated rabbits ( $P = 0.006$ ). This protective effect of DHEA treatment was seen

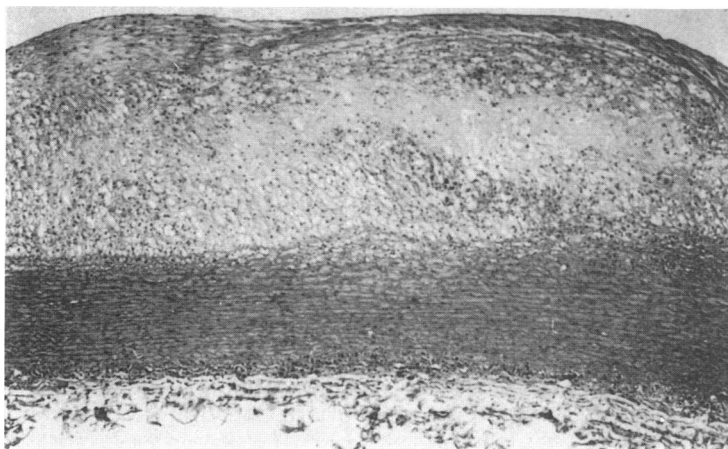
in all aortic regions (Fig. 4). We next examined whether this protective effect against atherosclerosis was directly related to elevated DHEA or DHEAS plasma levels or secondary to other possible DHEA actions on food intake, body weight, or plasma lipids.

#### *Body weight and food intake*

One of the most generalized protective actions against cancer and aging in rodents is decreased body weight secondary to food restriction (31, 32). Several workers have shown that DHEA in some settings can result in limitation of weight gain in freely fed animals, either as a result of decreased food intake or other poorly understood mechanisms (8). It was therefore of interest to determine if food intake or weight gain were decreased by DHEA supplementation. As shown in Fig. 5 all groups gained weight during the experiment ( $P < 0.001$ ). Both cholesterol-treated groups gained less weight than the groups not receiving cholesterol supplementation ( $P = 0.001$ ) and this reduction was unaffected by DHEA supplementation. Weight gain in the group receiving DHEA supplementation alone was not significantly different than that of the control group. Thus, DHEA did not independently limit weight gain or alter the

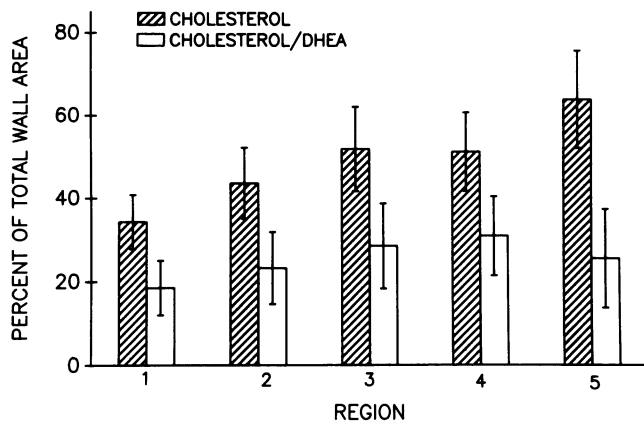


A



B

**Figure 3.** Representative histologic findings. Shown are hematoxylin-eosin stained aortas from DHEA/CHOL/INJ (A) and CHOL/INJ (B) treated animals. Cross-sectional plaque area was noticeably less in the DHEA-treated animals.



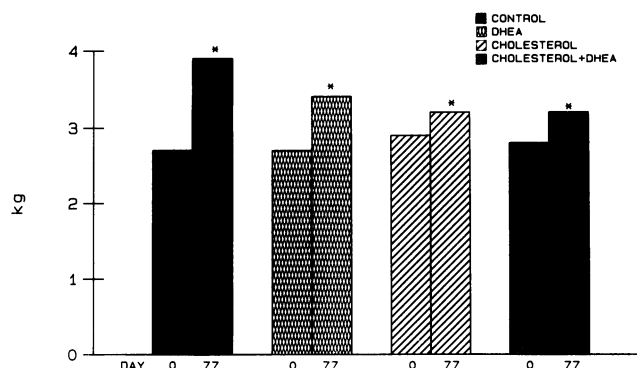
**Figure 4.** Extent of atherosclerosis as measured by percent of total cross-sectional wall area composed of plaque. Bars represent mean  $\pm$  95% confidence limits of the percent of total cross-sectional wall area involved with atherosclerosis from each of the five aortic regions studied in the CHOL/INJ and DHEA/CHOL/INJ groups. DHEA (0.5%) added to the diet markedly reduced the extent of atherosclerosis in all regions ( $P = 0.006$ , overall SEM = 8.5%).

cholesterol effect on weight gain. Cholesterol fed animals likewise ate less ( $P < 0.001$ ) without an independent DHEA effect (Fig. 6). Therefore, the observed protective effects of DHEA were not mediated by alterations in food intake or weight gain.

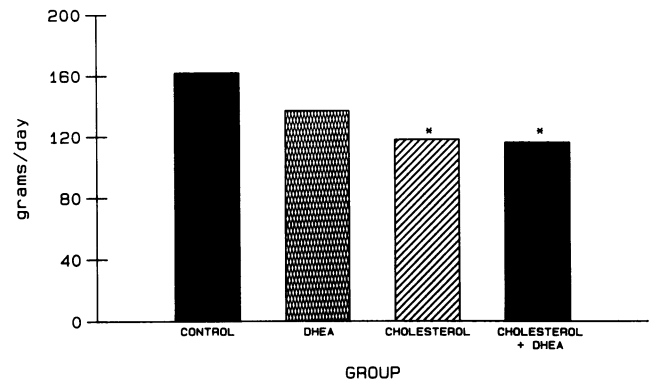
#### Plasma lipids

**Triglyceride.** Plasma triglyceride levels at baseline were similar in the four groups with an overall mean of 86.6 (68.7–104.5) mg/dl. There was a nonsignificant trend to increased plasma triglyceride concentrations in the cholesterol treated groups, but DHEA treatment did not effect plasma triglyceride concentrations (Table I).

**Cholesterol.** Addition of cholesterol to the diets raised total plasma cholesterol concentration  $\sim$  30-fold. Before treatment, mean plasma cholesterol was 38.3 (31.1–45.5) mg/dl with no significant differences between groups. Terminal cholesterol values were not significantly different from baseline levels in the CONTROL and DHEA only groups (Table I). However, cholesterol supplementation raised plasma ches-



**Figure 5.** Initial and final (day 77) average body weight of rabbits. All groups increased their average weight over the course of the experiment ( $P < 0.001$ ). Addition of 2% cholesterol to the diet significantly decreased weight gain ( $P < 0.001$ ) but there was no significant effect of DHEA on weight gain. Overall SEM = 0.07 kg.



**Figure 6.** Average daily food intake. Average daily food intake was determined by measuring the amount of food remaining in pre-weighed food bins three times per week. Addition of 2% cholesterol to the diet reduced food intake compared to control ( $P < 0.001$ ). DHEA (0.5%) added to the diet did not alter food intake in the DHEA or DHEA/CHOL/INJ groups. Overall SEM = 3.2 g/d.

terol concentration ( $P < 0.001$ ) to 1162 (804–1,522) mg/dl in the CHOL/INJ group and to 893 (605–1,182) mg/dl in the DHEA/CHOL/INJ group. The trend toward lower final cholesterol concentration with DHEA supplementation was not statistically significant ( $P = 0.46$ ). Moreover, within groups, there was no significant relationship between plasma cholesterol level and DHEA level (CHOL/INJ,  $r = 0.45$ ,  $P = 0.193$ ; DHEA/CHOL/INJ,  $r = 0.50$ ,  $P = 0.212$ ).

We also examined the changes in the distribution of cholesterol in VLDL, LDL, and HDL fractions in relation to treatment. The increase of total serum cholesterol in the animals fed a 2% cholesterol diet was accompanied by significant increases in HDL, LDL, and VLDL cholesterol fractions, with no significant effect of DHEA treatment on these increases (Table I). However, when viewed in terms of the ratio of the concentration of specific lipoprotein cholesterol to total cholesterol concentration a different pattern emerged. The increases in total serum cholesterol and lipoprotein subfractions in the two cholesterol fed groups were accompanied by borderline increases in the VLDL/total cholesterol ratio ( $P = 0.053$ ) and LDL/total cholesterol ratio ( $P = 0.083$ ) (data not shown), but the HDL fraction was significantly decreased in all animals fed a 2% cholesterol diet ( $P < 0.01$ , Fig. 7). DHEA dietary supplementation did not alter the distribution of the specific lipoprotein cholesterol/total cholesterol ratios among the three fractions in either the normal chow fed or cholesterol supplemented animals (DHEA effect for HDL ratio,  $P = 0.57$ ; for LDL ratio,  $P = 0.76$  and for VLDL ratio,  $P = 0.63$ ).

#### Relation of plasma DHEA, DHEAS, and Cholesterol levels to plaque size

Regression analysis using a general linear model was used to correlate plasma DHEA, DHEAS and total cholesterol with the extent of atherosclerosis in the two high cholesterol-aortic intimal injury groups. Univariate analysis showed that final plasma cholesterol level correlated with average plaque size (mean plaque size in the five sections from each aorta;  $r = 0.49$ ,  $P = 0.037$ ) while final DHEA plasma level correlated negatively with mean plaque size ( $r = 0.68$ ,  $P = 0.001$ ), (Fig. 8). A negative correlation of final DHEAS concentration with



atherosclerosis (35–37). The hyperlipidemia produced in these rabbits is characterized by a profound elevation in serum very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol as opposed to the more modest elevations of LDL observed in human atherosclerosis (38, 39). The pattern of distribution of atherosclerotic lesions is also different than that in human disease and lipid deposition in other organs is unique to this model (37, 38). However, recent work has shown that all aspects of the formation of atherosclerotic lesions, including cell types involved and the initiation and maturation processes, are identical in cholesterol-fed New Zealand White rabbits and the Watanabe Heritable Hyperlipidemic rabbit (39, 40). The disease seen in the Watanabe rabbit closely mimics the accelerated atherosclerosis seen in type IIa human hypercholesterolemia (35). Thus, despite its limitations, the New Zealand White rabbit model is a useful experimental system for study of the antiatherogenic actions of DHEA.

Supplementation of the diet of these animals with 0.5% DHEA reduced the extent of atherosclerosis by almost 50%. This dramatic effect was accompanied by decreased deposition of fat in the heart and liver. Although cholesterol feeding depressed food intake and decreased weight gain, addition of DHEA to the cholesterol diet did not alter these effects of cholesterol supplementation. Plasma DHEA concentration was predictive of the extent of disease independently of plasma cholesterol concentration.

Our data indicate that the actions of DHEA are not caused by alterations in food intake, body weight gain, or plasma lipid profile. Therefore, other modes of action must be considered. From the data we have obtained it is not possible to identify a specific mechanism of action for this compound. It has further not been established if the antiatherogenic actions are caused by DHEA itself or a metabolite of the parent compound. From our data it is clear that both cholesterol fed groups had similar marked increases in plasma cholesterol and in lipoprotein profiles. Thus, it seems unlikely that changes in absorption can explain the antiatherogenic effect of DHEA.

DHEA is a weak androgen that can be converted into many other steroids including more potent androgens and estrogens (41). There is limited information on the conversion of DHEA to estrogens in male rabbits. Rabbit testes have been shown to secrete DHEA and to convert it to testosterone and testosterone precursors (42). Rabbit spermatozoa also can metabolize DHEA to 5- $\alpha$ -androstane-3,17-dione and 4-androstene-3,17-dione, which can be converted to other androgens and estrogens (43). Weekly intramuscular injections of 17- $\beta$ -estradiol given to female New Zealand white rabbits fed a cholesterol and fat supplemented diet reduced the development of atherosclerosis (44, 45). The mechanism of action of estrogens in these studies is not clear. In one study estrogens dramatically blunted the rise of plasma cholesterol concentration due to the added dietary cholesterol (44). This effect could not explain our results because there was no significant relationship between plasma cholesterol level and the beneficial effect of elevated plasma DHEA. Therefore, although there is a possibility that DHEA is converted to estrogens or other steroids, the antiatherogenic effects of these compounds, as with DHEA itself, would have to be independent of cholesterol lowering effects.

The "response to injury hypothesis" of atherosclerosis proposed by Ross (1) provides several possible sites at which DHEA might interfere with the atherogenic process. He pro-

poses that the initial step in atherosclerosis is injury to the endothelial surface of the vessel wall as a result of elevated levels of circulating LDL. This is followed by the local accumulation of platelets and macrophages in the exposed vessel wall. These cells release various chemoattractants and growth factors that cause migration and proliferation of smooth muscle cells and macrophages in the intima. The proliferating cells take up lipids and cholesterol. Progression of this process eventually leads to the formation of the mature atherosclerotic plaque. The initial intimal injury was part of our model so that the beneficial effect of DHEA had to occur beyond this first step of atherogenesis. Thus, DHEA could interfere with atherogenesis by affecting adherence of platelets and macrophages, the release of chemoattractants and growth factors, the proliferation of cellular elements or the uptake of cholesterol into the atheroma. In addition, it has recently been proposed that superoxide production by macrophages promotes local conditions that maintain and enhance the atherogenic process (46). Because DHEA has been shown to interfere with the generation of superoxide radicals by phorbol ester-stimulated human granulocytes, it is possible that DHEA also protects against atherogenesis by reducing local free radical generation. A unique property of DHEA that could explain these various possible antiatherogenic effects is the inhibition of mammalian glucose-6-phosphate dehydrogenase (23, 24). This enzyme is the entry point into the pentose phosphate pathway. The central location of the pathway and the nature of its products makes it a potentially attractive unified, central site of action of DHEA.

In our study, wide variation in response to the administration of DHEA among animals was observed. This variation of response appeared to be secondary to variation in plasma level of DHEA attained. High levels in our study predicted decreased atherosclerosis. The reason some animals achieved high protective plasma DHEA concentrations and others did not requires further study. The variability was independent of daily food intake and body weight. However, metabolic degradation and conversion to other less active compounds is a possibility.

The clinical relevance of our findings is further suggested by several studies in humans. Kask (21) in 1959 used orally administered DHEA to treat six patients with intermittent claudication. Clinical improvement was noted, but body weight, serum glucose and serum cholesterol levels did not change. Zumoff et al. (47) found that men with abnormal coronary arteriograms had slightly, but not significantly lower plasma concentrations of DHEA and DHEAS than did men with normal arteriograms. Most convincing is the recent epidemiological work of Barrett-Connor and colleagues (10). In this study, it was shown that low plasma concentrations of DHEAS were inversely related to death from cardiovascular disease in men over age 50. Although these investigators did not measure plasma DHEA concentrations, DHEAS can be converted to DHEA by widely distributed sulfatases (48).

In summary, our results indicate that dietary supplementation with DHEA can limit the extent of atherosclerosis in male rabbits fed cholesterol and subjected to aortic intimal injury by almost 50%. This limitation is not caused by alterations in food intake, body weight gain or plasma lipid profile. The extent of protection against atherosclerosis is directly and significantly correlated with the plasma concentration of DHEA achieved. Thus, our results coupled with the epidemio-

logic findings of Barrett-Connor et al. (10) suggest that DHEA or a DHEA-like compound may play a role in moderating the development of atherosclerosis.

## Acknowledgments

The authors wish to thank Michelle Kraft and Mark Tinker for their technical assistance in conducting this study.

Supported by grants P50-HL-17655-13 and RO1-HL-28792-03 from the National Institutes of Health; grant 1-PO1-CA44530 from the National Cancer Institute and an Institutional Research Grant from the Johns Hopkins Medical Institutions. Dr. Weisman is the recipient of a Clinician-Scientist Award from the Johns Hopkins Medical Institutions.

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