Inhibition of Accelerated Coronary Atherosclerosis With Dehydroepiandrosterone in the Heterotopic Rabbit Model of Cardiac Transplantation

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Background. Accelerated coronary atherosclerosis has become a critical problem in cardiac transplantation. Although the pathogenesis of this disease is unknown, hypercholesterolemia has been shown to be a major risk factor.

Methods and Results. To study this problem, a hypercholesterolemic rabbit model of heterotopic cardiac transplantation was developed to study accelerated graft atherosclerosis. Based on suggestions in the literature, it was hypothesized that dehydroepiandrosterone (DHEA) may retard the progression of the disease. Using semiquantitative light microscopy, a predilection for the development of small vessel occlusive disease in the transplanted hearts was found. Chronic DHEA administration produced a 45% reduction in the number of significantly stenosed vessels in the transplanted hearts (p<0.05) compared with controls and a 62% reduction in the nontransplanted hearts (p<0.05), yielding an overall 50% reduction in the number of significantly stenosed vessels in both the transplanted and nontransplanted hearts. This reduction in luminal stenosis was observed in the absence of any significant alterations in lipid profiles.

Conclusions. It is concluded that chronic DHEA administration in a hypercholesterolemic rabbit model of heterotopic cardiac transplantation significantly retards the progression of accelerated atherosclerosis in both the transplanted heart and in the native heart. (Circulation 1993;87:261-269)

KEY WORDS • dehydroepiandrosterone • atherosclerosis, cardiac • transplantation, cardiac • hypercholesterolemia

Accelerated atherosclerosis is the leading cause of death in cardiac transplant recipients who survive more than 1 year after transplantation. The incidence of clinically significant coronary artery disease increases in parallel with survival, approaching an incidence of 44–50% at 5 years after the procedure, with a resultant 25% mortality rate.1 Although a variety of factors have been implicated in the pathogenesis of accelerated atherosclerosis, no single mechanism has been clearly identified. Immune-mediated injury and hyperlipidemia are believed to be key components.2,3

To study accelerated atherosclerosis, we used the rabbit heterotopic heart transplant model of Alonzo and coworkers.4 This model uses immune-mediated vascular injury in association with hypercholesterolemia induced by diet supplementation to stimulate athrogenesis and therefore may simulate the accelerated atherosclerosis of human cardiac transplantation.

Using this rabbit model, we tested the hypothesis that the adrenal steroid dehydroepiandrosterone (DHEA, 3β-hydroxy-5-androsten-17-one) exerts a protective effect against the development of accelerated atherosclerosis in the transplanted heart. This hypothesis was based on the fact that DHEA has been shown to reduce serum low density lipoprotein cholesterol levels in normal humans5 and epidemiological evidence showing that adult men with high serum DHEA sulfate levels are less likely to die from cardiovascular disease.6 Recently, young male survivors of acute myocardial infarction were shown to have lower serum DHEA levels than healthy age-matched control subjects.7

These observations in humans were supported further by two studies in animals. Gordon et al8 reported a 50% reduction in aortic atherosclerosis in rabbits that underwent balloon aortic injury and were then fed a high-cholesterol diet when the diet was supplemented with DHEA. Arad and colleagues9 reported a 30–40% reduction in the development of aortic fatty streaks in cholesterol-fed rabbits without vascular injury by supplementing their diet with DHEA. However, neither

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Supported by grants from the Virginia Tissue Bank, HL-24917, Virginia Affiliate of the American Diabetes Association, and a grant-in-aid from Virginia Commonwealth University.

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Received April 24, 1990; revision accepted September 24, 1992.
study examined coronary atherosclerosis, and to date, the effect of DHEA on the accelerated atherosclerosis of cardiac transplantation has not been addressed.

Methods

Surgical Procedure

Hearts from juvenile 2–3-kg Dutch Belted male rabbits (Myrtle Rabbitry, Thompson Station, Tenn.) were transplanted heterotopically into juvenile 3–4-kg recipient New Zealand White male rabbits (Blue & Grey Rabbitry, Aylett, Va.). The details of the surgical technique have been described previously by Dworkin et al. Briefly, all recipients were administered intramuscularly 1.0 ml of an equal mixture of 150,000 units/ml penicillin G benzathine and penicillin G procaine 150,000 units/ml (Vedco, Overland Park, Kan.) and 5 mg/kg cyclosporine (Sandoz, Basel, Switzerland) prophylactically before surgery. Both donor and recipient animals were given induction anesthesia with ketamine (50 μg/ml) in a 2:1 mixture with acepromazine (10 μl) followed by spontaneous inhalation anesthesia with a 2% halothane mixture with oxygen at 3 1/min. Using sterile technique, a midline laparotomy incision was performed in the recipient animal, with exposure of the abdominal aorta and inferior vena cava. Concurrently, the anesthetized donor animal was administered 300 units of heparin via the inferior vena cava, and a sternotomy and pericardiotomy were performed. The great vessels of the donor animal were transected, and the heart was removed by tying off and cutting en bloc the cavae and pulmonary veins. The removed donor heart was immediately placed into a 4°C heparinized saline bath. After arrest of the heart and brief cooling, the distal aorta of the recipient animal was clamped, and an aorta–aortic anastomosis was performed. Venotomy of the recipient inferior vena cava was completed, with anastomosis of the donor pulmonary artery to this vein. Clamps were removed followed by brief fibrillation of the donor heart and spontaneous reversion to normal sinus rhythm in 1–4 minutes. To conclude the surgery, the bowels were replaced over the beating intra-abdominal heterograft, and the fascia was closed by running sutures.

Treatment Groups

After an initial observation period, transplanted rabbits were assigned randomly to one of four treatment groups: group 1: Purina rabbit chow only (Purina Mills, Richmond, Ind.); group 2: Purina rabbit chow with 0.5% DHEA (Sigma Pharmaceuticals, St. Louis, Mo.); group 3: 1% cholesterol rabbit chow (ICN, Cleveland, Ohio); and group 4: 1% cholesterol chow with 0.5% DHEA. Animals were provided feed ad libitum, and the specific dietary regimens were continued for weeks before the operation. Animals were housed separately in cages where temperature and humidity were controlled, and light and dark cycles were regulated at 12-hour intervals. Nine animals were transplanted in group 1, nine animals in group 2, seven animals in group 3, and 20 in group 4, for a total of 45 animal preparations.

Complications leading to exclusion of a specific preparation could be classified according to technical intraoperative problems, hind limb paralysis precipitated by spinal cord ischemia, and Pasteurella sepsis. In group 1, three rabbits died secondary to surgical complications, one was excluded because of hind limb paralysis, one succumbed to Pasteurella sepsis, and four survived. Group 2 exclusions included three animals that died as a consequence of surgical complications and one that had postoperative paralysis, leaving a total of five survivors. In group 3, two animals died after surgery, leaving five survivors. In group 4, eight animals died in the perioperative period; five were excluded because of hind limb paralysis and three that succumbed to Pasteurella sepsis, leaving a total of seven survivors. The higher perioperative mortality in group 4 was due to the fact that, as a result of random assignment, six of these animals were the first to undergo transplantation.

All transplant recipients received maintenance immunosuppression with 5 mg/kg i.m. cyclosporine on a daily basis and 1.0 mg/kg azathioprine (Burroughs Wellcome, Research Triangle Park, N.C.) dissolved in 40 ml of water. Azathioprine dosing was dependent on the quantity of water consumed by the animal during the course of a day, which was usually the entire amount.

Laboratory Analyses

Baseline recipient weights and blood samples for DHEA levels and lipid determinations were obtained immediately after induction anesthesia. After surgery, body weight, lipid profiles, and DHEA levels were determined at weekly intervals for weeks 1–4. Collected rabbit sera were stored at −70°C in cryogenic vials. Lipid profiles were performed on a Roche Cobas Bio (Roche Diagnostic Systems, Nutley, N.J.). Total cholesterol and high density lipoprotein cholesterol were determined enzymatically (Boehringer Mannheim Diagnostics, Indianapolis, Ind.). Triglycerides were measured enzymatically using Stat-Pack Enzymatic Triglyceride (Boehringer Diagnostics, Sommerville, N.J.). DHEA levels were determined using a Coat-A-Count 125I-radioimmunoassay (Diagnostic Products Corp., Los Angeles), with each assay performed in duplicate. Cyclosporine levels were measured by high-performance liquid chromatography assays.

Removal and Preparation of Hearts and Coronary Arteries

At 5 weeks, the rabbits were anesthetized, opened by midline laparotomy, and allowed to exsanguinate through transection of the great vessels. Both native and donor hearts were removed and placed into a heparinized saline bath. A warm colloid mixture of Knox gelatin (7.5 g) and 60 g Renografin (Squibb Diagnostics, New Brunswick, N.J.) in 80 ml of normal saline was prepared and slowly injected at 90 mm Hg pressure into the cannulated aorta to distend the donor and recipient coronary arteries. Injection was stopped once the coronary arteries were flushed, distended, and the colloid had begun to solidify. Both hearts were then placed into a 10% buffered formalin solution. After adequate fixation, one 3–4-mm section was obtained from the distal, mid, and proximal portions of the left anterior descending, circumflex, and right coronary arteries and prepared for histology. Two complete transverse sections of heart approximately 5 mm in thickness were obtained for evaluation of intramyocardial arteries. Tissue sections processed for histology were embedded in paraffin, and 4-μm-thick sections were stained with hematox-
ylin and eosin, Verhoeff van Gieson stain for elastic tissue, and Alcian blue with and without hyaluronidase predigestion at pH 2.5 for acid mucopolysaccharides and hyaluronic acid. Slides of coronary arteries and myocardium were examined under light microscopy by one investigator (D.E.J.) who was blinded to treatment group. There were 1,027 sections of arteries examined. The severity of acute myocardial rejection present was graded according to the scheme of Billingham for human cardiac allografts.11

Morphometry

Morphometric analysis of coronary artery sections, including small intramyocardial branches, was performed using a Zeiss Videoplan computer (Thornwood, N.Y.). Only complete cross sections of arteries were measured, which included 374 sections from native hearts and 653 from transplanted hearts. Tangentially cut arteries were excluded, and every traceable vessel was examined. The area encompassed by the lumen and internal elastic lamina (IEL) was calculated for each vessel, and the cross-sectional area luminal stenosis was calculated by the formula A=IEL area−lumen area/IEL area. Epicardial and intramyocardial arteries were arbitrarily divided into small, medium, and large vessels based on the area encompassed by the IEL (small, 5,000 μm²; medium, 5,000−25,000 μm²; large, >25,000 μm²). Small vessels were those with a diameter <80 μm, medium vessels had diameters ≥80 μm but <178 μm, and large vessels had diameters ≥178 μm. The “circularized” luminal area (A) was computed by assuming A=P²/4 (where P is the parameter determined by the digitizer with use of MICROMAP computer software) to compensate for collapse in underperfused vessels. Morphometric data were entered into a computer data base by an operator blinded to treatment group.

Statistical Analysis

A regression analysis was performed to obviate the problem of variance, which is greatest in the largest and smallest vessels. The regression curve for each animal was obtained by fitting the quadratic curve for the percent stenosis on the log-transformed IEL areas. One function of the analysis was to identify the diseased vessels that were significantly stenosed. To accomplish this goal, a regression curve was fitted to determine the normal percent stenosis for each animal. To avoid the influence of outlying values, Rousseuw’s least median of squares estimate12 was used as a starting value, then a weighted least squares estimate was performed using Andrew’s biweight function.13 If the percent stenosis was greater than the upper 1% normal quantile from the regression line, we identified the vessel as being significantly stenosed. The percentage of significantly stenosed vessels for each heart in each animal was calculated. A one-sided t test was used to compare groups 3 and 4 as well as groups 1 and 2. A paired t test was used to compare native and transplanted hearts. The mean percent stenosis for each range of vessel size (small, medium, and large) was calculated, and comparisons were performed using the Student’s t test. A value of p<0.05 was considered significant.

Results

General Characteristics

Daily food intake was monitored and approximately equal for each rabbit. In each group, animals lost weight progressively, with an average loss of 0.1±0.06 kg over the entire study. Average total ischemic time was 72±4 minutes, with a range of 45–94 minutes, and did not differ significantly among groups. Cyclosporine levels ranged from 106 ng/ml to 467 ng/ml and did not differ significantly among groups. Mean serum total cholesterol, triglyceride, and high density lipoprotein cholesterol levels for each group are listed in Table 1. Serum cholesterol levels were significantly increased in groups 3 and 4 compared with groups 1 and 2 but did not differ between groups 3 and 4. There was no difference between serum triglyceride levels in groups 3 and 4, but group 4 triglyceride levels were greater than groups 1 and 2. There was no difference between high density lipoprotein cholesterol levels among groups.

The mean preoperative serum DHEA level for all of the animals was 396±63 ng/ml, and no significant difference was noted between groups. The mean serum DHEA level for all DHEA-treated animals (groups 2 and 4) was 2,101.1±298.5 ng/ml. Mean DHEA level for all animals not treated with DHEA (groups 1 and 3) was 494.1±51.3 ng/ml, which was not significantly different from preoperative levels.

Morphometric Analysis

Figure 1 (panels A–D) shows representative arterial sections from the transplanted heart for each treatment group. Figure 1A and 1B demonstrate normal intramyocardial arteries from group 1 and group 2. Figure 1C presents a representative intramyocardial artery sample from group 3 (1% cholesterol diet), demonstrating intimal proliferation and lipid deposition. Figure 1D presents a small intramyocardial artery from group 4 with fibrous intimal thickening and proliferation but no apparent lipid deposition.

To attempt to quantify the severity of stenosis present in the different animals, the regression curve for each animal was calculated by fitting the quadratic sum for

### Table 1. Lipid Profiles

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (normal diet)</th>
<th>Group 2 (0.5% DHEA)</th>
<th>Group 3 (1% cholesterol diet)</th>
<th>Group 4 (1% cholesterol diet+0.5% DHEA)</th>
</tr>
</thead>
<tbody>
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<td>(n=4)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=7)</td>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>48±4</td>
<td>126±38</td>
<td>1,846±156*</td>
<td>1,509±269*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>53±2</td>
<td>39±12</td>
<td>102±99*</td>
<td>348±94*</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>17±6</td>
<td>16±3</td>
<td>19±0</td>
<td>15±7</td>
</tr>
</tbody>
</table>

DHEA, dehydroepiandrosterone; HDL, high density lipoprotein.

*p=NS when compared with one another in same group.
FIGURE 1. This and facing page. Representative arterial cross sections from transplanted rabbit hearts for groups 1-4. Panel A: Normal diet (group 1): A medium-sized intramyocardial artery from a transplanted heart free of disease (hematoxylin and eosin; original magnification, ×510). Panel B: Normal diet+0.5% dehydroepiandrosterone (DHEA) (group 2): Normal medium-sized intramyocardial artery from a transplanted heart showing lymphocytic infiltration and myocyte injury (hematoxylin and eosin; original magnification, ×645). Panel C: 1% Cholesterol diet (group 3): Small intramyocardial artery demonstrating marked intimal thickening with accumulation of pale-staining lipid (hematoxylin and eosin; original magnification, ×1,020). Panel D: 1% Cholesterol+0.5% DHEA (group 4): Small intramyocardial artery showing mild concentric fibrous intimal thickening, smooth muscle cell proliferation, and no apparent lipid deposition (hematoxylin and eosin; original magnification, ×645).
the percent stenosis on the log-transformed IEL for each heart of each animal. Figure 2 (panels A–D) presents representative graphs of data from individual animals in each group depicting the percent stenosis versus the log of vessel size. The percent stenosis in group 3, defined as the number of arteries exhibiting stenosis greater than the upper 1% of the normal quantile from the regression line, contrasts markedly with that in group 4. The mean numbers of significantly stenosed vessels for group 3 (1% cholesterol) and group
4 (1% cholesterol+0.5% DHEA) are listed in Table 2. In the transplanted hearts, there was a 45% reduction in the number of significantly stenosed vessels in group 4 compared with group 3 ($p<0.05$). In native hearts, there was a 62% reduction in significantly stenosed vessels in group 4 compared with group 3 ($p<0.05$). Overall, for all hearts (both transplanted and nontransplanted), the number of significantly stenosed vessels was reduced by 50% in group 4 compared with group 3 ($p<0.05$).

For each treatment group, 76–94% (mean, 80%) of large arteries (nm > 25,000 µm²), 91–100% (mean, 97%) of medium-sized arteries (5,000–25,000 µm²), and 98–100% (mean, 99.5%) of small arteries (<5,000 µm²) were located within the myocardium. Figure 3 presents the distribution of atherosclerosis in transplanted hearts, expressed as percent stenosis for each protocol and sorted according to the range of vessel size. In groups 1 and 2, the mean percent stenosis was <15% for vessels of all sizes. In group 3, the greatest burden of atherosclerosis was present in small vessels of transplanted rabbit hearts.

**Table 2.** Mean Percentage of Significantly Stenosed Vessels in Cholesterol-Fed Rabbits and Percent Reduction in Atherosclerosis Effected by DHEA Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Transplanted hearts</th>
<th>Native hearts</th>
<th>Total hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3 (1% cholesterol diet)</td>
<td>44%</td>
<td>25%</td>
<td>34.5%</td>
</tr>
<tr>
<td>Group 4 (1% cholesterol diet + 0.5% DHEA)</td>
<td>25%</td>
<td>9%</td>
<td>17.1%</td>
</tr>
<tr>
<td>Difference between group 3 and group 4</td>
<td>-45%</td>
<td>-62%</td>
<td>-50%</td>
</tr>
</tbody>
</table>

DHEA, dehydroepiandrosterone.

*p < 0.05 when compared with group 3.

**Figure 2.** Representative scatterplots depicting data from individual rabbits in groups 1–4 presenting the percent stenosis vs. the log of the vessel size. IEL, internal elastic lamina.

**Figure 3.** Bar graph shows percent luminal stenosis of transplanted rabbit hearts as a function of vessel size and treatment group. Normal diet is group 1; 0.5% dehydroepiandrosterone (DHEA) diet is group 2; 1% cholesterol (Chol) diet is group 3; 1% cholesterol + 0.5% DHEA diet is group 4. *p < 0.05 for medium-sized arteries in group 3 (1% cholesterol) vs. group 4 (1% cholesterol and 0.5% DHEA). *p < 0.05 for small arteries in group 3 (1% cholesterol) vs. group 4 (1% cholesterol and 0.5% DHEA). n = 653 arterial sections.
planted hearts, and, as shown in group 4, atherosclerosis of these vessels was significantly reduced by treatment with DHEA (p<0.05). Likewise, a significant reduction of atherosclerosis was observed in medium-sized arteries of transplanted hearts from animals treated with DHEA (p<0.05). The large coronary arteries appeared to be relatively spared in this model of accelerated coronary artery disease.

The effects of hypercholesterolemia and DHEA intervention in coronary arteries of native hearts are presented in Figure 4. In contrast to the distribution of atherosclerosis in coronary arteries of transplanted hearts, the greatest burden of the disease process was not seen in the small coronary arteries. Instead, the atherosclerotic process affected primarily medium and large coronary arteries of group 3 animals. Again, as demonstrated by group 4, treatment with DHEA significantly inhibited the atherosclerotic process in these arteries (p<0.05). No significant difference was found in any size of artery among groups 1, 2, and 4.

In addition to vessel size, vessels were also compared according to location (i.e., intramyocardial versus epicardial). However, no meaningful data were gained for the small and medium-sized vessels because there were so few small and medium-sized epicardial arteries. For large arteries, no morphological differences and no differences in percent luminal stenosis existed for epicardial versus intramyocardial arteries. Most of the large intramyocardial arteries were located within the middle to outer zones of the myocardium.

Spectrum of Pathology

**Group 1 (normal diet).** The coronary arteries from three of the four transplanted hearts were entirely normal. Mild concentric fibrous intimal thickening focally involved two small intramyocardial branches of the left anterior descending artery from the fourth rabbit. The endothelium of all vessels appeared to be intact without light microscopic abnormalities. Two grafts had evidence of mild acute rejection, including the one with mild intimal thickening. Arteries from the native hearts were normal in all animals.

**Group 2 (normal diet + 0.5% DHEA).** Two of five transplants showed evidence of vascular disease. One had moderate to severe fibrosis thickening and acid mucopolysaccharide accumulation in the intima of a medium-sized intramyocardial artery and mild fibrous intimal thickening of two small papillary muscle intramyocardial vessels. The other had minimal concentric fibrous intimal thickening of two small intramyocardial arteries. This second graft as well as one with no vascular pathology had features of mild acute rejection. The endothelium of all vessels was intact, without cellular swelling. None of the grafts had myocardial infarcts. Arteries from the native hearts appeared to be microscopically normal in this group.

**Group 3 (1% cholesterol diet).** All transplanted hearts in this group showed significant coronary artery disease. Vascular pathology was characterized by intimal accumulation of smooth muscle and lipid-rich foam cells within a stroma of loose fibrous tissue and some acid mucopolysaccharide-rich ground substance. The endothelium was intact, without endothelial cell swelling or vacuolization. Arterial lesions were most frequently concentric and diffuse rather than focal stenoses. Disease preferentially involved the small secondary and tertiary intramyocardial branches located within the inner two thirds of the myocardium, although some large epicardial arteries were also diseased. All three major coronary artery distributions were affected, with the most severe changes concentrated within the interventricular septum and papillary muscles as assessed by light microscopy. None of the vessels was thrombosed or exhibited features of necrotizing vasculitis. Four of the five grafts showed acute rejection (two with mild rejection and two with moderate rejection and patchy myocyte necrosis). The severity of rejection did not correlate with the extent or severity of coronary vascular disease. One heart with mild rejection had widespread epicardial and intramyocardial disease, and one heart with moderate rejection showed narrowing of only four small intramyocardial branches. Two hearts had small healing myocardial infarcts (one septal, one subendocardial involving the left ventricle in the distribution of severe small vessel disease).

The five native hearts also showed some arterial pathology, although the number of branches affected and severity of stenosis were less than for graft coronary lesions as estimated by light microscopy. Native heart changes consisted largely of intimal foam cell deposits. None of these hearts had evidence of ischemic myocardial injury.

**Group 4 (1% cholesterol diet + 0.5% DHEA).** One graft had normal coronary arteries. Six transplants had evidence of coronary disease that, similar to group 3 animals, involved the small intramyocardial arteries. Compared with the 1% cholesterol-fed animals (group 3), the grafts in this group qualitatively had less widespread and severe disease, and there were fewer lipid-laden foam cells.

Three transplants showed acute rejection (two with mild rejection and one with moderate rejection and...
myocardial necrosis). All grafts with rejection had vascular disease, as did one with no evidence of acute rejection. Two hearts had small healing subendocardial infarcts in the left ventricular free wall.

Four native hearts showed mild to moderate disease of some small intramyocardial arteries. The remainder of the native hearts had no disease.

Discussion

We used a previously described heterotopic rabbit heart transplant model in which animals were fed a high-cholesterol diet to mimic the accelerated atherosclerosis observed in human heart transplant recipients. In transplanted hearts, intramyocardial arteries were affected primarily, with the atherosclerosis consisting of concentric, lipid-laden lesions rich in smooth muscle cells and stromal elements. One speculation is that the predilection for small vessel involvement could be a function of the increased surface area relative to blood flow through distal arteries, which might expose these arteries to the greatest risk of immune-mediated injury. The probable importance of immune-mediated injury in the pathogenesis of accelerated atherosclerosis is underscored by the relatively lesser degree of disease in the coronary arteries of native hearts compared with transplanted hearts (Table 2).

In this model, DHEA was shown to markedly inhibit the development of accelerated coronary atherosclerosis. In the transplanted hearts from rabbits whose high-cholesterol diet was supplemented with DHEA, only 24.8% of the arterial sections demonstrated significant coronary arterial narrowing compared with 44.0% in rabbits fed a high-cholesterol diet alone. On detailed pathological examination, DHEA treatment was shown to reduce the number of diseased arterial branches, the severity of luminal stenosis, and the intimal content of lipid-laden foam cells. It should be noted that DHEA treatment also reduced the degree of atherosclerosis present in coronary arteries of native hearts, suggesting that the antiatherogenic actions of DHEA may not be specific for or restricted to the transplant situation.

It should be noted that inhibition of atherosclerosis by DHEA was demonstrated in two ways. First, the overall amount of luminal narrowing or percent stenosis was calculated for each treatment group. Because we were concerned about possible sampling errors, a second method was used. Each vessel was defined as being significantly stenosed if the lumen was 3 SD smaller than the norm for that heart. The percentage of significantly stenosed vessels for each heart was calculated and meaned to derive a percentage of significantly stenosed vessels for that treatment group.

DHEA is the most abundantly produced adrenal steroid, and serum concentrations of its sulfate ester, DHEA sulfate, are approximately 20-fold higher than those of any other circulating steroid hormone. Peak serum levels of DHEA and DHEA sulfate occur at age 25 years, decrease progressively thereafter, and diminish by 95% by age 85–90 years. The rise in the incidence of atherosclerosis as serum levels of DHEA and DHEA sulfate decline suggests that higher levels of these steroids may be protective against the development of atherosclerosis. In support of this possibility, numerous studies have reported beneficial effects of DHEA administration in animal models of obesity, hyperlipidemia, and diabetes, which are known risk factors for premature atherosclerosis in humans.

Human epidemiological studies have demonstrated an inverse correlation between serum DHEA sulfate levels and death from cardiovascular disease in adult men as well as lower serum DHEA sulfate levels in young men who suffered a myocardial infarction compared with age-matched healthy men. Most recently, Herrington and colleagues have shown that an independent and inverse dose–response relation exists between serum DHEA sulfate and angiographically defined coronary atherosclerosis in men.

The putative “antiatherogenic” action of DHEA has been previously examined in animals by other investigators using different experimental models. Gordon et al demonstrated that dietary DHEA supplementation reduced the development of aortic atherosclerosis by 50% in a balloon aortic injury rabbit model, whereas Arad et al reported that DHEA administration reduced the development of aortic fatty streaks in cholesterol-fed rabbits by 30–40%. The present study differs from these in that mechanical vascular injury was not used, coronary atherosclerosis was studied rather than aortic atherosclerosis, and an immunosuppressed heart transplant model was used to examine the possible effects of DHEA specifically on the accelerated atherosclerosis associated with cardiac transplantation. In our study, DHEA administration conferred significant and marked protection against the development of typical accelerated atherosclerosis.

The mechanism(s) whereby DHEA exerts its antiatherogenic effects remain unknown. Because it had been shown by Nestler and coworkers that the administration of DHEA to young healthy men for 1 month results in a significant reduction in serum low density lipoprotein cholesterol levels, we presumed that DHEA would inhibit atherosclerosis in our rabbit transplant model by exerting a hypolipidemic effect. This was not the case, which is consistent with observations by Gordon et al and Arad et al, who also noted that antiatherogenic effects of DHEA were not due to a reduction in serum lipids. Thus, it seems likely that antiatherogenic actions of DHEA are exerted through other mechanisms.

A specific cellular receptor for DHEA has been reported, and it is possible that DHEA functions primarily through activation of this receptor. Alternately, DHEA can be metabolized to other androgens or estrogens, and it may be that a DHEA metabolite is responsible for the apparent beneficial properties of DHEA. In this regard, Foegh and colleagues have shown that feminizing doses of estradiol can retard accelerated atherosclerosis in a similar heart transplant model. It should be noted, however, that although we did not measure serum estrogen levels, our rabbits did not become feminized, and other investigators have reported antiatherogenic actions of DHEA to be independent of its conversion to estrogen.

Other possible mechanisms for the antiatherogenic effect of DHEA include but are not limited to inhibition of smooth muscle cell proliferation, regulation of the immune system, inhibition of platelet aggregation, and such receptor-independent processes as interpolation into cellular membranes or lipoproteins (thereby altering physicochemical properties). Suppression of
superoxide radical generation,\textsuperscript{36} and modulation of enzymatic processes.\textsuperscript{37}

**Summary**

Our results indicate that DHEA administration significantly inhibits the development of accelerated atherosclerosis in both transplanted and native hearts of a rabbit heart transplant model. It is possible that the addition of DHEA treatment to the medical regimen of human heart transplant recipients might impede the development of this serious complication.

**Acknowledgments**

The authors would like to acknowledge the following individuals for their contributions to this work: Cynthia Alolen, Bettie Duke, Carolyn Fortner, and Terre Williams. We also wish to thank the clinical chemistry lab and Dr. Tetrault for his contribution in performing the serum lipid levels.

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_Circulation_. 1993;87:261-269
doi: 10.1161/01.CIR.87.1.261
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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