Abstract—The effect of natural androgens on serum lipids and atherosclerosis is controversial. We therefore studied this important issue prospectively in an animal model of atherosclerosis. Eighty male rabbits were randomized to bilateral castration, and 20 animals were sham operated. The castrated rabbits were randomized to 500 mg oral dehydroepiandrosterone (DHEA) daily, 80 mg oral testosterone undecanoate (TU) daily, or 25-mg intramuscular injection of testosterone enanthe (TE) twice weekly, whereas the fourth castrated group (placebo) and the sham-operated rabbits did not receive any hormones. All animals were fed a cholesterol-rich diet during the 30-week treatment period. Average serum lipids and atherogenic lipoproteins were higher in the placebo group than in the other groups (ANOVA, P<0.0001). Aortic atherosclerosis, as evaluated by the cholesterol content (mmol/mg protein), was also highest in the placebo group (308±39) and lowest in the TE group (61±12), but was intermediate in the DHEA (155±30), TU (191±43), and sham operation (162±29) groups (ANOVA, P<0.0001). ANCOVA indicated that the androgen effect on aortic atherosclerosis was only in part explained by the changes in lipoproteins. Aortic estrogen receptor contents were significantly lower in the androgen-treated groups than in the control groups, whereas there was no difference in aortic androgen receptor contents between groups. Natural androgens inhibit aortic atherosclerosis in castrated male rabbits only partly through a lipid-mediated effect. (Circ Res. 1999;84:813-819.)

Key Words: atherosclerosis ■ androgen ■ rabbit ■ testosterone

Cardiovascular disease (CVD) is the primary cause of death and disability in the Western countries, and several risk factors, such as cigarette smoking, diabetes, hypertension, and elevated serum lipids, have been shown to accelerate the progression of the disease. Androgens in general and testosterone in particular are widely believed to be associated with a higher risk of CVD in men. There are probably 2 reasons for this view. First, the abuse of synthetic androgens in high doses by (male) athletes sometimes leads to premature CVD, and second, male gender itself is an independent risk factor. Some, therefore, regard testosterone as detrimental in terms of CVD. A few prospective clinical trials, some cross-sectional studies, and experimental studies suggest, however, that these steroids have either a neutral or a beneficial effect on atherosclerosis or its clinical manifestations in men. The effect of testosterone on CVD in men has never been investigated in a clinical, double-blind, placebo-controlled study. We hypothesized that natural androgens in general have a preventive effect in men with regard to CVD, similar to that of estrogens in women. To investigate this, we used a well-recognized and useful experimental model for the study of atherosclerosis, namely, the cholesterol-fed rabbit model.

Materials and Methods

Study Design

One hundred sexually mature male rabbits of the Danish Country strain (SSC:CPH) were used in the study. They were obtained from Statens Serum Institute, Copenhagen, Denmark, and kept in the animal facilities belonging to the Center for Clinical & Basic Research (Ledoeje, Denmark). The animals were housed individually with a 12-hour light cycle, 55±5% relative humidity, and room temperature of 20±2°C. Before and after their arrival, the animals were fed 100 g per day of a standard commercial rabbit chow (Altromin 2123, Brogaarden) and had free access to water. The animals received a standard diet without cholesterol during the 7-week “pretreatment period” for adaptation to the environment and baseline determinations. During this period, 80 rabbits were randomly assigned to bilateral castration (week 7), whereas 20 received a sham operation (week 7). The castrated animals were then randomized to receive 500 mg oral dehydroepiandrosterone (DHEA) daily, 80 mg oral testosterone undecanoate (TU) daily, or 25-mg intramuscular injections of testosterone enanthe (TE) twice a week, whereas the fourth castrated group received placebo, as did the sham operation group. On surgery, the rabbits were fed a cholesterol-rich diet (125 mg/day for 14 weeks [weeks 8 to 21] followed by 320 mg/day for 16 weeks [weeks 22 to 37]) to induce aortic atherosclerosis (“treatment period”). Treatment with cholesterol/androgens thus lasted 30 weeks. The rabbits were killed in week 38. The study was approved by the Danish Ministry of Justice. All procedures complied with the Danish guidelines for experimental studies. The androgen doses were evaluated in a pilot study with 10 castrated male rabbits. Testosterone (Schering AG) was given orally...
in daily doses of 40, 80, and 120 mg (each dose for 1 week) and intramuscularly as TE (Schering AG) (25 mg twice a week for 2 weeks), and DHEA was given in daily oral doses of 250 or 500 mg (each dose for 1 week). Blood samples were taken at 8:00 AM (before androgen administration), 12:00 AM (noon), 4:00 PM, and again at 8:00 AM (the next morning).

**Body Weight**
The rabbits were weighed every 4 weeks throughout the study, and the weight was used as a marker of general health status.

**Rabbit Chow**
Each rabbit was fed 100 g of chow per day throughout the study. To prepare the hormone-containing chow, the androgen was dissolved in maize oil (Unikem, Copenhagen, Denmark). Cholesterol (SIGC-8503, Bie & Berntsen A/S) was also dissolved in maize oil (total amount, 8 mL per animal per day), and the 2 maize oil solutions were mixed manually with the pellets (Altromin 2123, Brogaarden). A similar procedure was used for the placebo chow, except that no hormone was added. The chow was produced for a period of 5 weeks at a time, labeled, and stored in daily portions at −20°C. All animals had free access to water.

**Serum Lipids and Lipoproteins**
Serum lipids (ie, serum total cholesterol [TC]) and serum triglycerides [TG]) were determined at baseline (week 4), and serum lipids and ultracentrifuged lipoproteins (HDL-cholesterol [-C], LDL-C, IDL-C, and VLDL-C) were determined in weeks 12, 20, 29, and 34, always after a 24-hour fast. For logistic reasons, lipoproteins were not determined later than week 34. TC and TG were measured enzymatically, with routine kinetic colorimetric methods (Cobas Mira Plus), according to the manufacturer’s instructions (Roche Diagnostic Systems, F. Hoffmann-La Roche). Determination of ultracentrifuged serum lipoproteins has been described in detail elsewhere.22

**Serum Testosterone**
Serum concentrations of testosterone were measured in weeks 4 and 34, with a specific RIA (Diagnostic Products Corp) after extraction of the hormone from serum. The assay had an intra-assay and an interassay imprecision of ≈7% and 8%, respectively, and a detection limit of 0.14 nmol/L. Blood samples were collected after a 24-hour fast (main study) or after 4, 8, and 24 hours (pilot study). The assay was based on polyclonal anti-rabbit antibodies that are highly specific for testosterone, with very little cross-reactivity to other compounds (eg, the cross-reactivity to dihydrotestosterone is <5%). According to the manufacturer, lipemia does not interfere with the assay.

**Aortic Cholesterol Content**
At the end of the study (week 38), the rabbits were killed with an intravenous injection of 0.06 to 0.12 mg/kg of Mebumal (SAD; pentobarbital) solution. The isolation of the aortic intima has previously been described.22 The thoracic aorta was dissected free and fixed to a piece of paper on a cork board, and the surface area was determined. Aortic tissue was minced and determined chemically by extraction of lipids with chloroform and methanol (2:1, vol/vol) for 24 hours.20 The amount of aortic protein was determined as described elsewhere.22 The weight of the heart was recorded.

**Adrenal Glands and Prostate**
The adrenal glands were bilaterally dissected free, the capsule was removed, and the combined weight of the glands was recorded. Similarly, the prostate was removed from the surrounding connective tissue, the bladder (caudally), and the seminal vesicles (cranially). The prostatic weight was thus used as a marker of the accumulated androgenic effect in the animal.

**Aortic Androgen and Estrogen Receptor Content**
A ring section of the distal part of the thoracic aorta just above the first intercostal arteries was used for determination of the androgen and estrogen receptor content. During necropsy, the aortic tissue was immediately placed on solid carbon dioxide and stored at −85°C until analyzed. For the biochemical analysis, the aortic tissue was homogenized 10% (wt/vol) (Pottier-Elvethjem) and centrifuged at 800g.23 The 800g supernatant was further centrifuged at 105 000g, and the resulting supernatant was used for the determination of cytosolic receptors and protein content. The 800g pellet was washed, and nuclear receptors were extracted by 0.6 mol/L KCl.24 Briefly, the androgen receptor content was measured by dextran-coated charcoal separation.25 In the assay, the sample was incubated with 10 nmol/L [H]mibolerone (NET919, DuPont NEN) for 20 hours at 4°C. Nonspecific binding was assessed with parallel incubation in 1000 nmol/L mibolerone (NLP024, DuPont NEN). Estrogen receptor content was measured by a commercially available enzyme immunosay according to the manufacturer’s instructions (Abbott Laboratories). The cytosolic protein content was measured according to Bradford.26 The results of the receptor contents were normalized by the cytosolic protein content and expressed as fmol/mg protein. The interassay imprecisions for androgen receptor, estrogen receptor, and protein were, respectively, 11%, 6%, and 5%, and the detection limit for both the androgen and the estrogen receptor was ≈5 fmol/mg.

**Statistics**
The time-averaged levels of serum cholesterol and lipoproteins during the treatment period were calculated as the area under the curve divided by the duration of the study. ANOVA was used to test for statistically significant differences between the treatment groups with respect to baseline values such as age, body weight, TC and TG, and food consumption and, furthermore, for organ weights (prostate, heart, and adrenal glands), the average serum cholesterol and lipoprotein levels, and aortic accumulation of cholesterol. The placebo group was a priori selected as the control group. If statistical significance was indicated by ANOVA, then post hoc comparisons versus placebo were done using Dunnett test, whereas the Scheffé test was used for further post hoc comparisons to the sham operation group. The relation between the aortic accumulation of cholesterol and the average serum lipoprotein levels, prostatic weight, and final serum testosterone concentrations was determined for each treatment group by linear correlation analysis after logarithmic (ln) transformation. ANCOVA was then used to test the independent influence of baseline and average lipid and lipoprotein levels, final serum testosterone levels (or prostatic weight), and treatment (independent variables) on aortic accumulation of cholesterol (dependent variable). Independent variables in the ANCOVA with P > 0.05 (default value) were omitted. Data were logarithmically transformed when appropriate due to lack of normal distribution. All statistical analyses were performed with SAS software, with a level of significance of 5%.27

**Results**

**Pilot (Kinetic) Study**
One animal died (week 6) because of pyloric stenosis. Baseline serum testosterone was 5.9 ± 2.3 nmol/L (mean ± SEM), and baseline serum DHEA was 1.9 ± 0.3 nmol/L. During treatment with 40, 80, and 120 mg of oral testosterone, the 24-hour mean serum concentrations revealed a dose-response effect: 14.8 ± 2.2, 19.0 ± 3.2, and 24.7 ± 5.8 nmol/L, respectively. In comparison, the 24-hour fasting testosterone concentrations for the 3 oral testosterone doses were 0.8 ± 0.1, 2.3 ± 0.4, and 2.2 ± 0.7 nmol/L, respectively. Treatment with intramuscular testosterone resulted in higher mean 24-hour levels (43.5 ± 22.2 nmol/L). Serum testosterone levels were low during DHEA treatment (4.8 ± 0.4 nmol/L), during which the 24-hour mean DHEA levels increased to...
Main Study
Table 1 gives the baseline values of the 100 rabbits and shows the comparability of the 5 groups. Four animals did not complete the study period. One animal in the sham operation group was killed (week 9) because of an eye infection, and 1 in the DHEA group died (week 10) from choking on chow. In the TE group, 1 was killed (week 28) because of universal progressive tremor of unknown cause and another (week 29) because of a systemic infection. In all groups, body weight tended to increase slightly during the study period (NS, data not shown). All of the food was consumed in all groups (100 g/rabbit per day). For all treatment groups, serum TC (Figure 1), serum TG, IDL-C, and VLDL-C increased during the study period. With regard to TC, TG, IDL-C, and LDL-C, and VLDL-C, there was a statistically significant difference in the time-averaged concentrations between the groups (ANOVA, 0.0001<P<0.05) (Figure 2). The placebo group had significantly higher values as compared with the sham operation and the androgen-treated groups (P<0.05). The aortic accumulation of cholesterol adjusted for aortic protein in the sham operation group was only about half as much as that in the placebo group (162.0±28.5 versus 307.6±43.8 nmol/mg, P=0.005) (Figure 2), and the accumulation in the groups treated with oral testosterone (TU) and DHEA was virtually the same as that in the sham operation group (191.3±43.3 and 155.1±29.7 nmol/mg, respectively). The intramuscular testosterone (TE) group had accumulated only about half as much as the sham operation group (61.4±12.1 nmol/mg, P<0.05). Aortic accumulation of cholesterol adjusted for aortic surface area, or for wet weight of aortic tissue, gave similar results (Table 2). The aortic cholesterol values correlated with the mean average TC, TG, LDL-C, IDL-C, and VLDL-C levels (0.40≤r≤0.64, P<0.0001) but not with the HDL-C levels (r<0.05). The aortic cholesterol values correlated negatively with prostatic weight for the placebo group (r=0.70, P<0.001) and the intramuscular testosterone group (r=0.51, P<0.05), but not for the other groups (–0.16≤ r≤0.05). Combining all treatment groups for this correlation was not justified because of heterogenic slopes. Similarly, it was not statistically justified combining the groups for the correlation between aortic atherosclerosis and serum testosterone. On the basis of the significant inverse relationship between atherosclerosis and prostatic weight for the TE group, the effect of physiological testosterone replacement can be estimated by extrapolating the relationship to physiological values of prostatic weight (sham operation group). Using the linear regression line for the TE group, the mean physiological content can be estimated: exp[a*ln(prostatic weight)]=exp(–0.93*0.90+5.16) nmol/mg=75 nmol/mg.

ANOVA showed that only treatment (P<0.01) and IDL-C (P<0.01) were significant independent predictors of aortic atherosclerosis. On the basis of this analysis, there thus seemed to be a significant relationship between estimated atherosclerosis, IDL-C, and treatment, as given by the following equation: ln(atherosclerosis)=ln(estimateTREAT)+ln(estimateIDL–C)+ln(estimatePLACEBO)+βTREAT, where βTREAT indicates the intercept.

Prostatic weight was found only to be a borderline significant predictor (P=0.052). When compared with placebo, the logarithmic estimates of aortic atherosclerosis for the various treatment groups were as follows (mean±SEM): sham operation, –0.3±0.2; DHEA, –0.2±0.3; TU, –0.1±0.3; and TE, –1.0±0.3; and for IDL-C, 0.8±0.2, the intercept being 4.2±0.3. Using these estimates, the estimated extent of atherosclerosis with respect to the placebo group (reference group) can be calculated from the above equation, with the IDL-C level set at 5.2 nmol/mg, whereas the mean estimated extent of atherosclerosis in the intramuscular testosterone group is exp(0.8*ln[5.2+4.2])+(-1.0±0.3)] nmol/mg=92 nmol/mg (68 to 124 nmol/mg), and likewise for the other groups (Figure 2, right). Estimated atherosclerosis thus refers to aortic atherosclerosis calculated by the above equation.

For the population of rabbits in the main study, the final 24-hour fasting serum testosterone levels in the placebo group were significantly lower as compared with the initial (ie, preoperative, week 4) levels (–74%, P<0.01) (from 2.60

### Table 1. Baseline Values and Total Food Consumption of the Male Rabbits*

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=20)</th>
<th>Sham (n=20)</th>
<th>DHEA (n=5)</th>
<th>TU (n=20)</th>
<th>TE (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>3.6±0.1</td>
<td>3.7±0.1</td>
<td>3.6±0.1</td>
<td>3.6±0.1</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Age, months</td>
<td>6.6±0.3</td>
<td>7.0±0.2</td>
<td>7.1±0.2</td>
<td>6.9±0.2</td>
<td>7.1±0.2</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>0.65±0.05</td>
<td>0.73±0.08</td>
<td>0.75±0.07</td>
<td>0.67±0.06</td>
<td>0.70±0.06</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.38±0.02</td>
<td>0.39±0.02</td>
<td>0.45±0.08</td>
<td>0.38±0.02</td>
<td>0.42±0.03</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. ANOVA did not show significance for any value tested.

Figure 1. Time-related changes in total serum cholesterol for the 5 treatment groups during the study period. Values are mean±SEM. At week 22 the cholesterol content in the diet was increased from 125 to 320 mg/day.

### Table 1. Baseline Values and Total Food Consumption of the Male Rabbits*

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=20)</th>
<th>Sham (n=20)</th>
<th>DHEA (n=5)</th>
<th>TU (n=20)</th>
<th>TE (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>3.6±0.1</td>
<td>3.7±0.1</td>
<td>3.6±0.1</td>
<td>3.6±0.1</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Age, months</td>
<td>6.6±0.3</td>
<td>7.0±0.2</td>
<td>7.1±0.2</td>
<td>6.9±0.2</td>
<td>7.1±0.2</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>0.65±0.05</td>
<td>0.73±0.08</td>
<td>0.75±0.07</td>
<td>0.67±0.06</td>
<td>0.70±0.06</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.38±0.02</td>
<td>0.39±0.02</td>
<td>0.45±0.08</td>
<td>0.38±0.02</td>
<td>0.42±0.03</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. ANOVA did not show significance for any value tested.

Figure 1. Time-related changes in total serum cholesterol for the 5 treatment groups during the study period. Values are mean±SEM. At week 22 the cholesterol content in the diet was increased from 125 to 320 mg/day.
TABLE 2. Average Values of Serum Lipids and Lipoprotein Levels During the Study Period; Aortic Accumulation of Cholesterol; Aortic Areas and Weights; and Weights of the Prostate, Adrenal Glands, and Heart at the Time of Killing*  

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=20)</th>
<th>Sham (n=19)</th>
<th>DHEA (n=19)</th>
<th>TU (n=20)</th>
<th>TE (n=18)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>21.5±1.6†</td>
<td>13.5±1.4*</td>
<td>11.0±1.3*</td>
<td>10.8±1.1*</td>
<td>9.0±1.0*</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>2.2±0.2†</td>
<td>1.4±0.1*</td>
<td>1.3±0.2*</td>
<td>1.3±0.2*</td>
<td>0.9±0.1*</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.7±0.0</td>
<td>0.6±0.0</td>
<td>1.1±0.1†</td>
<td>0.9±0.1†</td>
<td>0.6±0.0</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.1±0.3</td>
<td>2.8±0.3*</td>
<td>2.6±0.4*</td>
<td>2.9±0.3*</td>
<td>3.1±0.3</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>IDL-C, mmol/L</td>
<td>5.2±0.4</td>
<td>4.1±0.1*</td>
<td>3.4±0.4*</td>
<td>3.2±0.3*</td>
<td>2.9±0.3*</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>VLDL-C, mmol/L</td>
<td>16.8±1.4†</td>
<td>10.1±1.3*</td>
<td>7.0±1.2*</td>
<td>6.8±1.0*</td>
<td>4.6±0.8†</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Aortic area, cm²</td>
<td>5.91±0.17</td>
<td>6.29±0.23</td>
<td>5.87±0.14</td>
<td>6.00±0.21</td>
<td>6.22±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Inner aortic layer, mg</td>
<td>311±20</td>
<td>282±15</td>
<td>281±17</td>
<td>307±21</td>
<td>282±18</td>
<td>NS</td>
</tr>
<tr>
<td>Outer aortic layer, mg</td>
<td>227±14</td>
<td>202±13</td>
<td>187±13</td>
<td>235±15</td>
<td>216±21</td>
<td>NS</td>
</tr>
<tr>
<td>Ratio inner layer/outer layer</td>
<td>1.44±0.12</td>
<td>1.51±0.13</td>
<td>1.55±0.09</td>
<td>1.38±0.10</td>
<td>1.41±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>AA (adjusted for wet weight), nmol/mg</td>
<td>20.0±2.1</td>
<td>11.6±1.7*</td>
<td>11.3±2.0*</td>
<td>14.4±2.6</td>
<td>4.3±0.7*</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>AA (adjusted for area), μmol/cm²</td>
<td>1.91±0.24†</td>
<td>0.88±0.3*</td>
<td>0.98±0.21*</td>
<td>1.39±0.31</td>
<td>0.36±0.06*</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Prostate, g</td>
<td>1.1±0.1†</td>
<td>2.6±0.2*</td>
<td>2.5±0.3*</td>
<td>3.4±0.3*</td>
<td>4.2±0.3†</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Adrenals, g</td>
<td>1.0±0.0</td>
<td>1.2±0.1</td>
<td>0.9±0.1</td>
<td>0.8±0.1†</td>
<td>0.7±0.1†</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Heart, g</td>
<td>8.8±0.2</td>
<td>9.0±0.2</td>
<td>9.0±0.2</td>
<td>9.2±0.2</td>
<td>9.3±0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are mean±SEM. AA indicates aortic accumulation of cholesterol. Ratio given expresses the thickness of the inner aortic layer compared with that of the outer aortic layer. Wet weight indicates inner and outer layer combined; NS, not significant. †P<0.05, Dunnett test (vs placebo); ‡P<0.05, Scheffé test (vs sham operation).

nmol/L [95% confidence interval, 1.92 to 3.53 nmol/L] to 0.68 nmol/L [0.62 to 0.75 nmol/L] and in the DHEA group (–36%, P<0.01) (from 2.96 nmol/L [2.26 to 3.96 nmol/L] to 1.89 nmol/L [1.59 to 2.26 nmol/L]), the oral testosterone group (–25%, P<0.01) (from 3.34 nmol/L [2.68 to 4.16 nmol/L] to 2.50 nmol/L [2.09 to 3.00 nmol/L]), and the sham operation group (–45%, P<0.01) (from 2.32 nmol/L [1.65 to 3.27 nmol/L] to 1.28 nmol/L [1.03 to 1.58 nmol/L]) but increased significantly in the intramuscular testosterone group (+1156%, P<0.0001) (from 3.01 nmol/L [2.30 to 3.95 nmol/L] to 37.81 nmol/L [34.68 to 41.23 nmol/L]).

The aortic cytosolic estrogen receptor content, shown in Table 3, was decreased in the testosterone-treated groups as compared with the sham operation group (P<0.05). The estrogen receptor content in the nuclear compartment was lower (around the detection limit of the assay) and significantly lower in the testosterone groups and the sham operation group than in the placebo group (P<0.05). Low levels of aortic androgen receptor contents were observed in both the cytosolic and nuclear compartments (Table 3), and there was no statistical significance between groups for either of these compartments.

**Discussion**

The present prospective, placebo-controlled study for the first time directly compares the impact of endogenous androgens with that of exogenous androgens on aortic atherosclerosis in castrated, cholesterol-fed male rabbits. An important finding is that androgens inhibit atherosclerosis, as illustrated by the substantial reduction in aortic cholesterol accumulation in the testosterone-treated groups compared to the control and placebo groups. This finding underscores the critical role of androgens in regulating atherogenesis and highlights the potential therapeutic implications of targeting androgen signaling for the prevention and treatment of cardiovascular disease.
was that castration per se resulted in a statistically significant increase of ≈100% in aortic atherosclerosis as compared with sham operation, which suggests that endogenous testosterone has a strong preventive effect on male atherosclerosis. The principal finding was that both testosterone and DHEA replacement therapy markedly inhibited male rabbit aortic atherosclerosis compared with placebo. The route of administration or dose of testosterone, or both, seems to be of importance, as the intramuscular testosterone group had even less aortic atherosclerosis than the oral testosterone group, but the intramuscular testosterone group also had higher 24-hour fasting serum testosterone levels than the oral testosterone and sham operation groups at the end of the study. Nevertheless, our results clearly suggest that both oral testosterone and DHEA replacement may substitute endogenous testosterone in the prevention of atherosclerosis, although there is no evidence that either endogenous or oral exogenous androgens in the present doses have any effect on atherosclerosis beyond that which can be accounted for by differences in IDL-C. Intramuscular testosterone (25 mg twice weekly) seems to be even more efficacious. It is at present unclear why only the intramuscular testosterone treatment in this study showed a significant lipid-independent effect, but the arterial exposure to testosterone over time (as reflected by the final serum testosterone concentration or the prostatic weight in this group) may be crucial and add to the antiatherogenic effect. The androgen doses in this study were chosen from the pilot study and are comparable with those used in other experimental studies.15–17 Atherosclerosis in animals treated with physiological replacement, as estimated from extrapolation of the inverse relationship between atherosclerosis and prostatic weight for the TE group, would thus suggest less atherosclerosis for the sham operation group than observed, if a non–lipid-mediated mechanism were also involved at physiological serum testosterone concentrations. It may be speculated that modulation of lipoproteins, therefore, probably only is 1 of several methods by which natural androgens in pharmacological doses prevent atherosclerosis. For both the testicular and the adrenal androgens, several non–lipid-mediated mechanisms of action have been suggested,18 although these are not known in detail. For example, testosterone may interfere with CVD risk factors (eg, fibrinogen),28 and there is evidence that DHEA may act directly on the vascular wall or by preventing platelet aggregation.29 In contrast to the present study, most previous experimental studies16,17,30 have found androgens to mediate the antiatherogenic effect largely through nonlipid mechanisms. An in vitro study of both male and female rabbit coronary arteries and aortas has recently indicated that testosterone induces vasorelaxation partly through a direct effect on the potassium channels of the vascular smooth muscle cells.31 This effect was supported by the demonstration of an in vivo vasodilatory capacity of short-term administration of testosterone in canine coronary arteries.32

To further explore the non–lipid-mediated mechanism of action for the intramuscular administration of testosterone in our study, we determined the aortic androgen and estrogen receptor concentrations. Interestingly, our study demonstrated lower aortic estrogen receptor levels in the hormone-treated groups than in the control animals. On the other hand, there was no overall difference in androgen receptor levels between the groups, which seems to indicate that the aortic androgen receptor perhaps does not play a major role in atherogenesis. However, the values for the androgen receptors were generally very low and close to the limit of detection, but of the same order of magnitude as those reported by others.32,33 Given that chronic estrogen stimulation is known to downregulate the cytoplasmic estrogen receptor levels,34 our data suggest that the conversion of testosterone to estrogens by aromatase may play a role in the protective effects of androgen replacement on atherogenesis. That this mode of action may be relevant at the level of the arterial wall is supported by demonstration of significant aromatase activity in cultured rat aortic smooth muscle cells.35,36 Further studies are, however, needed to confirm this hypothesis.

The influence of androgens on serum lipids and lipoproteins in men remains controversial. Some studies reported a negative relation between testosterone (particularly exogenous testosterone) and HDL-C,37 whereas others suggested the opposite (particularly of endogenous testosterone levels).28,38,39 Differences in study design may also be an important reason for this discrepancy. Our animal data, which are consistent with those of other experimental studies in terms of atherosclerosis inhibition,16,17,40 clearly support a beneficial impact of natural androgens on serum lipids and lipoproteins. Nevertheless, compared with this study, previous studies with DHEA are of markedly shorter duration, and also the dietary cholesterol dose differs in the various animal studies of DHEA. These methodological differences may account for the significant DHEA effect on lipids in the present study. Previous clinical intervention studies have reported a neutral effect on serum TG by testosterone.41,42 Differences in species, gender, and study design, including dose and route of administration, may be essential for these discrepancies in the changes in triglycerides. The somewhat high increase in serum TG in the placebo group in this study is higher than expected, although it is in agreement with that seen in other rabbit studies.19,22 Because of important basic

---

**TABLE 3. Aortic Estrogen and Androgen Receptor Contents (Cytosolic and Nuclear Compartments)**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Sham</th>
<th>DHEA</th>
<th>TU</th>
<th>TE</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic estrogen receptor content (cytosolic)</td>
<td>4.0 (3.2–5.0)</td>
<td>5.8 (4.6–7.4)</td>
<td>2.2 (1.8–2.8)</td>
<td>1.8 (1.4–2.3)†</td>
<td>1.6 (1.2–2.1)†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aortic estrogen receptor content (nuclear)</td>
<td>1.6 (1.4–1.7)</td>
<td>1.1 (0.9–1.2)‡</td>
<td>1.5 (1.4–1.6)</td>
<td>0.9 (0.8–1.0)‡</td>
<td>1.0 (0.9–1.1)‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic androgen receptor content (cytosolic)</td>
<td>3.7 (3.2–4.3)</td>
<td>6.2 (5.1–7.7)</td>
<td>4.2 (3.5–5.2)</td>
<td>4.9 (4.2–5.8)</td>
<td>4.5 (3.6–5.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Aortic androgen receptor content (nuclear)</td>
<td>4.7 (4.5–5.0)</td>
<td>6.0 (5.4–6.6)</td>
<td>5.0 (4.7–5.4)</td>
<td>5.8 (5.3–6.2)</td>
<td>5.1 (4.8–5.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values, in fmol/mg protein, are geometric mean. Values in parentheses are mean SEM to mean + SEM. Scheffé test (vs sham operation); †P<0.05. Dunnett test (vs placebo), ‡P<0.05.
differences between rabbits and humans with respect to lipid and lipoprotein metabolism, extrapolation of the present experimental data to humans should be done with caution. A beneficial effect of testosterone on clinical manifestations of CVD has also been reported in multiple case histories with middle-aged men treated with testosterone replacement. These observations are supported by data from several epidemiological and case-control studies, plus a single intervention study. In the latter study, angina pectoris and ambulatory ischemia (Holter monitoring) improved significantly in testosterone-replaced men compared with those receiving placebo. These findings are also in agreement with most, but not all, previous experimental studies with male animals. In 2 studies, cholesterol-fed rabbits given oral DHEA had significantly less cholesterol in the aorta and coronary arteries than the rabbits given placebo. However, a 17-week study in which intramuscular testosterone was administered to castrated, cholesterol-fed rabbits with a plasma cholesterol clamped at ≈20 mmol/L, suggested that testosterone had a neutral effect on aortic atherosclerosis, although the testosterone-treated animals tended to have less atherosclerosis than the controls. Recently, an interesting study demonstrated a beneficial effect of testosterone injections on atherogenesis in male but not female rabbits (unless combined with estrogen). Testosterone replacement may also have adverse effects, as it can cause prostatic hyperplasia, which can lead to infravesical urinary obstruction in the elderly male. Therefore, the search for new "androgen-like substances" that ideally would preserve the substantial antiatherogenic properties of natural androgens and at the same time have a neutral effect on the prostate is extremely important. This therapeutic situation is similar to that of developing designer estrogens for postmenopausal women, which ideally prevent osteoporosis and CVD but leave the endometrium and breast unaffected.

In conclusion, this study strongly indicates that natural androgens have a significant antiatherosclerotic effect in male rabbits. Experimental and clinical human studies are clearly needed to explore further the antiatherogenic effect of natural androgens in men.

Acknowledgments

This study was supported financially by a grant from the European Organization for the Control of Circulatory Diseases (Copenhagen, Denmark), and Snedkermeister Sophus Jacobsens og hustru Astrid Jacobsens Fond. We thank Organon Denmark A/S and Schering A/S for supporting the hormone products.

References

Natural Androgens Inhibit Male Atherosclerosis: A Study in Castrated, Cholesterol-Fed Rabbits
Peter Alexandersen, Jens Haarbo, Inger Byrjalsen, Henrik Lawaetz and Claus Christiansen

Circ Res. 1999;84:813-819
doi: 10.1161/01.RES.84.7.813

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/84/7/813

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/