Androgen Inhibition of Atherogenesis in Pullets

By D. L. Cook, Ph.D., R. A. Edgren, Ph.D. and T. W. Harris, D.V.M.

Continuous testosterone propionate administration, but not pretreatment, inhibited cholesterol-induced coronary atherogenesis in pullets. This inhibition was associated with depression of the plasma total cholesterol, phospholipids, e/p ratio and turbidity. Aortic atherogenesis was not affected.

The higher incidence of coronary atherosclerosis in young male as compared with young female humans is well established as are similar sex differences in susceptibility in some other species. The administration of female sex hormones to cholesterol-fed cockerels has been reported to have beneficial prophylactic as well as therapeutic effects on coronary atherosclerosis. In one study reported by Pick, Stamler, Rodbard and Katz, comparison of plasma lipid concentrations and coronary lesions showed a close association between depression of the cholesterol-lipid phosphorus ratio and inhibition of coronary atherosclerosis. Reduction in cholesterol-lipid phosphorus ratios following administration of estrogens has been reported to occur in rats and in man as well as in cockerels. On the other hand, testosterone, given in a dosage adequate to reverse the feminizing effect of estrogen on combs, has been reported to have no effect on cholesterolemia, phospholipidemia, e/p ratios or atherogenesis in cholesterol-fed cockerels. Testosterone has also been reported to have no influence on lipids in a group of non-cholesterol-fed cockerels and pullets over a 48-hour period. Both estrogens and androgens have been reported to inhibit the hypercholesterolemia and aortic atherosclerosis of intact female rabbits, but had no protective action either in intact males or spayed females. In a recent report, Fillios and Mann found that testosterone treatment led to a decrease in the cholesterolemic response before and after cholesterol feeding in both male and female rabbits, but was without effect following castration. Data obtained by Moskowitz and colleagues suggested a decreased incidence of coronary lesions in cholesterol-fed rats chronically treated with testosterone propionate that was similar to that observed with chronic estrogen administration. Several workers have reported that androgens in humans have effects on plasma lipids which were the exact opposite of estrogens, i.e., increased cholesterolemia and elevated e/p ratios.

How androgens are integrated into the whole picture of atherosclerosis is unclear. Whether they are active prosclerotic agents or perhaps indirectly involved, remains to be elucidated. In an attempt to clarify this problem experimentally, the influence of androgen administration on atherogenesis of the coronary arteries and aorta was determined in cholesterol-fed pullets. The androgens could exert their effect through two possible mechanisms. Either circulating androgens could sensitize tissues to affect responsiveness to subsequent hypercholesterolemia, or they could play an active role in cholesterol uptake. To differentiate between these alternatives the experimental design included treatment with androgen prior to cholesterol feeding and simultaneous androgen treatment. Measurements of plasma lipid levels were made to ascertain if any observed effects on the blood vessels might be related to any alterations in circulating lipids.

METHODS

Twenty-one 1-day-old Hy-line pullets were reared in a battery brooder and fed chick starter mash until 6 weeks of age. The experiment was divided into a 2 week pretreatment period followed by an 8 week period during which cholesterol (2 per cent) and cottonseed oil (5 per cent) were added to the diet. Three groups of pullets were used. Group I (control) was given daily (5 days per week) subcutaneous injections of corn oil. Group II was given daily doses (3 mg.) of testosterone propionate in corn oil for the pretreatment period only. Group III was given testosterone propionate in corn oil throughout.
the 10 weeks of study. Each bird was weighed weekly. Plasma cholesterol and phospholipid concentrations, plasma turbidity, comb indices and food consumption were determined initially, at the start of the cholesterol diet and at 1, 4 and 8 weeks during the cholesterol feeding. Total cholesterol was determined by the method of Zlatkis, Zak and Boyle. The lipid phosphorus, determined by Sperry's method, was multiplied by the factor 25 to give the amount of total phospholipids. Plasma turbidity was graded into 5 groups (0 to 4) by gross observation.

All birds were sacrificed and autopsied at the end of the experimental regimen. The aortas were removed and cut open longitudinally. Following fixation in 10 per cent Formalin each aorta was stained with Sudan IV. The degree of aortic atherosclerosis was graded into 5 groups (0 to 4), according to the area of intima involved by lesions. The hearts were removed, weighed and fixed in Formalin. Four frozen sections, 400 µ apart, were cut from each heart and stained with Sudan IV and hematoxylin for histologic examination of the arteries. A count was made of the number of arteries that showed presence of lipid in the intima as well as the total number of arteries observed. The ratio of these two counts was used as the index of coronary atherogenesis. The gross grading of the aortas and the coronary counting were done under blind experimental conditions.

The statistical significance of differences between means was tested by Student's t test.

RESULTS

The incidence of aortic atherosclerosis was similar for all three groups (table 1). The severity of the lesions as judged by gross observation of the stained intima was greatest for the testosterone-treated groups, however, they did not differ significantly from the controls. In all groups the atheromatous lesions were confined primarily to the arch with occasional plaques surrounding the orifices of arterial branches. The hearts appeared grossly normal. On microscopic examination all except one of group I, showed lipid infiltration of the intima of some of the arteries. Group III (testosterone-treated throughout the experimental regimen) exhibited a significantly lower index of coronary atherosclerosis compared to the controls, while those of the pretreatment group did not differ significantly from the controls (table 1).

During the 2 week pretreatment period prior to cholesterol feeding, the mean cholesterol remained unchanged while the phospholipid level decreased significantly, resulting in elevated c/p ratios in each group (fig. 1). Administration of testosterone propionate (groups II and III) did not alter the plasma lipids during this period, however, the con-

![Fig. 1. Relation between plasma lipids, body weight, comb index and time in pullets on high cholesterol diet and treated with testosterone propionate. Arrow, cholesterol diet started: I, controls; 2, testosterone propionate pretreatment; 3, testosterone propionate continuous treatment.](http://circres.ahajournals.org/Downloaded from)
TABLE 2.—Effects of Testosterone Propionate on Plasma Lipids in Cholesterol-Fed Pullets After Eight Weeks of Feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma total cholesterol (mg. per cent) mean ± S.E. (range)</th>
<th>Plasma phospholipids (mg. per cent) mean ± S.E. (range)</th>
<th>C/P ratio mean ± S.E. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>1433 ± 157.3 (890 - 2034)</td>
<td>446 ± 41.0 (330 - 628)</td>
<td>3.21 ± 0.16 (2.72 - 3.99)</td>
</tr>
<tr>
<td>II. Testosterone pretreatment</td>
<td>1633 ± 217.3 (813 - 2500)</td>
<td>505 ± 55.8 (343 - 740)</td>
<td>3.18 ± 0.14 (2.37 - 3.46)</td>
</tr>
<tr>
<td>III. Testosterone continuous treatment</td>
<td>451 ± 86.0* (222 - 747)</td>
<td>204 ± 30.8* (127 - 308)</td>
<td>2.16 ± 0.16* (1.43 - 2.44)</td>
</tr>
</tbody>
</table>

*Statistically significant.

Fig. 2. Relation between cholesterol/phospholipid ratio (ordinate) and coronary index (abscissa) in pullets on high cholesterol diet and treated with testosterone propionate. Open circles, controls; half-filled circles, testosterone propionate pretreatment; solid circles, testosterone propionate continuous treatment.

Continuous administration of testosterone during cholesterol feeding had a marked inhibitory effect on the rise in plasma cholesterol and phospholipid and their ratio (table 2 and fig. 1). This inhibitory effect was not apparent in pullets given testosterone prior to cholesterol feeding. The increase in plasma turbidity which accompanied cholesterol feeding was also inhibited by continuous testosterone administration.

Testosterone treatment did not appear to inhibit ovarian growth since ovary weights for the treated groups did not differ significantly from controls (table 3). Histologic study showed no effect on primordial or primary follicles. Likewise there were no differences in body weight, liver weight or food consumption. Continuous testosterone treatment appeared to increase heart weight. The comb index was markedly increased by testosterone and cessation of the drug was followed by a gradual decrease in comb size approaching that of the normal pullet (fig. 1).

DISCUSSION

It is apparent from these studies that treatment of pullets with androgen prior to cholesterol feeding did not sensitize the coronary arteries to deposition of lipid. Continuous androgenization, however, appeared to protect the pullet against coronary lesions. This protection was associated with a marked reduction in circulating lipids. A close relationship existed between depression of c/p ratio and inhibition of coronary atherosclerosis (fig. 2). The reduction in plasma lipids could not be accounted for by decreased cholesterol intake since food consumption and body

TABLE 3.—Effects of Testosterone Propionate in Cholesterol-Fed Pullets After 8 Weeks of Feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (Kg.) mean ± S.E. (range)</th>
<th>Liver weight (Gm.) mean ± S.E. (range)</th>
<th>Heart weight (Gm.) mean ± S.E. (range)</th>
<th>Ovary weight (Gm.) mean ± S.E. (range)</th>
<th>Comb index length + height (mm.) mean ± S.E. (range)</th>
<th>Food intake Gm./bird/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>1.16±0.043 (1.06 -1.27)</td>
<td>36.1± 2.38 (27.2 -44.9)</td>
<td>4.05±0.090 (3.52 -4.80)</td>
<td>0.61±0.07 (0.39 -0.89)</td>
<td>0.61±0.07 (0.39 -0.89)</td>
<td>66</td>
</tr>
<tr>
<td>II. Testosterone pretreatment</td>
<td>1.13±0.040 (1.00 -1.33)</td>
<td>44.0± 1.74 (38.4 -51.4)</td>
<td>4.57±0.24 (3.71 -5.67)</td>
<td>0.72±0.12 (0.51 -0.88)</td>
<td>0.72±0.12 (0.51 -0.88)</td>
<td>65</td>
</tr>
<tr>
<td>III. Testosterone continuous treatment</td>
<td>1.10±0.057 (0.94 -1.28)</td>
<td>35.0± 3.28 (25.3 -43.5)</td>
<td>6.05±0.401 (5.00 -7.00)</td>
<td>0.60±0.05 (0.47 -0.75)</td>
<td>0.60±0.05 (0.47 -0.75)</td>
<td>67</td>
</tr>
</tbody>
</table>

* Statistically significant at the 5 per cent level.
† Statistically significant at the 1 per cent level.
weights were essentially similar (table 3). A similar relationship has been reported to exist in cockerels treated with estrogen; however, the depressed c/p ratio resulted from a greatly augmented phospholipid concentration without appreciable effect on cholesterolemia. In the present experiment a decrease in c/p ratio resulted from a marked inhibition of cholesterolemia and a lesser effect on phospholipidemia.

**Summary**

Continuous testosterone propionate administration inhibited coronary atherogenesis in cholesterol-fed pullets. This inhibition was associated with a reduction in plasma total cholesterol, phospholipids and turbidity. The reduction in cholesterol was greater than for phospholipid thereby decreasing the cholesterol-phospholipid ratio. A close relationship existed between depression of the cholesterol-phospholipid ratio and inhibition of coronary atherogenesis. Aortic atherogenesis was not affected.

Pretreatment with testosterone propionate did not affect significantly the course of cholesterol-induced coronary or aortic atherogenesis.

**Summario in Interlingua**

Continue administrationes de testosterona propionate inhibivaba atherogenese coronari in gallinettas a dieta cholesterolic. Iste inhibition esseva associate con un reduction del nivellos plasmatic de cholesterol total, de phospholipidos, e del turbiditate. Le reduction del nivellos de cholesterol esseva plus marcate que le reduction del nivellos de phospholipido, de manera que le proportion de cholesterol a phospholipido esseva abbassato. Un relation nette existeva inter le depression del proportion de cholesterol a phospholipido e le inhibition del atherogenese coronari. Le atherogenese aortic non esseva afficide.

Pretreatment con testosterona propionate non afficeva significativamente le curso del atherogenese coronari o aortic a induction cholesterolic.

**REFERENCES**

Androgen Inhibition of Atherogenesis in Pullets
D. L. COOK, R. A. EDGREN and T. W. HARRIS

Circ Res. 1957;5:54-57
doi: 10.1161/01.RES.5.1.54

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1957 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/5/1/54