Benefit from Testosterone and Hydrocortisone on Coronary Atherogenesis in Cockerels on a Low Protein Atherogenic Diet

By Savitri Jain, M.D., Ruth Pick, M.D., and Louis N. Katz, M.D.

During the course of our studies on the role of hormones in atherosclerosis, we have become increasingly aware of the importance of the protein level in the diet upon the action of hormones. In the present study, therefore, the lipid and atherogenic responses to the administration of an anabolic hormone, testosterone propionate, and a catabolic hormone, hydrocortisone (compound F), were analyzed at two different levels of protein in a cholesterol-oil supplemented diet.

**Methods**

Two series (S72, S82) of experiments were done and involved a total of six groups and 230 birds. The established procedures of our department for chronic atherosclerosis experiments in chickens were used throughout. All the chicks were from a H-line hybrid strain, obtained at one day of age from a commercial hatchery. They were reared in a battery brooder on commercial chick starter mash until they were nine weeks of age when the experimental regimen was started.

During the experiment all the cockerels were fed chick starter mash supplemented with 0.5% cholesterol and 5% cottonseed oil. For three groups of cockerels the protein content of the mash was kept at the "normal" level of 20%; for the other three groups the protein content was reduced from 20 to 12% by an appropriate amount of sucrose supplement (table I). In view of our previous observations that in mash diluted with sucrose, vitamin and mineral supplementation did not have any appreciable effect on growth, hypercholesterolemia or atherogenesis, no vitamins or minerals were added to the sucrose-diluted mash used in these experiments. Cockerels of groups 1, 2, and 3 received the low protein diet (12% protein) and the remaining cockerels, groups 1', 2', and 3' received the "normal" protein diet (20% protein).

Testosterone propionate* was given in a dose of 10 mg/chick/day in sesame oil, intramuscularly, five days a week for five weeks to groups 2 and 2'. Hydrocortisone† was given in a dose of 1 mg/chick/day intramuscularly, to groups 3 and 3' for five days a week for five weeks.

The parameters recorded in this study were body weight, food intake, comb index, testes weights, total plasma cholesterol, together with gross thoracic aorta and microscopic coronary artery atherosclerotic involvement. Each bird was weighed at the start and at two and five weeks of the experimental period. Food intake was determined for each group every week. Total plasma cholesterol was determined at two and five weeks of the experimental period by the method of Schoenheimer and Sperry. The comb index (height/width of the comb) was determined for each bird at the time the birds were killed.

All birds were autopsied at the end of the experimental period (five weeks). The aortas were removed and cut open longitudinally. The degree of aortic atherosclerosis was graded from 0 to 4 according to the degree of elevation of the lesions and the area of intima involved by the atheromata. For this study, only atherosclerosis in the thoracic aorta (which included the brachiocephalic arteries) was evaluated. In cockerels, the abdominal aorta is markedly susceptible to spontaneous atherosclerosis and, therefore, is not reported upon here.

The hearts were removed, and fixed in formaldehyde. Two blocks were taken from each heart.

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*Oretone propionate, generously supplied by Dr. Wesley Herndon, Schering Corporation, Bloomfield, New Jersey.
†Cortef acetate, generously supplied by Dr. Thomas J. Vechhio, Upjohn Company, Kalamazoo, Michigan.
Experimental Diets*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protein content of diet</th>
<th>Mash†</th>
<th>Sucrose</th>
<th>Cottonseed oil</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, and 3</td>
<td>%</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td>12</td>
<td>54.8</td>
<td>39.7</td>
<td>5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1′, 2′, and 3′</td>
<td>20</td>
<td>91.3</td>
<td>3.2</td>
<td>5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* All values are grams per 100 g of diet.
† Composition of mash: ground corn 40%, pulverized oats 5%, wheat bran 5%, wheat middlings 10.5%, alfalfa meal 5%, 55%-meat scraps 3.7%, dry buttermilk 1%, soybean meal 26.8%, bone meal 1%, CaCO₃ with Mn and KI 1.5%, salt 0.5%; plus procaine penicillin and B complex vitamins and vitamins A and D.

TABLE 2
Effect of Testosterone and Hydrocortisone on Cockerels Fed High and Low Protein Atherogenic Diets

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protein content of mash</th>
<th>Experimental regimen*</th>
<th>Daily food intake</th>
<th>Gain of body weight†</th>
<th>Testes weights</th>
<th>Comb index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>%</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>82</td>
<td>+463 ± 21;</td>
<td>1.97 ± 0.32†</td>
<td>40.8 ± 1.7;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Testosterone 10 mg/chick/day, IM</td>
<td>77</td>
<td>+401 ± 16</td>
<td>0.23 ± 0.01</td>
<td>72.6 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hydrocortisone 1 mg/chick/day, IM</td>
<td>73</td>
<td>+213 ± 17</td>
<td>3.67 ± 0.40</td>
<td>52.8 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>1′</td>
<td>20</td>
<td>Control</td>
<td>90</td>
<td>+666 ± 15</td>
<td>3.41 ± 0.42</td>
<td>49.8 ± 2.4</td>
</tr>
<tr>
<td>2′</td>
<td>Testosterone 10 mg/chick/day, IM</td>
<td>81</td>
<td>+505 ± 15</td>
<td>0.27 ± 0.02</td>
<td>67.5 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>3′</td>
<td>Hydrocortisone 1 mg/chick/day, IM</td>
<td>77</td>
<td>+141 ± 11</td>
<td>4.72 ± 0.44</td>
<td>53.8 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>

* All groups received 0.5% cholesterol and 5% cottonseed oil in chick starter mash.
† Gain in body weight over five-week experimental period.
‡ Standard error.

and two frozen sections were cut from each of these two blocks and stained with Sudan IV and hematoxylin. A count was made of the number of arteries that showed sudanophilic intimal thickening as a positive finding. Not infrequently the intima or media of a vessel retained some Sudan IV but showed no structural abnormality. This was not counted as atheroma, but was regarded as only lipid infiltration. The percentage of arteries with lesions was used as the index of coronary atherosclerosis. The gross grading of the aortas and the counting of coronary lesions were done without knowledge as to the group to which the animal belonged.

For evaluation of results, a simple analysis of variance after a Bartlett test of equal variability determined whether specific differences would be t-tested. In all tests, the accepted confidence level was $P < 0.05$. The central tendency, and variability statistics used were the mean, the range, and the standard error.

Results
The food intake was slightly higher in all groups on 20% protein than in comparable groups on 12% protein diet (table 1). Both the control and the testosterone-treated groups...
on mash containing 20% protein gained more weight than comparable groups on mash containing 12% protein. Hydrocortisone-treated birds gained very little weight, probably because of the catabolic action of hydrocortisone (table 2).

The weights of testes in the control groups were significantly lower on 12% than on 20% protein. Hydrocortisone-treated cockerels had significantly higher testes weights than the controls while testosterone-treated birds, as expected, had significantly lower testes weights (table 2). The comb index was significantly increased by hydrocortisone and even more by testosterone (table 2).

Figure 1 and table 3 show the effects on plasma cholesterol and atherosclerosis. In all three groups on 20% protein, plasma cholesterol levels were lower than in comparable groups on low protein. However, plasma cholesterol was highest in the groups on hydrocortisone and lowest in those receiving testosterone. The difference in plasma cholesterol values between the control and testosterone-treated groups was 1622 mg% in group 2 versus 2187 mg% in group 1 (P < 0.05) on 12% protein and 425 mg% in group 2' versus 577 mg% in group 1' (P < 0.05) on 20% protein mash.

There were no significant differences between the average grades for thoracic aorta atherosclerosis in the various groups.

**Figure 1**

Effect of testosterone and hydrocortisone (compound F) on plasma cholesterol and coronary atherosclerosis in cockerels fed high and low protein atherogenic diets (572,582). Solid bars represent serum cholesterol in mg% and hatched bars represent coronary arteries showing atheroma. The numerator, + coronaries, refers to coronary vessels with lesions.
TABLE 3
Effect of Testosterone and Hydrocortisone on Cholesterol and Atherosclerosis in Cockerels Fed High and Low Protein Atherogenic Diets*

<table>
<thead>
<tr>
<th>Groups and regimen</th>
<th>Protein content of mash</th>
<th>Plasma cholesterol</th>
<th>Number with lesions and incidence</th>
<th>Average grade of lesions</th>
<th>Thoracic lesions</th>
<th>Coronal lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>12</td>
<td>2187 ± 86†</td>
<td>39/39</td>
<td>100</td>
<td>1.28 ± 0.08†</td>
<td>39/39</td>
</tr>
<tr>
<td>2 Testosterone</td>
<td>12</td>
<td>1622 ± 106</td>
<td>38/39</td>
<td>97</td>
<td>1.07 ± 0.08</td>
<td>38/38</td>
</tr>
<tr>
<td>3 Hydrocortisone</td>
<td>12</td>
<td>2893 ± 92</td>
<td>36/39</td>
<td>92</td>
<td>0.91 ± 0.08</td>
<td>29/33</td>
</tr>
<tr>
<td>1' Control</td>
<td>20</td>
<td>577 ± 51</td>
<td>36/39</td>
<td>92</td>
<td>0.96 ± 0.08</td>
<td>36/38</td>
</tr>
<tr>
<td>2' Testosterone</td>
<td>20</td>
<td>425 ± 34</td>
<td>31/37</td>
<td>84</td>
<td>0.79 ± 0.09</td>
<td>35/37</td>
</tr>
<tr>
<td>3' Hydrocortisone</td>
<td>20</td>
<td>2041 ± 117</td>
<td>39/40</td>
<td>98</td>
<td>1.11 ± 0.09</td>
<td>38/40</td>
</tr>
</tbody>
</table>

*All groups received 0.5% cholesterol and 5% cottonseed oil in chick starter mash.
†Standard error.
Both the incidence and the severity of coronary atherosclerosis were significantly higher in the control groups on 12% protein diet than in those on 20% protein diet. It is noteworthy that in cockerels fed a low protein diet, testosterone as well as hydrocortisone administration lowered markedly the percentage of coronary arteries with atherosclerotic lesions. For groups 2 and 3 the figures were 13.0 and 7.4%, respectively, as compared to 20.8% in group 1 (P < 0.05), while no significant difference between the 3 groups was found when the dietary protein was "adequate" (20%). Also, there is no significant difference in the degree of coronary atherogenesis between the testosterone-treated birds on 12% protein (group 2) and the controls on 20% protein diets (group 1') despite the fact that the level of plasma cholesterol in group 2 was almost three times that of group 1'. Protein level in the diet thus has a clearly demonstrable effect and must be taken into account in designing such experiments.

**Discussion**

These results are in accord with our previous studies on the effect of various protein levels in growing cockerels.\(^5\) It has been shown by various workers that, in chickens, high protein levels inhibit, whereas low protein levels enhance, both hypercholesterolemia and coronary atherosclerosis induced by feeding diets that are high in fat and cholesterol.\(^5\),\(^7\),\(^10\),\(^11\)

Lowering of serum cholesterol levels and coronary atherogenesis by testosterone was reported in cockerels by Cook,\(^12\) and in pullets by Cook, Edgren, and Harris.\(^13\) However, the level of protein in the diet in their experiments was not stated. In our present study, testosterone significantly lowered coronary atherogenesis and plasma cholesterol levels in cockerels fed a low protein diet; but when the dietary protein was "adequate" only a lowering of plasma cholesterol levels was noted with no significant reduction in coronary atherogenesis. On the basis of the present results, the previously reported absence of protection by testosterone and hydrocortisone against coronary atherosclerosis\(^2\)-\(^4\) can now be attributed to the "adequate" dietary protein level used in those experiments.

Our results with testosterone administered during "normal" and low protein intakes agree with the studies of Mosbach, Abell, and Kendall\(^14\) in dogs with 17-α-methyl testosterone, which also showed that androgens caused hypocholesterolemia in animals fed a high cholesterol, high fat diet; an effect which disappeared when this diet was supplemented with horsemeat. Furman et al.\(^15\) have shown that an interrelationship exists between methyltestosterone and dietary protein intake with respect to serum lipoprotein levels in men. On a low protein or protein-free diet both α- and β-lipoproteins were depressed significantly by methyltestosterone, beyond the depression produced by the protein-free diet alone. They also noted that there was no creatinuria in the presence of testosterone, even on protein-free diets.\(^16\) These findings have been confirmed by Olson and Vester.\(^17\)

Despite approximately threefold higher serum cholesterol levels in the testosterone-treated birds on low protein diet, coronary atherogenesis was suppressed to an extent that made the severity of atherosclerosis equal to that in control birds on 20% protein. Thus, the protective action of testosterone in the low protein group in the present study may be explained also by its "protein sparing" effect. This conclusion is supported further by similar results obtained in cholesterol-fed cockerels by Campbell et al. with 19-norandrostenolone, which is anabolic but has only a weak androgenic effect.\(^18\)

Our results with hydrocortisone in the present study are in agreement with those of Adlersberg in cholesterol-fed cockerels.\(^19\) How a "catabolic" hormone like hydrocortisone protects cockerels against diet-induced atherosclerosis, despite the markedly enhanced hypercholesterolemia, is still unsettled. Hormonally-induced diminished permeability of the vessel wall was suggested to explain the experimental findings.\(^20\)-\(^22\) It is also possible that the specific effects on other endocrine organs, elicited by hydrocortisone exhibition,
may tend to prevent aggravation of atherogenesis despite increased lipemia. It is thus apparent that the actions of anabolic and catabolic agents on blood cholesterol and atherosclerosis are complex.

Summary

In the presence of high fat and high cholesterol in the diet, growing cockerels showed more marked hypercholesterolemia and atherosclerosis when their protein intake was low. Testosterone counteracted both the increased hypercholesterolemic and the enhanced atherogenic effects of a low protein diet. Hydrocortisone markedly aggravated the hypercholesterolemia in these cockerels but offered significant protection against coronary atherogenesis when associated with a low protein intake. When the dietary protein level is adequate neither testosterone nor hydrocortisone lowered coronary atherogenesis, despite modification of plasma cholesterol levels. Protein level in the diet must be considered in evaluating hormonal atherogenic effects.

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References


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