Effect of Insulin in the Induction and Regression of Atherosclerosis in the Chick

By JEREMIAH STAMLER, M.D., RUTH PICK, M.D., AND LOUIS N. KATZ, M.D.

During the last 3 decades—the insulin era in the treatment of diabetes mellitus—atherosclerotic vascular complications have become the major causes of morbidity and mortality in diabetic persons, doubtless due, in part, to their increased life span with insulin therapy. Diabetics, as a group, undoubtedly have an increased susceptibility to atherosclerotic disease.1,2 The causative and pathogenetic factors responsible for this phenomenon remain obscure.

In view of this problem, experiments were undertaken to explore the effects of exogenous insulin on coronary and aorta atherogenesis in intact chicks.

Methods

Twelve series of chronic experiments, utilizing a total of 401 cockerels, were accomplished over a 5-year period (1954 to 1959). The established control technics of this department for experimentation on atherosclerosis were employed throughout.1 Overall, 3 different types of experiments were done to assess the influences of exogenous insulin: (1) regression or "unloading" experiments, in which birds were first fed an atherogenic diet for several weeks (mash + cholesterol + oil), then transferred to plain mash for 2 weeks, with administration of insulin during this regression period (tables 1 and 2); (2) "loading" experiments, in which birds were fed mash + cholesterol + oil, and simultaneously given insulin (table 3); (3) experiments on the effects of insulin on estrogen antatherogenesis, in which chicks fed an atherogenic diet were simultaneously given estrogen and insulin (table 3).7,8

In accordance with established procedure,1 a record of feed intake and rate of weight gain was maintained in all experiments. Since groups receiving insulin exhibited decreased feed intake and rate of weight gain, paired-feeding methods were used to assess the possible role of these nonspecific effects in accounting for changes observed with insulin.9,10

Blood glucose concentration was measured as an index of insulin effect.

Results

In control birds, transferred from an atherogenic diet to plain mash for a terminal 2-week period, significant regression of coronary atherosclerosis occurred, in accordance with previous observations, while aortic lesions showed no change during this short period.8,9

In contrast, cockerels given insulin during this period exhibited no regression of lesions (table 1). This marked, significant difference between the 2 groups prevailed, although their patterns of cholesterolemia and phospholipemia were very similar, i.e., both exhibited a decline during these terminal 2 weeks from hyperlipemic to virtually normolipemic levels.

The control and insulin groups differed significantly in feed intake, weight and blood glucose level during these 2 weeks. The insulin-treated group had sustained periods of hypoglycemia, ate much less, and lost (rather than gained) weight (table 1).

Since previous work had shown that undernutrition inhibited regression of atherosclerosis,9,10 further pair-feeding experiments were undertaken to assess the role of this variable (table 2). The data of this experiment demonstrated that the insulin administration—and not matched underfeeding alone—was associated with complete inhibition of regression of coronary lesions. Thus, this phenomenon in birds receiving insulin cannot be attributed to the accompanying low feed intake, i.e., it is not basically an undernutrition effect.

In experiments on the effects of insulin on cholesterol-oil fed and estrogen-treated birds, no consistent influences of the islet hormone were noted. In 4 of the 9 series of experiments,
Effects of Insulin on Regression of Atherosclerosis Composite Tabulation—Series 33, 38, 48, and 57

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>Terminal weight Gm.</th>
<th>Δ weight Gm.</th>
<th>Feed intake Gm./bird/day</th>
<th>Blood glucose mg. %</th>
<th>Terminal plasma cholesterol mg. %</th>
<th>C/P ratio</th>
<th>Percentage Grade</th>
<th>Gross thoracic aorta arteriosclerosis</th>
<th>Microscopic coronary arteriosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-0</td>
<td>36</td>
<td>1443±47*</td>
<td>+578±24</td>
<td>114±6</td>
<td>—</td>
<td>907±90</td>
<td>2.00±0.09</td>
<td>75%</td>
<td>1.5±0.2</td>
<td>94%</td>
</tr>
<tr>
<td>C-0 →</td>
<td>RM‡</td>
<td>34</td>
<td>1610±62</td>
<td>+181±30§</td>
<td>136±41§</td>
<td>158±3</td>
<td>0.55±0.02</td>
<td>91%</td>
<td>1.1±0.1</td>
<td>56%</td>
</tr>
<tr>
<td>C-0 →</td>
<td>RM ‡</td>
<td>38</td>
<td>1268±42</td>
<td>-144±27§</td>
<td>57±8§</td>
<td>54±10†</td>
<td>0.74±0.07</td>
<td>79%</td>
<td>1.6±0.2</td>
<td>97%</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

*C-0 is mash + cholesterol + 5% cottonseed oil; the per cent of cholesterol was 2, 2, 2, and 1% in series 33, 38, 48, and 57, respectively.

‡C-0 → RM is mash + cholesterol + oil for 5 weeks, then plain mash for the terminal 2 weeks. Birds were 12, 12, 9, and 8 weeks of age at the beginning of the experiment in series 33, 38, 48, and 57, respectively.

§Feed intake and Δ weight during terminal 2 weeks of experiment, on RM.

Glucose data collected on series 48 and 57 birds only; Somogyi method.

**Schonheimer-Sperry method.

†C/P Ratio is the ratio of plasma total cholesterol to total phospholipids.

‡‡Glucose values 3 hours after injecting 10 units Lente insulin; glucose values at 6, 18, and 24 hours were 64±14, 151±63, and 202±18 mg. %, respectively.

§§Per cent involvement is an estimate of severity of coronary arteriosclerosis; it is a count of coronary vessels exhibiting arteriosclerosis on microscopic examination, in relation to the total number of vessels visualized in standardized sudan IV-hematoxylin stained sections. The figure given represents involvement of vessel in birds with lesions. Negative birds are not included.

††10 units of Lente insulin daily per bird.

Discussion

These experiments are supplementary to previous studies in this department on peroxisomal enzymes in birds fed a diet low in cholesterol. They also demonstrated that peroxisomal enzymes in birds fed a diet low in cholesterol were suppressed, suggesting that insulin suppressed arteriosclerosis in the unsuppressed diet. These experiments did not differ significantly from other series. Analyses of the over-all data revealed that the 2 groups—C-0 and C-0 + insulin—did not differ significantly (table 3).
Effect of Underfeeding vs. Insulin on Regression of Atherosclerosis—Series 57

Table 2

<table>
<thead>
<tr>
<th>Group*</th>
<th>Weeks of age</th>
<th>Terminal weight Gm.</th>
<th>Δ weight Gm.</th>
<th>Feed intake Gm./bird/day</th>
<th>Blood glucose mg.%</th>
<th>Terminal plasma cholesterol mg.%†</th>
<th>Terminal C/P ratio††</th>
<th>Gross thoracic aorta atherogenesis</th>
<th>Microscopic coronary atherogenesis % Involvement‡‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 C-O</td>
<td>8-15</td>
<td>1420±33</td>
<td>+578±24</td>
<td>108±7</td>
<td>—</td>
<td>1481±140</td>
<td>2.42±0.08</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>3 C-O</td>
<td>RM*</td>
<td>8-15</td>
<td>1663±46</td>
<td>+269±26††</td>
<td>169±86††</td>
<td>—</td>
<td>131±11</td>
<td>0.54±0.03</td>
<td>100%</td>
</tr>
<tr>
<td>Ad Lib</td>
<td>1 C-O</td>
<td>RM</td>
<td>8-15</td>
<td>1239±55</td>
<td>56±9††</td>
<td>158±3</td>
<td>289±30</td>
<td>1.12±0.08</td>
<td>100%</td>
</tr>
<tr>
<td>Pair Fed§</td>
<td>1 C-O</td>
<td>RM + Insulin</td>
<td>8-15</td>
<td>1173±56</td>
<td>-186±23††</td>
<td>57±9††</td>
<td>243±51</td>
<td>0.96±0.19</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Number of birds per group: 10, 9, 18, and 15, respectively.
††Per cent involvement is an estimate of severity of coronary atherosclerosis; it is a count of coronary vessels exhibiting atherosclerosis in microscopic examination, in relation to the total number of vessels visualized in standardized Sudan IV hematoxylin stained sections. The figure given represents involvement of vessel in birds with lesions. Negative birds are not included.
§§Pair fed to insulin group.

Summary

Insulin administration to intact cockerels made atherosclerotic by the feeding of a cholesterol-oil diet prevented regression of coronary atherosclerosis. In some experiments, insulin slightly inhibited estrogen-induced protection of the coronary arteries when the birds were on a cholesterol-rich diet. Insulin administration during the induction of atherosclerosis had no effect on the development of lesions. In some experiments, insulin slightly inhibited cholesterol-aortic atherogenesis. Insulin administration prevented the development of lesions in the cholesterol-oil feeding experiment, whereas in the chick studies, no atherogenesis-intensifying effects of insulin were noted in the cholesterol-oil feeding experiments.

Acknowledgment

We gratefully acknowledge the generous supply of cottonseed oil made available by Mr. M. Di Stone, Procter and Gamble Company, Chicago, Ill., and of cholesterol by Dr. N. S. Bitter of Merck and Company. Dr. W. R. Kirtley, of the Lilly Research Laboratories, kindly made available the insulin used in these experiments. Mr. Grady Crowley.

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Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Birds</th>
<th>Terminal weight Gm.</th>
<th>Blood glucose mg. %</th>
<th>Terminal plasma cholesterol mg. %</th>
<th>Terminal C/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>82</td>
<td>1699±43</td>
<td>166±9</td>
<td>770±54</td>
<td>1.83±0.07</td>
</tr>
<tr>
<td>Insulin</td>
<td>65</td>
<td>1476±28</td>
<td>94±8</td>
<td>540±43</td>
<td>1.56±0.07</td>
</tr>
<tr>
<td>Estrogen</td>
<td>78</td>
<td>1701±82</td>
<td>185±14</td>
<td>982±43</td>
<td>0.75±0.01</td>
</tr>
<tr>
<td>Insulin + Estrogen</td>
<td>76</td>
<td>1499±40</td>
<td>94±4</td>
<td>982±53</td>
<td>0.83±0.04</td>
</tr>
</tbody>
</table>

* Three hours after injection of Lente insulin, 10 units. Hypoglycemia was also noted in both insulin-treated groups at 1%, 4, and 6 hours. Blood glucose values were at normal levels, 24 hours after insulin injection.

Estrogen was 25 mg. per bird, per day, of conjugated equine estrogens (Premarin) in the drinking water.

By further explanation, see table 1.

**Summary in Interlingua**

Le administration de insulina a intakte gallottos que habeva essite ruddle atherosclerose per un dieta a contenuto de cholesterol e oleo preveniva le regression de atherosclerosis coronari. Le administration de insulina durante le induction de atherosclerosis habeva nullo effecto super le diavellomento del lesionos. In pluro experimentos, insulina inhibiva levemente le protection estrogenogenie del arterias coronari durante que le aves recipera un dieta ric in cholesterol e oleo.

**References**

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