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. 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 461 cockerels, were accomplished over a 5-year period (1954 to 1959). The established control techniques of this department for experimentation on atherosclerosis were employed throughout. Over-all, 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 antiatherogenesis, in which chicks fed an atherogenic diet were simultaneously given estrogen and insulin (table 3).

In accordance with established procedure, 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. 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. 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, 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,
### Table 1

**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 Gm. %*</th>
<th>Terminal C/P ratio†</th>
<th>Incidence Grade</th>
<th>Microscopic coronary atherogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-O†</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>
</tr>
<tr>
<td>C-0 →</td>
<td>34</td>
<td>1610±63</td>
<td>181±30$</td>
<td>136±41$</td>
<td>158±3</td>
<td>137±6</td>
<td>0.55±0.02</td>
<td>91%</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>RM‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56%</td>
</tr>
<tr>
<td>C-0 →</td>
<td>34</td>
<td>1610±63</td>
<td>181±30§</td>
<td>136±41§</td>
<td>158±3</td>
<td>137±6</td>
<td>0.55±0.02</td>
<td>91%</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>RM‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56%</td>
</tr>
<tr>
<td>Insulin‡</td>
<td>38</td>
<td>1268±42</td>
<td>-144±27§</td>
<td>57±8$</td>
<td>54±10†</td>
<td>18±18</td>
<td>0.7±0.07</td>
<td>79%</td>
<td>1.6±0.2</td>
</tr>
</tbody>
</table>

*Standard error of the mean.
†C-O is mash + cholesterol + 5% cottonseed oil; the per cent of cholesterol was 2, 1%, and 1% in series 33, 38, 48, and 57, respectively.
‡C-O → 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.
**Schoenheimer-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 atherosclerosis; it is a count of coronary vessels exhibiting atherosclerosis 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 influences of insulin and atherogenesis. They also demonstrated that peroxisomal influences of insulin and atherogenesis in birds fed a diet high in cholesterol and fat. They further showed that diabetes and fat fed a diet high in cholesterol and fat. They further showed that diabetes and fat fed a diet high in cholesterol and fat. They further showed that diabetes and fat fed a diet high in cholesterol and fat.

The results were inconsistent, being negative in the other series. Analysis of the over-all data revealed that the 2 groups—oestrogen-and-insulin—did not differ significantly (table 3).
Effect of Underfeeding vs. Insulin on Regression of Atherosclerosis—Series 57

<table>
<thead>
<tr>
<th>Group*</th>
<th>Weeks of age</th>
<th>Terminal 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 athrogenesis</th>
<th>Microscopic coronary athrogenesis</th>
<th>% Incidence</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CO</td>
<td>8-15</td>
<td>1420±33</td>
<td>578±24</td>
<td>108±7</td>
<td>1461±149</td>
<td>2.42±0.08</td>
<td>100%</td>
<td>2.8±0.2</td>
<td>100%</td>
<td>30.8±3.0</td>
</tr>
<tr>
<td>3 CO</td>
<td>8-15</td>
<td>1003±46</td>
<td>269±26††</td>
<td>108±86††</td>
<td>131±11</td>
<td>0.54±0.03</td>
<td>100%</td>
<td>1.6±0.2</td>
<td>89%</td>
<td>16.8±2.3</td>
</tr>
<tr>
<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>
<td>2.0±0.2</td>
<td>94%</td>
<td>17.4±1.9</td>
</tr>
<tr>
<td>Pair Fed§</td>
<td>8-15</td>
<td>1173±36</td>
<td>-186±23‡‡</td>
<td>57±9††</td>
<td>72±4‡</td>
<td>0.96±0.19</td>
<td>100%</td>
<td>2.2±0.3</td>
<td>100%</td>
<td>32.5±4.4</td>
</tr>
</tbody>
</table>

*Number of birds per group: 10, 9, 18, and 15, respectively.
†110 units Lente insulin daily.
‡Chick starter mash + 3% cholesterol + 5% cottonseed oil + 20% sucrose (to reduce dietary protein to 15% by weight).
§Pair fed to insulin group.
"Three hours after insulin.
**1 CO → RM is a diet of 1 CO for 5 weeks, followed by a diet of chick starter mash (RM) for 2 weeks (the regression period).

Summary

Insulin administration to intact cockerels made atherosclerotic by the feeding of a high cholesterol-oil diet prevented regression of coronary lesions. In no effect on the development of atherosclerosis. In some experiments, insulin slightly inhibited atherosclerosis by the feeding of a cholesterol-oil-contain diet, prevented reatherosclerosis. Inhibiting effects of insulin on the development of atherosclerosis, but apparently none in the high cholesterol-oil feeding experiments.

Acknowledgment

We gratefully acknowledge the generous supply of our experimental animals. Our technical assistant, Mrs. Mary Thompson, Mrs. Evelyn Miller, Mrs. Martha Thompson, Mrs. Helen Stenger, Mrs. Joan Thompson, Mrs. Mary Thompson, and Mr. Grady Crowley.

Circulation Research, Volume VIII, May 1960

Table 2
Table 3

Effects of Insulin on Cholesterol-Lipid, Atherogenesis, and on Estrogen Antiatherogenesis

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Insulin</th>
<th>Estrogen</th>
<th>Insulin + Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of birds</td>
<td>82</td>
<td>65</td>
<td>78</td>
<td>76</td>
</tr>
<tr>
<td>Terminal weight Gm.</td>
<td>1699±43</td>
<td>1476±28</td>
<td>1701±82</td>
<td>1499±40</td>
</tr>
<tr>
<td>Feed intake Gm./bird/day</td>
<td>108±5</td>
<td>87±4</td>
<td>104±5</td>
<td>83±4</td>
</tr>
<tr>
<td>Blood glucose mg.%</td>
<td>166±9</td>
<td>94±8</td>
<td>185±14</td>
<td>94±8</td>
</tr>
<tr>
<td>Terminal plasma cholesterol mg.%</td>
<td>770±54</td>
<td>540±43</td>
<td>982±43</td>
<td>982±53</td>
</tr>
<tr>
<td>Terminal C/P ratio</td>
<td>1.83±0.07</td>
<td>1.56±0.07</td>
<td>0.75±0.01</td>
<td>0.83±0.04</td>
</tr>
<tr>
<td>Gross thoracic aorta atherosclerosis</td>
<td>Incidence</td>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72%</td>
<td>1.5±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62%</td>
<td>1.4±0.1</td>
<td></td>
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<tr>
<td>87%</td>
<td>1.5±0.1</td>
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<td></td>
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</tr>
<tr>
<td>83%</td>
<td>1.3±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopic coronary atherosclerosis</td>
<td>Incidence</td>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>86%</td>
<td>20.6±1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>77%</td>
<td>20.4±1.9</td>
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<tr>
<td>15%</td>
<td>14.0±3.6</td>
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<tr>
<td>39%</td>
<td>9.4±1.2</td>
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</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.

For further explanation, see table 1.

**Summario in Interlingua**

La administration de insulina a intacte gallottos que habeva esite randee atherosclerose per un dieta a contento de cholesterol e oleo preveniva le regression de atherosclerosis coronari. La administration de insulina durante le induction de atherosclerosis habeva nullo effetto supe le diasvelopamento del lesionos. In pluro experimentos, insulina inhibitiva levemente la protection estrogenogenica del arterias coronari durante que le aves recuperava un dieta ric in cholesterol e oleo.

**References**


13. **Duff, G. L., and McMillan, G. C.**: Effect of alloxan diabetes on experimental cholesterol atherosclerosis in the rabbit. I. Inhibition of


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Circ Res. 1960;8:572-576
doi: 10.1161/01.RES.8.3.572

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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