Effects of Beta-Sitosterol on Regression of Cholesterol Atherosclerosis in Rabbits

By William T. Beheh, Ph.D., William L. Anthony, B.S., and Gizella D. Baker, B.S.

Feeding β-sitosterol to atherosclerotic rabbits slightly increased the regression of plasma and liver cholesterol. However, β-sitosterol had no effect on aorta lipid, cholesterol or plaque regression.

Among the various substances tested on experimental animals, β-sitosterol was found to be one of the most effective in minimizing hypercholesterolemia and in retarding atherosclerotic plaque formation. Very few studies have been done on regression of established hypercholesterolemia and atherosclerosis. The substances used in regression studies were choline, potassium iodide, and heparin, none of which were effective.

The investigation reported here is concerned with the effects of β-sitosterol on the regression of cholesterol atherosclerosis in rabbits.

Methods

Procedures. The following procedures were utilized in the course of these experimental studies:

1. Periodic plasma cholesterol determinations were carried out according to the method of Sperry and Webb.

2. For liver cholesterol determinations, small samples of liver were removed from the major lobes, weighed and quick frozen. Total cholesterol was analyzed by a previously described method.

3. In order to estimate the extent of aortic plaque development, the aortic arches and 5 to 7 cm. of the thoracic aorta were removed, washed in distilled water, and fixed overnight in a 9:1 solution of formalin. Subsequently the aortas were stained with Sudan IV, and the plaque area visually estimated and graded.

4. Aorta total lipid and cholesterol were determined as follows: The stained aortas were shredded, dried and weighed. The total lipids were extracted with ether in a Soxhlet extraction apparatus and determined gravimetrically. The lipids were then dissolved in a 1:1 alcohol-acetone solution, and total cholesterol determined by the method of Sperry and Webb.

Development Study. Forty female albino rabbits, weighing 2200–2300 Gm., were divided into 4 groups of 10 and fed ad lib diets shown in table 1. In order to check the development of hypercholesterolemia in each rabbit, blood samples were drawn at intervals of 1 to 3 weeks for total cholesterol determination. At the end of a 3-month period, 5 rabbits from group 1 and all 10 rabbits in group 2 were sacrificed by intravenous injection of potassium chloride. The aortic arch with a segment of thoracic aorta, a liver sample, and a final blood sample were removed from each sacrificed animal for staining and analysis.

Regression Study. The remaining 25 rabbits were placed on the diets shown in table 2. As in the development study, total blood cholesterol was determined periodically. At the end of a 4-month period, all the animals were sacrificed as before and tissue samples were stained and analyzed.

Results and Discussion

Total Plasma and Liver Cholesterol. As shown in figure 1, dietary β-sitosterol (2.5 per cent) slightly accelerates the normal rate of total plasma cholesterol regression. At the end of the 4-month period, the extent of regression was somewhat greater in the group fed β-sitosterol than in the control group (table 3). Indeed, the final cholesterol value for group 4 approaches the value for group 1, the untreated controls.

The data in table 3 show that total liver cholesterol in both control and β-sitosterol-treated animals (groups 3 and 4) decreased considerably during the 4-month regression period. However, as in the case of plasma cholesterol, liver cholesterol approached nearer to the control levels in the β-sitosterol treated animals (group 4). At the termination of the experiment, liver cholesterol values closely
Table 1.—Development Period Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulverized Rockland rabbit ration</td>
<td>97%</td>
<td>96%</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>Mazola oil</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Table 2.—Regression Period Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulverized Rockland rabbit ration</td>
<td>97%</td>
<td>97%</td>
<td>94.5%</td>
<td>92%</td>
</tr>
<tr>
<td>Mazola oil</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
<td>2.5%</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

paralleled plasma cholesterol values in both the control and β-sitosterol treated rabbits, emphasizing the relationship between plasma and liver cholesterol.

In view of the proposed theory of hepatointestinal cholesterol recirculation, it is possible to account for these results if β-sitosterol acts by preventing the absorption of cholesterol from the intestinal tract. However, one might have expected faster regression of plasma and liver cholesterol in the β-sitosterol treated group. Since these data support the findings of a previous experiment, it must be concluded either that accumulated plasma and liver cholesterol do not appear as such in the intestinal tract or that, at the point and time of appearance, conditions are not favorable for the prevention of reabsorption. It should be noted that recirculation is a continuous process, while the ingestion of food containing β-sitosterol takes place at irregular intervals.

Aorta Total Lipid and Cholesterol. The analytical values for aorta total lipid and cholesterol (table 3) reveal that atherosclerotic animals fed either the cholesterol free control

![Fig. 1. Effect of β-sitosterol on regression of hypercholesterolemia in rabbit.](http://circres.ahajournals.org/Downloadedfrom)

Table 3.—The Effect of β-Sitosterol on Regression of Plasma, Liver, and Aorta Cholesterol Levels, and on Aorta Plaque Area

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>Total plasma cholesterol mg/100</th>
<th>Total liver cholesterol mg/Gm.</th>
<th>% Lipid in aorta</th>
<th>% Cholesterol in lipid</th>
<th>% Cholesterol in aorta</th>
<th>Aorta plaque area grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Untreated controls</td>
<td>9</td>
<td>119 ± 5.3</td>
<td>3.20 ± 0.50</td>
<td>12.0 ± 0.30</td>
<td>1.44 ± 0.66</td>
<td>0.132 ± 0.037</td>
<td>0</td>
</tr>
<tr>
<td>2. Developed atherosclerosis</td>
<td>9</td>
<td>1057 ± 188</td>
<td>21.10 ± 7.53</td>
<td>33.0 ± 5.26</td>
<td>33.4 ± 10.04</td>
<td>11.53 ± 4.68</td>
<td>2.66 ± 1.31</td>
</tr>
<tr>
<td>3. Regression of atherosclerosis controls</td>
<td>8</td>
<td>383 ± 104</td>
<td>5.07 ± 2.34</td>
<td>26.6 ± 8.86</td>
<td>30.7 ± 17.20</td>
<td>15.5 ± 6.18</td>
<td>2.65 ± 1.17</td>
</tr>
<tr>
<td>4. Regression of atherosclerosis treated with β-sitosterol</td>
<td>7</td>
<td>143 ± 62</td>
<td>3.60 ± 0.90</td>
<td>29.4 ± 6.57</td>
<td>43.7 ± 6.47</td>
<td>11.9 ± 2.50</td>
<td>3.01 ± 0.91</td>
</tr>
</tbody>
</table>

Numbers after ± are the standard deviations.
diet (group 3) or the diet supplemented with 2.5 per cent \( \beta \)-sitosterol (group 4), showed no significant regression in the 4-month period. This indicates that there is an extremely slow turnover rate in plaque lipids. This fact, supported by other studies, would seem to limit the possibility of plaque elimination by drugs that retard the absorption of cholesterol from the intestinal tract. \( \beta \)-sitosterol and dihydrocholesterol apparently function in this way.\(^8\)\(^9\)

**Aorta Plaques.** The last column in table 3 presents data on regression of plaque area. The grading system used by us was as follows: 0 = no plaque involvement; 1 = plaque involvement between 1 and 25 per cent of aorta area; 2 = between 25 and 50 per cent; 3 = between 50 and 75 per cent; and 4 = between 75 and 100 per cent. The figures in the table represent the averages of 7 to 9 animals in each case.

The data indicate that over a 4-month period \( \beta \)-sitosterol was ineffective in reducing plaque area. Further, it would appear that the treated animals (group 4) showed a higher degree of involvement than the control animals (group 3). While the values for aorta lipids and cholesterol do not seem to support this observation, differences in lipid composition or plaque thickness could account for the apparent discrepancy. This fact is especially interesting inasmuch as Curran and Costello\(^1\) have recently shown that feeding diets free of cholesterol but containing soybean sterols will produce atherosclerosis in rabbits.

**SUMMARY**

Beta sitosterol increased the rate and degree of plasma cholesterol regression.

During the development of atherosclerosis, liver cholesterol reached very high levels, which regressed to near control values in 4 months. \( \beta \)-sitosterol treated animals regressed more than the untreated ones.

At the end of the 4-month treatment period, there was no evident aorta lipid, cholesterol or plaque regression in either of the groups—\( \beta \)-sitosterol treated or control.

**SUMMARY IN ITALIAN**

Beta sitosterol accelerava e augmentava le regression de cholesterol del plasma.

Durante le disveloppamento de atherosclerosis, cholesterol hepatic attingeva altissime nivellos que regredeva in le curso de quatro meses usque a presso al valores de controlo. Animales tractate con beta sitosterol mostrava un plus marcate regression que le animals non tractate con beta sitosterol.

Al fin del periodo de quatro meses de tractamento, il non habeva un evidente regression del lipidio aortic, del cholesterol, o de placca in o le gruppo tractate con beta sitosterol o le gruppo de controlo.

**REFERENCES**

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