Effects of Sitosterol on the Cholesterol Concentration in Serum and Liver in Hypothyroidism

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The effects of the administration of the plant sterol, β-sitosterol, on the elevated serum lipids of hypothyroidism and on the cholesterol content of the liver have been investigated. In patients with hypothyroidism sitosterol administration, without dietary restriction, resulted in a reduction of serum cholesterol and other lipids. In the hypothyroid rat the addition of sitosterol to the diet prevented the increase in serum lipids and in the cholesterol content of the liver which otherwise occurs on a high-cholesterol diet.

The plant sterol, β-sitosterol, has been reported to exert a hypcholesterolemic effect in the cholesterol-fed chick,1 and rabbit,2 and in man on a free diet.3 It presumably acts by interfering with the intestinal absorption of cholesterol.4 In hypothyroidism, numerous observers have demonstrated that the plasma cholesterol and other plasma lipids tend to be elevated.5 In the rat fed thiouracil, Rosenman, Byers, and Friedman6 observed a much reduced rate of turnover of plasma cholesterol, both excretion and hepatic synthesis being reduced. These alterations in the handling of cholesterol in hypothyroidism might be expected to modify the effects on plasma lipids of sitosterol administration. The present study compares the effects on serum and liver lipids of sitosterol administration to normal and to hypothyroid rats. The effect on serum lipids of sitosterol administration to myxedematous human subjects has also been studied.

Methods

Rat Studies. Male white rats (Holtzman) of approximately 210 Gm. were maintained for 30 days on a commercial feed containing no added iodine (Pillsbury's mix-grain pellets), 1 per cent sodium chloride solution, and tap water, provided ad libitum. The animals were then divided into 6 groups of 4 to 7 each, the groups having similar mean weights. They were housed in mesh-bottomed cages and maintained in an air-conditioned animal room at approximately 75 F. for the duration of the experiment. At the end of this 30-day period of low-iodine intake, the 17 rats in 3 of the groups were subjected to radiation destruction of the thyroid by radioiodine. Each of the animals received 875 μc. of carrier-free 131I intraperitoneally. The 14 rats in the remaining 3 groups received no 131I and were considered to be euthyroid.

Three experimental diets were prepared as follows: low-cholesterol, Purina rabbit pellets plus 5 per cent cottonseed oil; high-cholesterol, Purina rabbit pellets plus 5 per cent cottonseed oil plus 1 per cent cholesterol—the cholesterol was dissolved in the warm cottonseed oil prior to its addition to the rabbit pellets; cholesterol-sitosterol, Purina rabbit pellets plus 5 per cent cottonseed oil plus 1 per cent cholesterol plus 5 per cent β-sitosterol—the rabbit pellets were impregnated with the β-sitosterol dissolved in chloroform, the chloroform was removed by evaporation prior to the addition of the cottonseed oil and cholesterol.

Each of the 3 diets was fed to 1 group of normal and to 1 group of 131I treated rats. The special diets were started 2 weeks after the administration of the radioiodine, and offered ad libitum for a period of 13 days. Tap water was provided at all times. The diets were well tolerated, none of the animals developed diarrhea, and all appeared healthy throughout the

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period of study. On the thirteenth day of the special diet, the animals were anesthetized with intraperitoneal amobarbital sodium, blood was obtained by cardiectomy, and the liver and thyroid were removed. The blood was permitted to clot, serum was separated by centrifugation, and total cholesterol, total lipid, and lipid phosphorus were determined within 24 hours. Cholesterol was determined by the method of Abell and associates,7 lipid phosphorus by that of Zilversmit and Davis,8 and total lipid by the turbidometric method of de la Huerca and coworkers.9 The livers were blotted dry, weighed, and digested over night in 20 per cent alcoholic potassium hydroxide. Total cholesterol was then determined by the method of Abell, and expressed as mg./100 Gm. of liver, wet weight.

The destruction of the thyroid glands was confirmed by microscopic examination. Portions of representative livers were fixed in formalin, embedded in gelatin, and stained for lipid with oil red O.

Human Studies. Six patients with myxedema were selected for study. Included were 2 patients with spontaneous myxedema, 2 with post-thyroidectomy myxedema, and 2 with radioiodine-induced myxedema. Beta sitosterol was administered before eating in a total daily dosage of 20 to 25 Gm.; there was no restriction as to the type or amount of food. A placebo preparation was administered during a portion but not all of the control periods. Fasting serum lipids were determined by the previously described methods at intervals of 1 to 3 weeks during control and sitosterol periods.

RESULTS

The results of the animal experiments are summarized in table 1. The addition of 1 per cent cholesterol to the diet resulted in a significant increase in liver cholesterol in both normal and hypothyroid animals. Some increase in serum total cholesterol and in total lipid was also observed with the 1 per cent cholesterol diet. The increases were greater in the hypothyroid than in the normal rats, but in neither group were they statistically significant.

The further addition of 5 per cent β-sitosterol to the 1 per cent cholesterol diet significantly reduced the liver cholesterol in both normal and hypothyroid rats. In addition to the reduction in liver cholesterol, the hypothyroid rats also displayed a significant lowering of serum cholesterol and serum total lipid as a result of the added sitosterol.

Comparison of the cholesterol-sitosterol fed groups with those on the low cholesterol diet reveals no significant differences in liver cholesterol or in serum lipids. This is true of both normal and hypothyroid groups.

Microscopic examination of the livers showed a pronounced increase in stainable lipid in the liver cells at the periphery of the lobules of the cholesterol-fed rats, both normal and hypothyroid. The further addition of sitosterol prevented this increase in stainable lipid, the sections being indistinguishable from those of animals on the low cholesterol diet (fig. 2).

For the final 13-day period during which the

* Values given are the mean, standard deviation (estimated using n - 1) and, in parentheses, the range.

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**Table 1.—Effects on Normal and Hypothyroid Rats of the Addition to the Diet of Cholesterol and of Cholesterol Plus Sitosterol**

<table>
<thead>
<tr>
<th>Thyroid state</th>
<th>Diet</th>
<th>Number of Rats</th>
<th>Weight change</th>
<th>Serum total cholesterol</th>
<th>Serum lipid phosphorus</th>
<th>Serum total lipid</th>
<th>Liver total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg./100 ml.</td>
<td>mg./100 ml.</td>
<td>mg./100 ml.</td>
<td>mg./100 Gm.</td>
</tr>
<tr>
<td>Normal</td>
<td>Low cholesterol</td>
<td>4</td>
<td>+33 ± 8.7</td>
<td>72 ± 5 (64-76)</td>
<td>7.6 ± 0.7 (6.8-8.5)</td>
<td>217 ± 27.3 (180-250)</td>
<td>303 ± 6 (297-311)</td>
</tr>
<tr>
<td>Normal</td>
<td>1% Cholesterol</td>
<td>5</td>
<td>+35 ± 5.0</td>
<td>75 ± 9 (59-81)</td>
<td>6.6 ± 1.5 (5.3-7.9)</td>
<td>254 ± 42.0 (192-310)</td>
<td>1109 ± 285 (872-1558)</td>
</tr>
<tr>
<td>Normal</td>
<td>5% Sitosterol</td>
<td>5</td>
<td>+29 ± 10.2</td>
<td>68 ± 6 (50-74)</td>
<td>7.2 ± 0.9 (5.8-8.0)</td>
<td>220 ± 18.5 (208-253)</td>
<td>271 ± 39 (237-333)</td>
</tr>
<tr>
<td>Hypo</td>
<td>Low cholesterol</td>
<td>4</td>
<td>-21 ± 2.7</td>
<td>88 ± 16 (74-111)</td>
<td>7.6 ± 0.6 (7.1-8.4)</td>
<td>246 ± 63.5 (192-236)</td>
<td>270 ± 28 (230-293)</td>
</tr>
<tr>
<td>Hypo</td>
<td>1% Cholesterol</td>
<td>7</td>
<td>-24 ± 9.3</td>
<td>119 ± 25 (81-151)</td>
<td>7.0 ± 1.1 (6.0-8.8)</td>
<td>312 ± 53.8 (250-397)</td>
<td>1625 ± 296 (1044-1914)</td>
</tr>
<tr>
<td>Hypo</td>
<td>5% Sitosterol</td>
<td>6</td>
<td>-27 ± 6.5</td>
<td>80 ± 13 (66-95)</td>
<td>7.2 ± 0.9 (5.9-8.3)</td>
<td>233 ± 26.6 (189-266)</td>
<td>281 ± 28 (260-321)</td>
</tr>
</tbody>
</table>

* Difference of the means considered significant when p < 2.5 per cent as estimated by the t test.
SITOSTEROL AND CHOLESTEROL CONCENTRATION IN HYPOTHYROIDISM

Table 2.—Effects on Serum Lipids of the Administration of Sitosterol to Six Hypothyroid Patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Observation (weeks)</th>
<th>Total cholesterol (mg./100 ml.)</th>
<th>Lipid phosphorus (mg./100 ml.)</th>
<th>Total Lipid (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sitosterol</td>
<td>Control</td>
<td>Sitosterol</td>
</tr>
<tr>
<td>S.M., 131 Induced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>42</td>
<td>(±11.5)</td>
<td>305</td>
<td>(±33.2)</td>
</tr>
<tr>
<td>V.S., Surgical</td>
<td>14</td>
<td>315</td>
<td>(±14.3)</td>
<td>266</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td>(±13.1)</td>
<td></td>
</tr>
<tr>
<td>G.K., Spontaneous</td>
<td>8</td>
<td>563</td>
<td>(±95.4)</td>
<td>415</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td>(±117)</td>
<td></td>
</tr>
<tr>
<td>C.M., 131 Induced</td>
<td>17</td>
<td>246</td>
<td>(±26.8)</td>
<td>216</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td>(±23.6)</td>
<td></td>
</tr>
<tr>
<td>L.H., Surgical</td>
<td>54</td>
<td>216</td>
<td>(±52.3)</td>
<td>(±14.9)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>(±14.0)</td>
<td></td>
</tr>
<tr>
<td>J.W., Spontaneous</td>
<td>3</td>
<td>257</td>
<td>(±10.8)</td>
<td>(±5.0)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>(±0.7)</td>
<td></td>
</tr>
</tbody>
</table>

* Values given are the mean and, in parentheses, the standard deviation (estimated using n—1).

Fig. 1. Changes in the serum total cholesterol in C. M. Following the administration of radioactive iodine the patient developed clinical manifestations of myxedema and elevation of serum cholesterol. Upon 3 separate occasions the administration of sitosterol was accompanied by a reduction in serum cholesterol (cross-hatched line).

special diets were fed, all euthyroid animals gained weight (mean 2.47 Gm./day) while all the 131 treated rats lost weight (mean 1.88 Gm./day). These weight changes were not significantly influenced by the addition of cholesterol or sitosterol to the diet.

The patient observations are summarized in table 2. The administration of sitosterol was accompanied by a fall in the mean values of serum total cholesterol, lipid phosphorus, and total lipid. The etiology of the hypothyroidism had no apparent effect on the response to sitosterol. Figure 1 depicts the effects on serum cholesterol of 3 separate periods of sitosterol administration to patient C.M., in whom hypothyroidism was induced by 131 in an attempt to alleviate intractable congestive heart failure due to rheumatic heart disease with mitral insufficiency.

DISCUSSION

The administration of β-sitosterol to 6 hypothyroid patients was accompanied by a consistent reduction in serum cholesterol. The mean fall of 20.1 per cent of control level compares with the fall of 15.6 per cent observed in 10 euthyroid hypercholesterolemic patients. Human studies thus indicate that the hypercholesterolemia associated with hypothyroidism is reduced by the administration of sitosterol without dietary modification.

That the greater fall in serum cholesterol in the hypothyroid patients was not due purely to chance is suggested by the results of the rat studies. Under the experimental conditions employed, the addition of 1 per cent cholesterol to the diet of the rats resulted in a 3- to 6-fold increase in the cholesterol level of the liver, the value being significantly higher in the hypothyroid than in the normal animal. If liver cholesterol is referred to total body weight instead of to liver weight, the relative values for the various groups are not appreciably changed.

Although in this small series the increase in serum cholesterol occurring when hypothyroid rats were fed a high cholesterol diet was not statistically significant, the report of Page and
Brown\textsuperscript{11} suggests that it would be with a larger series or more prolonged period of feeding. The euthyroid rat fed cholesterol demonstrated essentially no effect on serum cholesterol level. The greater accumulation of cholesterol in serum and liver occurring in the hypothyroid as compared to the euthyroid rat when fed a high cholesterol diet is presumed to be due to the decreased rate of cholesterol excretion or destruction.\textsuperscript{6}

The further addition of 5 per cent $\beta$-sitosterol to the diet prevented the increases in liver and serum cholesterol levels, in both normal and hypothyroid rats on a high cholesterol diet. This effect was more pronounced in the hypothyroid rat in which cholesterol feeding produces greater increases. The effect was also much more pronounced in the liver than in the serum.

In the hypothyroid rat, not only cholesterol excretion, but also hepatic synthesis of cholesterol is greatly reduced.\textsuperscript{6} Under these conditions of decreased rate of turnover of cholesterol, one would expect changes in the amount of cholesterol absorbed from the intestine to have an augmented effect on serum and liver levels. Since sitosterol interferes with the absorption of cholesterol from the digestive tract,\textsuperscript{4} its increased hypocholesterolemic effect in hypothyroidism is understandable.

**Summary**

In 6 patients with hypothyroidism the preprandial administration of $\beta$-sitosterol in the total daily dosage of 20 to 25 Gm. without dietary restriction, resulted in a mean reduction of serum total cholesterol of 20.1 per cent of the control level.

In both the normal and the hypothyroid rat, the addition of 5 per cent $\beta$-sitosterol to the diet prevented the increase in liver cholesterol and stainable lipid which otherwise occurred on a 1 per cent cholesterol diet. Sitosterol also prevented the rise in serum total cholesterol and total lipid which occurs in the hypothyroid rat fed a 1 per cent cholesterol diet.

The reduction in serum and liver cholesterol levels resulting from the interference by sitosterol with the intestinal absorption of cholesterol is augmented by the decreased rate of turnover of cholesterol in the hypothyroid state.

**Summario in Interlingua**

In sex patientes con hypothyroidismo le administration preprandial de beta-sitosterol in total dosages diurne de 20 a 25 g, sin restriction dietari, resultava in un reduction median del total cholesterol seral de 20,1 pro cento del nivello de controlo.

In rattos normal e in rattos hypothyroide, le
addition al dieta de 5 pro cento de beta-sitosterol preveniva le aumento de hepatic cholesterol e lipidio colorabile che ocorreva alternemente con un dieta a 1 pro cento de cholesterol. Sitosterol etiam preveniva le aumento del total cholesterol serum e del total lipid serum que ocorre in rattos hypothyroide recipiente un dieta a 1 pro cento de cholesterol.

Le reduction del nivellos serum e hepatic de cholesterol, resultante ab le obstruction per sitosterol in le absorption intestinal de cholesterol es augmentate per le reducida passage de cholesterol que es characteristic del stato hypothyroide.

REFERENCES


Four instances of situs inversus are described in which, although none was a twin, a strikingly high incidence of twinning occurred in the family: (1) both parents twins; (2) grandfather and grandmother twins; a sister and uncle begat 1 and 3 sets of twins; (3) father a twin, paternal grandmother had set of twins by each of 2 husbands; (4) mother twin, a first cousin begat 2 sets of twins.

Reference is made to the work of Ruud and Spemann (1922) who produced situs inversus through division of amphibian eggs. The twin derived from the right side showed a special tendency to develop this abnormality.

McKusick
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