Response of Serum Lipids and Lipoproteins of Man to Beta-Sitosterol and Safflower Oil
A Long-Term Study

By John W. Farquhar, M.D., and Maurice Sokolow, M.D.

Previous studies in man have indicated that administration of either plant sterols or unsaturated dietary fats causes decreases in certain serum lipids. This report presents the results of a long-term 7-phase study of the comparative effects of the single and combined administration of plant sterols (β-sitosterol) and a highly unsaturated vegetable oil (safflower oil) in 15 ambulatory subjects. The serum lipid changes observed indicate that both agents act on the same low-density lipoprotein fraction, that the changes produced by β-sitosterol and by safflower oil are similar in magnitude, but that the combination has a 55 per cent greater effect than either agent alone, that the mechanisms of action of these 2 agents probably differ, and that the action of the safflower oil is probably not due to the amount of sitosterol it contains.

The dietary factors that affect serum lipid levels of man have recently aroused considerable interest. This interest has stemmed in part from evidence linking premature complications of atherosclerosis with elevations of certain serum lipids. Two major dietary factors, plant sterols (phytosterols) and vegetable oils, have received particular attention because of their known effect of lowering the serum lipids of man.

Plant sterols are similar in structure to cholesterol. They are widely distributed as constituents of certain types of plants and are present in variable concentration in vegetable oils. The most common plant sterol is β-sitosterol; this substance significantly lowers the concentration of serum cholesterol when given to human subjects. A previous study demonstrated that β-sitosterol not only lowers the concentration of total serum cholesterol but also reduces the level of total β-lipoprotein, although it has little effect on α-lipoprotein. In addition, β-sitosterol decreases the intestinal absorption of cholesterol. It seems likely that the altered absorption is responsible for the changes in serum lipids, although the intermediate steps are not completely understood.

Vegetable oils of diverse composition, when substituted for the animal fats of the diet, have also been shown to lower serum cholesterol and β-lipoprotein cholesterol of man. There is disagreement, however, regarding the constituents of vegetable oils responsible for these effects.

Ahrens and associates, in a recent summary of their data, found that the degree of unsaturation of a wide variety of dietary fats correlated with the extent of fall in the serum cholesterol concentration after their ingestion. On the other hand, Keys and co-workers concluded that some factor other than the type or total amount of unsaturated fatty acid is responsible for part of the action of one vegetable oil (corn oil) on serum cholesterol of man. Recently, Beveridge and his associates suggested that much of the effect of corn oil on serum lipids can be explained on the basis of its content of β-sitosterol.

The purpose of the present study was to investigate the effects of β-sitosterol and a particular vegetable oil (safflower oil), alone and in combination, on serum lipid concentrations of a group of 15 subjects. The same 15 were studied throughout to allow more accurate evaluation of the dietary factors under study. Safflower oil was chosen because...
its unique composition (high content of linoleic acid and virtual absence of β-sitosterol, tocopherol, and linolenic acid) would lessen some of the experimental variables observed with other vegetable oils. Various internal controls were included in an attempt to clarify other points in question, such as the magnitude of the effects of the agents studied, the rapidity and consistency of the fall and subsequent rise in serum lipid concentrations, and the stability of the changes observed. To define more accurately the lipoprotein changes that occur after administration of these agents, the concentration of the following serum lipids were measured: total cholesterol, α- and β-lipoprotein cholesterol, phospholipid, total lipid, and triglyceride.

**Materials and Methods**

**Selection of Subjects**

Fifteen ambulatory, nondiabetic subjects (14 men, 1 woman), averaging 48 years of age (range 36 to 63) were studied. Thirteen had been diagnosed clinically as having either arteriosclerotic heart disease or arteriosclerosis obliterans; 1 had no apparent disease, and 1 had mild hypertension. In 3 individuals xanthelasma was present; no xanthomatous lesions were noted in the other 12 subjects. The subjects were selected on the basis of intelligence, desire to cooperate, and ambulatory status. All knew they were participating in a research project and agreed to the scheduled weekly interviews.

**Controls of Diet, Body Weight, and Activity**

The habitual diet of each subject was determined in detail prior to starting the study. This diet, with slight modifications if needed to maintain constant body weight, was adhered to during all control and sitosterol test periods. The average composition of the diets was 2380 total calories (range 1961 to 3000): 41 per cent of the calories were derived from fat, (range 33 to 53 per cent), of which 95 per cent was animal or solid vegetable fat.

The daily cholesterol intake* averaged 0.62 Gm. daily (range 0.26 to 0.96) during the control and sitosterol test periods. To maintain similar daily cholesterol intakes during the safflower oil test period each subject consumed from 3 to 7 eggs weekly; the cholesterol intake during this period averaged 0.80 per cent of that during the other periods.

**Experimental Design**

After a preliminary stabilization period the subjects underwent a consecutive 7-phase study, averaging 43 weeks in duration (range 35 to 54 weeks). The study was divided into 4 control periods of approximately 6 weeks each (phases I, III, V, and VII) alternated with 3 test periods (phases II, IV, and VI), averaging 8 weeks in duration (range 4 to 14 weeks).

In any study on the effect of multiple dietary variables on serum lipids, it is important to

| Table 1.—Composition of Safflower Oil* |

<table>
<thead>
<tr>
<th></th>
<th>Edible (degummed, deodorized)</th>
<th>Hydrogenated (degummed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatty acid composition†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt. % of total fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>73</td>
<td>none</td>
</tr>
<tr>
<td>95% as cis-cis isomer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>&lt;0.05</td>
<td>none</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>21</td>
<td>55.3</td>
</tr>
<tr>
<td>Total saturated</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>Free fatty acids (as oleic)</td>
<td>0.04</td>
<td>—</td>
</tr>
<tr>
<td>Conjugated dienes</td>
<td>0.82</td>
<td>—</td>
</tr>
<tr>
<td>Conjugated trienes</td>
<td>none</td>
<td>—</td>
</tr>
<tr>
<td>Iodine value (Wijc)</td>
<td>143 (142-144)</td>
<td>49.7</td>
</tr>
<tr>
<td>Phospholipid, wt. %</td>
<td>0.008</td>
<td>—</td>
</tr>
<tr>
<td>(lipid phosphorus X 25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol, wt. %</td>
<td>0.059</td>
<td>—</td>
</tr>
<tr>
<td>Unsaponifiable material, wt. %</td>
<td>0.60</td>
<td>0.40</td>
</tr>
<tr>
<td>Sterol content, ‡ wt. %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>0.09</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>0.15</td>
<td>—</td>
</tr>
<tr>
<td>Preservatives added, wt. %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propylgallate</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Melting point</td>
<td>115 C</td>
<td></td>
</tr>
</tbody>
</table>

*Analyses performed in the laboratories of Pacific Vegetable Oil Corporation, San Francisco, and Durkee Famous Foods, Berkeley. Values in italics are an average of many serial determinations. The range of these repeat determinations was narrow.
†Standard AOCS spectrophotometric analysis of KOH-isomerized oil and infrared spectroscopic methods.
‡Determined by both digitonin and infrared spectroscopic methods.
TABLE 2.—Average Body Weights (Kg.) of Subjects

<table>
<thead>
<tr>
<th>Phases of the study—consecutive in time</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean*</td>
<td>72.9</td>
<td>72.9</td>
<td>72.9</td>
<td>72.8</td>
<td>73.0</td>
<td>72.9</td>
<td>72.8</td>
</tr>
<tr>
<td>S.D.†</td>
<td>±9.2</td>
<td>±8.8</td>
<td>±9.1</td>
<td>±9.4</td>
<td>±9.6</td>
<td>±9.0</td>
<td></td>
</tr>
</tbody>
</table>

* Represents the mean of the average individual weights during period.

† Standard deviation

\[ \frac{\sqrt{\sum (x-x)²}}{n-1} \]

ensure that the agent given first in time does not affect the response to a subsequent agent. This objective was approached in our project by placing control periods between randomized test periods. This alternating design allowed close comparison of the lipid levels during the test and control phases.

During the control periods, the subjects consumed their habitual (control) diet, and in addition were given a placebo* similar in taste and consistency to the sitosterol preparation. During phases II and IV the subjects received either the control diet plus 18 Gm. (90 ml.) of \( \beta \)-sitosterol* daily, taken in 3 equal portions orally immediately before meals, or a total of 81 Gm. (90 ml.) of safflower oil daily, substituted for a computed equicaloric portion of the animal and solid vegetable fat of the control diet. The safflower oil was obtained from a single source,† and was stored at a temperature of less than 25° C. Frequent analyses performed during the study showed no changes in the composition of the oil (table 1). The oil was ingested in a variety of ways, most commonly as a suspension in skimmed milk or fruit juice, but also as an artificial ice cream or a salad oil. Use of the oil in frying or baking was forbidden. During phase VI the subjects received a combination of both \( \beta \)-sitosterol and safflower oil administered as in phases II and IV. In addition, 2 subjects were studied immediately after the safflower oil phase for an additional 2 weeks during which they ingested 81 Gm. of partially hydrogenated safflower oil daily (see table 1 for composition). A longer test period was not possible because of the relative unpalatability of the preparation.

Methods

During each phase serum was obtained from

*The placebo and the \( \beta \)-sitosterol (Cytellin) were generously supplied by the Eli Lilly Company, Indianapolis, Ind., courtesy of Drs. Robert Shipley and Kenneth Kohlstaedt.

†Purchased from the Pacific Vegetable Oil Corporation, San Francisco, Calif.

the subjects in the fasting state at weekly intervals. Each sample was analyzed for total cholesterol by the method of Abell et al. and for \( \alpha \) and \( \beta \)-lipoprotein cholesterol (ALPC and BLPC) by a modification of the method of Anderson and Keys. Our method differed chiefly in the technic of paper electrophoresis used for the initial separation. A brief description of our electrophoretic procedure follows: Six 33 cm. Whatman 3 MM paper strips (chromatography grade) were mounted in a vertical suspension electrophoresis apparatus similar to that described by Williams et al. Serum, 0.04 ml., was applied to 3 strips 2.5 cm. in width, and 0.1 ml. was applied to 3 paired strips 6 cm. in width. Electrophoresis was performed for 15 hours at 5 C. in barbital buffer of pH 8.6 and ionic strength 0.05. After oven-drying on a horizontal rack for 30 minutes at 100 C., the narrow strips were stained with Sudan black-B. The wide strips were cut into 2 segments, corresponding to the \( \alpha \) and \( \beta \)-lipoprotein fractions that appeared on the stained strip. The segments of the unstained strip were then analyzed for total cholesterol by the method of Anderson and Keys.

The coefficient of variation of differences* between 46 duplicates of total serum cholesterol in our laboratory was ± 1.2 per cent. Ninety replicates were analyzed for total serum cholesterol at intervals after storage of the serum in the frozen state for 1 to 12 months after the initial analysis. The coefficient of variation of these replicates was ± 3.1 per cent. The sum of ALPC and BLPC averaged 97.5 per cent of the total serum cholesterol, and represents the degree of recovery of cholesterol from the paper. The coefficient of variation of differences between 129 duplicates of total serum lipid was ± 1.1 per cent; for 133 duplicates of total serum phospholipid this value was ± 0.8 per cent.

Aliquots of each individual's serum samples were pooled for each phase and analyzed for total lipid by the method of Bragdon and for lipid phosphorus by the method of Stewart and Hendry. The factor 1.68 was used to convert ester cholesterol to cholesterol ester, a constant ratio of 0.27 was assumed for free to total cholesterol, and phospholipid was calculated from lipid phosphorus by use of the factor 25. Serum triglycerides were calculated by the method of Bragdon.

Results

The subjects' body weights in all phases and the serum lipid levels during the control phases served as internal experimental controls.

*Coefficient of variation = standard deviation of differences between duplicates divided by mean difference X 100 per cent.
### Table 3.—Summary of Mean Serum Lipid Changes During the Test and Control Phases

<table>
<thead>
<tr>
<th>Serum lipid fraction mg./100 ml.</th>
<th>Control*</th>
<th>β-sitosterol</th>
<th>Saﬄower oil</th>
<th>β-sitosterol and saﬄower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>279 ± 43†</td>
<td>283 ± 38</td>
<td>283 ± 33</td>
<td>204 ± 33</td>
</tr>
<tr>
<td>β-lipoprotein cholesterol</td>
<td>233 ± 42</td>
<td>186 ± 41</td>
<td>185 ± 32</td>
<td>157 ± 33</td>
</tr>
<tr>
<td>α-lipoprotein cholesterol</td>
<td>40 ± 10</td>
<td>40 ± 13</td>
<td>42 ± 10</td>
<td>42 ± 13</td>
</tr>
<tr>
<td>Phospholipid†</td>
<td>279 ± 30</td>
<td>248 ± 30</td>
<td>252 ± 42</td>
<td>239 ± 23</td>
</tr>
<tr>
<td>Total lipid‡</td>
<td>831 ± 106</td>
<td>711 ± 114</td>
<td>700 ± 112</td>
<td>653 ± 98</td>
</tr>
<tr>
<td>Triglyceride†</td>
<td>141 ± 46</td>
<td>124 ± 68</td>
<td>109 ± 51</td>
<td>119 ± 53</td>
</tr>
<tr>
<td>Cholesterol: phospholipid</td>
<td>1.00</td>
<td>0.94</td>
<td>0.93</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* Derived from average of all control periods.
† Mean ± standard deviation.
‡ Derived from pooled samples.

Values in italics differ from control signiﬁcantly ($p < 0.01$).

**Body Weight**

The maximum difference in the average weights of all subjects between any 2 phases was less than 1 per cent (table 2). Individual weights were also closely maintained; none of the subjects had a weight change of more than 3.5 Kg., and 9 subjects had a change of less than 2.0 Kg. during the entire study.

**Serum Lipid Concentrations**

The ﬁrst 2 weeks of each phase were required for stabilization of the serum lipid concentrations. Consequently, the ﬁgures for these periods were not included in the calculations of the average control and test values.

**During Control Periods.** The average serum lipid values for the control periods, phases I, III, V, and VII, were analyzed separately (ﬁg. 1); the results served as one check on the consistency of the return of the subjects to their control diets. As shown in ﬁgure 1 the average total serum cholesterol and the ALPC and BLPC levels were remarkably stable during all control periods. The maximum average difference in these serum lipid levels between any of the 4 phases was less than 4 per cent.

**During Test Periods.** A prompt and sustained fall in the concentration of serum total lipid, phospholipid, and BLPC occurred in all subjects during test phases II, IV, and VI (table 3). All the decreases were statistically signiﬁcant ($p = < 0.01$) when analyzed by a 2-way classiﬁcation analysis of variance. No signiﬁcant change in the level of ALPC was found during these phases, and serum triglyceride levels, although slightly decreased, did not differ signiﬁcantly from the control levels ($p = > 0.05$). However, since the triglycerides were calculated from each of the other 3 lipid moieties measured and a summation of errors may
occur in such calculations, the reliability of this measurement is reduced.

Although individual differences did occur, the average reductions in concentration of BLPC during the β-sitosterol and safflower oil phases were almost identical: 51 mg. (standard error of mean difference = ± 3.13) and 52 mg. per 100 ml. (standard error of mean difference = ± 5.82), respectively. Table 4 lists the BLPC values for each individual for all phases of the study. The average reduction in BLPC levels during administration of β-sitosterol and safflower oil combined was 80 mg. per 100 ml. (standard error of mean difference = ± 9.84), a 55 per cent greater decrease than that observed when either agent was given alone (fig. 2). The average reduction in BLPC concentration from control levels for the sitosterol, safflower oil, and combination phases was 22, 22, and 34 per cent, respectively.

Changes in serum cholesterol fractions (BLPC and ALPC) of 2 representative subjects are depicted in figure 3.

The 2 subjects who were given hydrogenated safflower oil for a 2-week period at the termination of the safflower oil phase showed a rise in serum BLPC concentration. This increase was from 165 mg. to 180 mg. per 100 ml. in 1 individual, and from 170 mg. to 205 mg. per 100 ml. in the second individual. A further rise in BLPC concentration occurred after return to the control diet (from 180 mg. to 204 mg. per 100 ml. and from 205 mg. to 222 mg. per 100 ml., respectively).

**Rate and Magnitude of Change in Serum Lipid Levels**

The fall in serum lipid levels during each test phase and their rise during the subsequent control phase were comparable in rate. All changes were rapid, taking place during the first 2 weeks of each phase, after which they leveled off and remained stable. Comparison of the BLPC levels at weekly intervals showed that the maximum effect occurred during the first week of each phase. The reduction in concentration during both the sitosterol and safflower oil phases averaged 50 mg. per 100 ml.; the magnitude of these reductions at the end of the first week was 49 mg. and 34 mg. per 100 ml., respectively (fig. 4). The difference between the means of these 1-week values was statistically significant (p = < 0.01). The rate of rise during the control period paralleled the rate of fall during the preceding test period, i.e.,

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control†</th>
<th>Sitosterol</th>
<th>Safflower oil</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB</td>
<td>244 ± 11‡</td>
<td>189 ± 6</td>
<td>185 ± 21</td>
<td>175 ± 6</td>
</tr>
<tr>
<td>WB</td>
<td>235 ± 7</td>
<td>180 ± 5</td>
<td>192 ± 13</td>
<td>153 ± 6</td>
</tr>
<tr>
<td>HC</td>
<td>265 ± 10</td>
<td>212 ± 13</td>
<td>221 ± 14</td>
<td>199 ± 10</td>
</tr>
<tr>
<td>MC</td>
<td>221 ± 13</td>
<td>183 ± 9</td>
<td>152 ± 6</td>
<td>126 ± 3</td>
</tr>
<tr>
<td>GD</td>
<td>193 ± 10</td>
<td>145 ± 11</td>
<td>158 ± 16</td>
<td>138 ± 7</td>
</tr>
<tr>
<td>RE</td>
<td>206 ± 13</td>
<td>162 ± 6</td>
<td>147 ± 11</td>
<td>122 ± 3</td>
</tr>
<tr>
<td>HF</td>
<td>212 ± 21</td>
<td>158 ± 7</td>
<td>181 ± 10</td>
<td>156 ± 13</td>
</tr>
<tr>
<td>WL</td>
<td>196 ± 10</td>
<td>171 ± 12</td>
<td>167 ± 4</td>
<td>149 ± 7</td>
</tr>
<tr>
<td>FP</td>
<td>281 ± 21</td>
<td>237 ± 15</td>
<td>228 ± 6</td>
<td>187 ± 10</td>
</tr>
<tr>
<td>RP</td>
<td>213 ± 13</td>
<td>166 ± 11</td>
<td>185 ± 5</td>
<td>130 ± 9</td>
</tr>
<tr>
<td>RS</td>
<td>332 ± 30</td>
<td>264 ± 9</td>
<td>225 ± 22</td>
<td>195 ± 6</td>
</tr>
<tr>
<td>HS</td>
<td>224 ± 11</td>
<td>195 ± 15</td>
<td>215 ± 12</td>
<td>180 ± 7</td>
</tr>
<tr>
<td>HV</td>
<td>171 ± 20</td>
<td>139 ± 13</td>
<td>124 ± 9</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>CW</td>
<td>217 ± 10</td>
<td>154 ± 11</td>
<td>169 ± 11</td>
<td>135 ± 10</td>
</tr>
<tr>
<td>JW</td>
<td>280 ± 22</td>
<td>229 ± 8</td>
<td>220 ± 18</td>
<td>207 ± 11</td>
</tr>
</tbody>
</table>

* Mean number of determinations per phase: control, 18; sitosterol, 7; safflower oil, 7; combination, 5.
† Mean of all control samples of each of the 4 control periods.
‡ Mean = standard deviation.

**Table 4**—Changes in β-Lipoprotein Cholesterol Concentrations in Each Phase of Study
BETA-SITOSTEROL AND SAFFLOWER OIL EFFECTS

Fig. 3. A comparison of the response of serum lipoprotein cholesterol concentrations in each of 2 patients.
the rise was somewhat slower after cessation of safflower oil than after sitosterol (fig. 4). The average reduction in concentration of BLPC during the combination phase was 80 mg. per 100 ml.; the amount of this fall at the end of the first week was 50 mg. per 100 ml. Again, the rate of return to control levels paralleled the rate of decrease during the test phase.

Clinical Data

All agents were well tolerated by the subjects, and no abnormal clinical signs or symptoms developed during the course of the experiment. There was no definite change in the frequency of the angina pectoris; in some subjects the exercise tolerance appeared to be greater. Photographs of the 3 subjects with xanthelasma taken at the beginning and end of the study showed a reduction in the size of the lesion in only 1 case.

Discussion

Ingestion of \( \beta \)-sitosterol resulted unequivocally in a prompt and significant fall in serum cholesterol in our subjects. The rate, magnitude, and stability of this decrease and the subsequent return to control values paralleled the results in a previous study on a different group of subjects.\(^7\)

The lipoprotein fraction affected during all 3 experimental periods appeared to consist exclusively of low-density (or beta) lipoproteins, since ALPC concentrations remained unchanged. On the basis of the known chemical composition of the various low-density lipoprotein classes and the distribution of cholesterol among them, it is evident that the lipoprotein fraction affected during all 3 experimental phases was predominantly S1 0-10.\(^{21,22}\)

The mechanisms by which serum cholesterol is lowered after ingestion of sitosterol in man are by no means clear. Hernandez and Chaikoff\(^8\) have shown that sitosterols decrease gastrointestinal absorption of C\(^{14}\) cholesterol in the rat; furthermore, experiments in man have demonstrated that sitosterols are poorly absorbed from the intestine.\(^{23}\) Since hepatic synthesis of cholesterol apparently can compensate for alterations in dietary cholesterol in the rat,\(^{24}\) it appears unlikely that sitosterol could lower the concentration of cholesterol in the serum simply by inhibiting its absorption. However, even if a similar hepatic control existed in man, the resultant compensation might not be sufficient to prevent sitosterol from inducing a temporary state of negative cholesterol balance. After re-establishment of a steady state, total body cholesterol might remain significantly reduced, and this reduction could be manifested by a decrease in the cholesterol content of the serum.

The recent experiments of Beveridge and associates\(^{25}\) indicate that as little as 1.5 Gm. of \( \beta \)-sitosterol daily will appreciably reduce serum cholesterol concentrations in human subjects ingesting a fat-free, cholesterol-free formula diet. These observations suggest that the action of sitosterol on endogenous cholesterol reabsorption is important and that the presence of relatively small amounts of these sterols in the diet of various peoples may influence their serum cholesterol concentrations.

In our study administration of safflower oil in a constant amount of 81 Gm. daily (supplying from 24 to 37 per cent of total calories, average 31 per cent) to ambulatory subjects consuming nonformula diets lowered certain \( \beta \)-lipoprotein cholesterol concentra-

![Image](http://circ.ahajournals.org/lookup/suppl/doi:10.1161/01.CIR.58.4.896/-/DC1/FIG4.jpg)

Fig. 4. Illustration of the delayed action of safflower oil compared to \( \beta \)-sitosterol. The solid line represents the changes with \( \beta \)-sitosterol, the dotted line the changes with safflower oil. The data are derived from 11 of the 15 subjects.
tions for periods up to 14 weeks. This decrease might have been caused simply by omission from the diet of animal and hydrogenated vegetable fat. Alternatively, the substituted vegetable oil itself could have been responsible, or both factors might have been involved. It is unlikely that the action of safflower oil is dependent on sitosterol, linoleic acid, or tocopherol (because of their low concentration in the oil), although it might be related to linoleic acid, which is present in high concentration. The rise in BLPC levels in 2 of our subjects after administration of linoleic acid-free hydrogenated safflower oil would support the assumption that serum cholesterol concentration is at least partly dependent on the degree of unsaturation of dietary fats. Since the observations on the action of hydrogenated safflower oil were limited in scope, further conclusions are not warranted.

The somewhat slower change in BLPC levels during and after administration of safflower oil than during and after \( \beta \)-sitosterol suggests that the 2 compounds differ in mechanism of action. It seems very unlikely that the 50 per cent greater decrease in serum cholesterol effected by the 2 agents combined results from the added action of the small amount of sitosterol present in safflower oil (approximately 0.12 Gm. per 81 Gm.); particularly since the effects of ingesting 9 Gm. and 18 Gm. of sitosterol daily are similar in magnitude.\(^7\)

Investigation of the effects of ingestion of pure fatty acids to which varying amounts of sitosterol can be added might aid in clarifying the role of sitosterol in the action of vegetable oils. Such studies are in progress. Since it is possible that the unsaturated fatty acid portion and the plant sterol fraction of plant oils will be found to have independent hypcholesterolemic actions, the effect of these agents on fecal cholesterol balance will also be studied.

**Summary**

Fifteen ambulatory subjects with clinical evidence of atherosclerosis were studied during a 7-phase experiment lasting 35 to 54 weeks. They were given in random sequence \( \beta \)-sitosterol, 18 Gm. per day in conjunction with the control diet which contained 39 per cent of calories derived from animal or hydrogenated vegetable fat; safflower oil, 81 Gm. per day as 31 per cent of total calories in equicaloric substitution for animal and hydrogenated vegetable fat of the control diet; and a combination of the 2. Placebos were administered during the control period that preceded and followed each test period. Serum lipids were measured weekly.

A rapid and sustained fall in \( \beta \)-lipoprotein cholesterol (BLPC), total lipid and phospholipid occurred during each of the 3 test periods. The average fall in BLPC after ingestion of the test agents was: sitosterol alone, 22 per cent; safflower oil alone, 22 per cent; the 2 combined, 34 per cent. Average body weights were constant during the study, and the average serum cholesterol concentrations were closely similar during the 4 control periods. The uniformity of serum lipid response to the test agents, coupled with the stability of average body weights and control BLPC concentrations, indicates that confidence can be placed in the results of long-term dietary studies using nonformula diets in well-instructed ambulatory subjects.

The fall and subsequent rise in BLPC were more rapid with sitosterol than with safflower oil, suggesting that the 2 agents have different mechanisms of action. The 55 per cent greater fall in BLPC after ingestion of sitosterol and safflower oil combined indicates that the action of safflower oil is not likely to be the result of the small amount of sitosterol it contains. Other possible explanations for the changes observed with safflower oil are discussed.

Since no significant changes in alpha lipoprotein cholesterol or triglyceride were observed during the study, it was concluded that the \( S_t \) 0-10 fraction of the serum \( \beta \)-lipoproteins was primarily affected by the test agents.

**Acknowledgment**

The authors wish to express their appreciation to Dr. Richard J. Havel for helpful suggestions given during the course of the study and to Dr.
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**SUMMARIO IN INTERLINGUA**

Dese-cinquante subjectos ambulatori con signos clinica de atherosclerosis esseva studiate in un experimento heptaphasic de un duration de 35 a 54 setmanas. Omne le subjectos esseva studiate durante tres periodos experimental e quatro periodos de controlo ante, inter, e post le periodos experimental. Durante le periodos experimental illes reci- peva (1) sitosterol beta, 18 g per die, in conjunction con le dieta de controlo que contineva grassia animal o hydrogenate grassia vegetal amontante a 39 pro cento del valor caloric, (2) oleo de carthamo, 81 g per die, representante 31 pro cento del total ingestion caloric e reimplacante un quantitate equicaloric de grassia animal o de hydrogenate grassia vegetal in le dieta de controlo, e (3) un combination del 2. Le ordine del periodos correspondent a iste 3 dictas experimental variava al hasardo inter le 15 subjectos. Supplementos fictitie esseva administrate durante le 4 periodos de controlo. Le lipidos serial esseva mesurate septimalmente.

Un rapide e sustenite reduction del cholesterol de lipoproteina beta (CLPB), del lipidio total, e del phospholipido occurreva durante cata un del 3 periodos experimental. Le reduction medie de CLPB post le ingestion del agentes experimental esseva 22 pro cento post sitosterol sol, 22 pro cento post oleo de carthamo sol, e 34 pro cento post le combination del 2. Le pesos corporee medie esseva constante durante le studio, e le concentraciones medie del cholesterol serial esseva multo simile durante le 4 periodos de controlo. Le uniformitate del responsa sero-lipidic al agentes experimental, insimul con le stabilitate del pesos corporee medie e del concentration de CLPB de controlo, indica que il es justificate haber confidentia in le resultatos de studios dietari a longe vista con dietas altere que per formula alimentari, providite que le subjectos (qui pote esser ambulatori) es ben instruite.

Le reduction e le subsequente re-aumento de CLPB esseva plus rapido con sitosterol que con oleo de carthamo. Isto pare indicar que le 2 agentes ha differente mechanismos de action. Le facto que le reduction de CLPB esseva 55 pro cento plus grande post le ingestion de sitosterol e de oleo de carthamo in combination indica que le action de oleo de carthamo non se explica facilmente per le presentia de micre quantitates de sitosterol in illo. Explicationes plus probable del alterationes observate con oleo de carthamo es discutite.

Viste le facto que nulle alterationes significative de cholesterol de lipoproteina alpha o de triglycerido esseva observate durante le studio, il esseva conclude que le agentes experimental afficceva primarmente le fraction S, 0-10 del lipoproteinas beta in le sero.

**REFERENCES**


BETA-SITOSTEROL AND SAFFLOWER OIL EFFECTS


Five different spectra of proteins were found by paper electrophoresis in the urine of patients with congestive heart failure. All contained albumin, only exceptionally isolated, usually in combination with 1 to 3 globulin fractions, the most common being alpha and beta globulin. Gamma globulins were found in protein-rich urines but also with low protein concentrations following ultrafiltration, suggesting a renal factor contributing to proteinuria in heart failure. The authors believe that the various spectra of globulin fractions may have different prognostic significance.
Response of Serum Lipids and Lipoproteins of Man to Beta-Sitosterol and Safflower Oil: A Long-Term Study
JOHN W. FARQUHAR and MAURICE SOKOLOW

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