Influence of Various Dietary Fats on Hypercholesterolemia and Atherogenesis in Cockerels

By Gregory B. Clarke, Ph.D., Savitri Jain, M.D., Ruth Pick, M.D., and Louis N. Katz, M.D.

With the assistance of P. Johnson and C. Kakita

It is evident that both dietary and metabolic factors influence plasma cholesterol levels and the development of experimental atherosclerosis. The type of fat normally consumed is one of the important dietary factors. Although the "polyunsaturated" fatty acids (consisting primarily of linoleic acid) are known to depress plasma cholesterol levels, it does not follow that an excess of dietary linoleic acid is necessarily more beneficial than a proper ratio of fatty acids of different chain length and saturation.

The literature indicates a striking disparity between the effects of various dietary fats on plasma cholesterol levels in human beings and in animals. We ourselves were unable, in earlier studies, to find consistent differences in hypercholesterolemia and atherogenesis in cockerels fed different saturated and unsaturated fats added to cholesterol supplemented chick starter mash.

In the present study, therefore, a fat-free, semi-synthetic diet was used as the basic ration, to which was added 1% cholesterol and 10% of one of the several fats used in the study. The hypercholesterolemic and atherogenic effects of these diets were observed in young growing cockerels.

Methods

Two experiments involving 388 cockerels were done. The chicks were from a hybrid strain obtained at one day of age from a commercial hatchery. They were fed a commercial chick starter mash and maintained in a battery brooder until the onset of the experiment. The experiments were started when the cockerels were 9 to 11 weeks old. For the first two weeks, to eliminate the influence of unknown fat in the mash, the chicks received the basal diet (table 1), including the experimental fat but without cholesterol supplementation. For the next five weeks 1% cholesterol was added to each of the diets. Birds were weighed at the beginning and end of the five-week experimental period and weekly food intakes were determined. The animals were killed at the end of this seven-week period.

EXPERIMENT 1

Three series of cockerels, totalling 348, were studied. In this experiment the hypercholesterolemic and atherogenic effects were compared for coconut oil, butter, cottonseed oil, cottonseed oil margarine,* safflower oil, safflower oil margarine,t corn oil and corn oil margarine.+ Allowance was made for the nonfat substances present in each margarine.

EXPERIMENT 2

This experiment, involving 40 cockerels, compared the hypercholesterolemic and atherogenic effects of corn oil margarine and corn oil margarine fat (i.e., partially hydrogenated corn, soy-
bean and cotton seed oil without the additives used in margarine manufacture).

The fatty acid composition of each of the fats and oils was determined by gas-liquid chromatography and the polyunsaturated/saturated ratios (P/S ratios) calculated (table 2). The 2S-1P (twice the saturated fatty acid content minus the polyunsaturated fatty acid content) values of all the fats and oils were calculated. The methyl esters of the fatty acids were prepared by transesterification and were analyzed on a Barber Coleman model 10 with an argon flow rate of 60 ml per minute. National Heart Institute fatty acid standards were used as reference compounds for all gas chromatographic analyses.

Plasma cholesterol levels were determined by the method of Sperry and Webb. Aortic atherosclerosis was evaluated according to the established methods of this department, by grading from 0 (no lesion) to 4+ (most severe). The extent of coronary atherosclerosis was determined by taking one frozen section stained with Sudan IV from each of two blocks of each heart and calculating the percentage of atherosclerotic coronary arteries.

Results
In all experiments food intakes were comparable within the series, as was gain in body weight. Therefore, they are not reported here.

EXPERIMENT 1
The data from the three series in this experiment were comparable and, therefore, are combined to yield the results presented in table 3. The serum cholesterol levels decline with a rise in P/S ratio for the coconut oil, butter, cottonseed oil, and corn oil fed groups.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Cottonseed Oil</th>
<th>Corn Oil</th>
<th>Safflower Oil</th>
<th>Coconut Oil</th>
<th>Butter</th>
<th>Cottonseed Oil Margarine</th>
<th>Corn Oil Margarine</th>
<th>Safflower Oil Margarine</th>
</tr>
</thead>
<tbody>
<tr>
<td>6:0</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:0</td>
<td></td>
<td>9.9</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>7.0</td>
<td></td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>0.2</td>
<td>38.5</td>
<td>3.7</td>
<td>0.2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>1.0</td>
<td>0.2</td>
<td>0.4</td>
<td>19.7</td>
<td>12.3</td>
<td>0.1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>20.3</td>
<td>11.4</td>
<td>9.8</td>
<td>9.9</td>
<td>25.8</td>
<td>18.8</td>
<td>13.6</td>
<td>8.8</td>
</tr>
<tr>
<td>16:1</td>
<td>0.9</td>
<td></td>
<td>0.3</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>3.1</td>
<td>2.3</td>
<td>2.9</td>
<td>3.9</td>
<td>13.6</td>
<td>8.5</td>
<td>8.4</td>
<td>9.0</td>
</tr>
<tr>
<td>18:1</td>
<td>22.0</td>
<td>27.0</td>
<td>16.3</td>
<td>8.8</td>
<td>28.4</td>
<td>43.0</td>
<td>46.5</td>
<td>44.2</td>
</tr>
<tr>
<td>18:2</td>
<td>52.7</td>
<td>57.9</td>
<td>68.9</td>
<td>2.3</td>
<td>2.9</td>
<td>29.3</td>
<td>30.1</td>
<td>38.0</td>
</tr>
<tr>
<td>18:3</td>
<td>1.0</td>
<td>1.4</td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td></td>
<td></td>
<td></td>
<td>1.8</td>
<td></td>
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</tbody>
</table>

*The first number indicates the number of carbon atoms; the second, the number of double bonds.
The margarines, in spite of having lower P/S ratios as compared to their parent oils, produced lower serum cholesterol levels. The difference in the latter was slight between groups fed cottonseed oil and cottonseed oil margarine, but highly significant between those fed corn oil and corn oil margarine (P < 0.001) and also between those fed safflower oil and safflower oil margarine (P < 0.05).

However, as can be seen from the data in table 3, when the oils are arranged according to their P/S ratios, the cholesterol values decrease as the P/S ratio increases with the exception of safflower oil. When all fats are calculated according to the 2S-1P formula (fig. 1), safflower oil falls in line with the other oils in its hypercholesterolemic action. The corn oil margarines and safflower oil margarine, however, still behave differently in this correlation as in the other one.

The incidence as well as the severity of aortic and coronary atherosclerosis followed generally the same trend as the serum cholesterol levels, being highest in fats with low P/S ratios; this was not true for the margarines. Safflower oil, again, in spite of having the highest P/S ratio of all fats in this experiment, did not have the lowest values for coronary atherosclerosis (table 3).

However, if the percentages of coronary lesions are plotted against the 2S-1P calculation (fig. 2), the same results as for cholesterol are obtained, i.e., all oils are atherogenic according to their 2S-1P ratios while the corn oil margarines and safflower oil margarine again behave differently.

**EXPERIMENT 2**

In order to determine whether the additives in the margarine were responsible for the lower degree of hypercholesterolemia and atherogenesis, the margarine and margarine fat were fed with the cholesterol supplemented diet used in experiment 1. As seen in table 4, the birds receiving the complete corn oil margarine had significantly lower serum cholesterol levels than those fed the same fat used in the margarine minus the additives. But between these two groups, there was no significant difference in the severity of aortic and coronary atherosclerosis, though—per cent—

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**TABLE 3**

| Dietary fat             | P/S* ratio | Number of birds | Plasma cholesterol mg % | Thoracic aorta lesions | Per cent of birds with lesions | Average grade | Per cent of birds with lesions | Per cent positive
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil</td>
<td>0.03</td>
<td>39</td>
<td>539 ± 33†</td>
<td>90</td>
<td>0.75 ± 0.07</td>
<td></td>
<td>85</td>
<td>6.6 ± 0.8</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>2.16</td>
<td>60</td>
<td>431 ± 22</td>
<td>67</td>
<td>0.49 ± 0.06</td>
<td></td>
<td>77</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.18</td>
<td>38</td>
<td>380 ± 21</td>
<td>47</td>
<td>0.24 ± 0.05</td>
<td></td>
<td>87</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>5.36</td>
<td>37</td>
<td>386 ± 19</td>
<td>70</td>
<td>0.54 ± 0.08</td>
<td></td>
<td>73</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>Butter</td>
<td>0.06</td>
<td>40</td>
<td>493 ± 27</td>
<td>88</td>
<td>0.51 ± 0.05</td>
<td></td>
<td>82</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>Cottonseed oil margarine</td>
<td>1.06</td>
<td>39</td>
<td>391 ± 20</td>
<td>72</td>
<td>0.66 ± 0.09</td>
<td></td>
<td>85</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>Corn oil margarine</td>
<td>1.34</td>
<td>58</td>
<td>273 ± 11</td>
<td>41</td>
<td>0.24 ± 0.05</td>
<td></td>
<td>60</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Safflower oil margarine</td>
<td>2.13</td>
<td>37</td>
<td>319 ± 17</td>
<td>40</td>
<td>0.30 ± 0.07</td>
<td></td>
<td>68</td>
<td>2.7 ± 0.5</td>
</tr>
</tbody>
</table>

*P/S: polyunsaturated fatty acids, saturated fatty acids
†±: standard error of the mean.
*Per cent positive: per cent coronary arteries with lesions, total no. of arteries
age incidence of lesions was slightly greater when margarine fat was fed.

**Discussion**

Most studies on the influence of dietary lipids on serum cholesterol levels in man and animals have shown that the hypocholesterolemic effect of certain of these dietary fats is related to their unsaturated fatty acid content. However, other reports have shown that oils with a very high linoleic acid content exert relatively little influence on either atherosclerosis or levels of serum cholesterol, when compared to dietary fats which contain smaller, but significant, quantities of linoleic acid.

![Figure 1](image1.png)

**Figure 1**

Effect of oils and fats with different 2S-1P values (see text) on plasma cholesterol levels. Note: the margarines are excluded from the regression line calculation.

![Figure 2](image2.png)

**Figure 2**

Effect of oils and fats with different 2S-1P values (see text) on incidence of coronary atherosclerosis. Note: the margarines are excluded from the regression line calculation.

*CLARKE, JAIN, PICK, KATZ*

*Circulation Research, Vol. XVIII, February 1966*
DIETARY FATS AND ATHEROGENESIS

TABLE 4
Plasma Cholesterol Levels and Thoracic Aorta and Coronary Lesions in Cockerels Fed Either Corn Oil Margarine or Corn Oil Margarine Fat

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma cholesterol (mg %)</th>
<th>Thoracic aorta lesions (%)</th>
<th>Average grade</th>
<th>Coronary arteries (Per cent positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mazola margarine</td>
<td>268 ± 14*</td>
<td>63</td>
<td>.47 ± 0.11</td>
<td>53</td>
</tr>
<tr>
<td>Margarine fat</td>
<td>388 ± 23</td>
<td>90</td>
<td>.55 ± 0.06</td>
<td>60</td>
</tr>
</tbody>
</table>

*±: standard error of the mean.
†See table 3 for explanation.

The data presented here indicate that while the degree of elevation of serum cholesterol levels and atherogenesis are related to the relative unsaturated fatty acid content of the dietary fat, this is not the only factor involved. Margarines, even with relatively lower unsaturated fatty acid content, were less hypercholesterolemic and less atherogenic than oils.

The additives used in the manufacture of margarine, such as plasticizers, emulsifiers, milk solids, etc. may offer a partial explanation. Animals fed corn oil margarine fat without these additives have significantly higher plasma cholesterol levels than animals fed the complete corn oil margarine, comparable to oils with similar fatty acid composition. However, the decrease in the atherogenic effect was preserved and remained at the degree observed with the complete margarine. The reason for this disparity is not yet explained.

The decreased atherogenicity of the margarines could be due to the relatively large amounts of trans-isomers formed during the hydrogenation of oils for margarine manufacture. It has been shown that elaidic acid, the trans-isomer of oleic acid, is incorporated into the lymph phospholipids to a greater extent than oleic acid during the peak of absorption and differences have been shown in plasma glycerides of human beings after ingestion of elaidic acid. Although no fat absorption studies were carried out, it is unlikely that decreased absorption of the margarine is responsible for the lower hypercholesterolemic and atherogenic effects, since weight gain was similar in oil- and margarine-fed birds.

The data presented here would indicate that there is no simple relationship between fatty acid content, hypercholesterolemia, and atherogenesis. In studying the effects of dietary fats on lipid metabolism and coronary atherosclerosis, structural modifications during processing must be considered.

Summary

When cockerels were given a cholesterol supplemented, fat-free, semi-synthetic diet as the basic ration, all oils and solid fats were atherogenic and caused elevated serum cholesterol levels. The degree of hypercholesterolemia and atherogenesis was related to fatty acid composition of the fat fed. Margarines were less atherogenic and less hypercholesterolemic than oils. The additives used in the preparation of margarine have some influence on maintaining lower cholesterol levels, but do not appear to have any significant effects on atherogenesis. The structural isomers formed during margarine manufacture may be responsible through an alteration of lipid metabolism.

Acknowledgment

We thank Dr. Dorothy Rathman of the Corn Product Company for generous supplies of corn oil, corn oil margarine, and corn oil margarine fats, Dr. E. Alpert of Merck and Company for cholesterol and Mr. E. M. Deck of Anderson Clayton and Company for cottonseed oil.

It is a pleasure to acknowledge the contribution of the Institute's atherosclerosis research team: Miss Birute Konce, Mrs. Charlene Thompson, Mrs. Beverly Wharton, Mrs. Dorothy Croom, Mrs. Dorothy Clark, Mr. Cecil Henderson, and Mr. Howard Walker.

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Circ Res. 1966;18:213-218
doi: 10.1161/01.RES.18.2.213

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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