Saturated and Unsaturated Fats

Effects on Cholesterolemia and Atherogenesis in Chicks on High-Cholesterol Diets

By J. Stamler, M.D., R. Pick, M.D., and L. N. Katz, M.D.

With the assistance of D. Century and P. Johnson

The effects of supplementary oils—saturated and unsaturated—were examined in groups of cholesterol-fed cockerels. Unsaturated oils failed to suppress hypercholesterolemia and atherogenesis. When various oils and fats high in oleic acid were added to the mash, slight lowering of serum cholesterol occurred. Oleic acid per se given to a group was accompanied by a diminution in atherosclerosis.

Recent studies in man have shown that substitution of unsaturated oils for saturated fats in the human diet is associated with significant decreases in serum cholesterol concentration.1-8 These studies were undertaken to explore the parameters and mechanisms of this phenomenon in the chick.

Methods

The established experimental atherosclerosis techniques of this department were utilized throughout.9,10 Ten series of experiments were carried out, involving a total of 39 groups and 390 cockerels. All birds were of the Hy-line hybrid strain, obtained from a commercial hatchery at one day of age, and reared in a battery brooder. They were fed commercial chick starter mash until the onset of the experiment. Experiments were done on young growing cockerels and varied in duration from 5 to 15 weeks. All birds received cholesterol (0.5 to 2.0 per cent) during the experimental period. In essence the design involved a comparison of hypercholesterolemia and atherogenesis with incorporation of different oils and fats in cholesterol-supplemented mashes. In some series a comparison was made among different unsaturated oils of vegetable and marine origin incorporated in the cholesterol-mash at the 5 or 10 per cent level. In other series, a comparison was made of unsaturated vegetable oil and saturated fat of either animal (lard, butter, chickenfat, egg yolk) or vegetable (coconut oil, hydrogenated coconut oil, hydrogenated cottonseed oil) origin. An analysis was also made of the effects of unsaturated oil supplementation at different levels (5 and 20 per cent, 10 and 20 per cent). Further, an assessment was made of the effects of supplementing an egg yolk containing mash with a linoleic acid concentrate. Finally an analysis was accomplished of the effects of substituting corn oil for butter in cockerels ingesting a cholesterol-supplemented mash.

Results

Effects of Different Oils and Fats Incorporated at the Same Level in Cholesterol-Supplemented Mashes

Polyunsaturated Oils (5 or 10 per cent). Over-all, no significant consistent differences in feed intake, weight gain, hypercholesterolemia or atherogenesis (aortic, coronary) were observed in cockerels receiving different unsaturated oils—cottonseed oil, linseed oil, corn oil, corn oil fractions, linoleic acid concentrate (with and without added vitamins B₆ and E) (representative experiments are presented in table 1).* Hypercholesterolemia and atherogenesis were less marked with safflower oil than with cottonseed oil, how-

* A more detailed form of table 1 has been deposited as Document number 5868 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C.
### TABLE 1.—Effects of Different Fats in Cholesterol-Fed Chicks

<table>
<thead>
<tr>
<th>Series</th>
<th>Type and amount of dietary fat* (%)</th>
<th>Plasma total cholesterol (mg. % ± S.E.)</th>
<th>Gross thoracic aorta lesions</th>
<th>Microscopic coronary lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Cholesterol Grade (%)</td>
</tr>
<tr>
<td>13-23</td>
<td>Cottonseed oil — 5</td>
<td>215 ± 13</td>
<td>40</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid conc. — 5</td>
<td>271 ± 26</td>
<td>83</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>1/2 C§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cottonseed oil — 5</td>
<td>189 ± 29</td>
<td>42</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid conc. — 5</td>
<td>211 ± 25</td>
<td>22</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>14-19</td>
<td>Oleic acid — 10</td>
<td>766 ± 78</td>
<td>100</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>1 C§</td>
<td></td>
<td>1010 ± 82</td>
<td>57</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Cottonseed Oil — 10</td>
<td>641 ± 49</td>
<td>89</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>12-18</td>
<td>Butter — 10</td>
<td>643 ± 66</td>
<td>80</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>1 C§</td>
<td>Fat from egg yolk — 11</td>
<td>384 ± 36</td>
<td>80</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>12-17 &amp; 13-23</td>
<td>Cottonseed oil — 5</td>
<td>1252 ± 76</td>
<td>90</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>1/2 C§</td>
<td>Linoleic acid conc. — 10</td>
<td>1628 ± 122</td>
<td>100</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>14-19</td>
<td>Olive oil — 10</td>
<td>650 ± 115</td>
<td>70</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>1 C§</td>
<td>Linoleic acid conc. — 10</td>
<td>704 ± 124</td>
<td>78</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Safflower oil — 10</td>
<td>839 ± 48</td>
<td>11</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>9-14</td>
<td>Neofat — 10</td>
<td>974 ± 109</td>
<td>90</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>1 C§</td>
<td>Chicken fat — 10</td>
<td>710 ± 43</td>
<td>70</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Coconut oil — 10</td>
<td>832 ± 81</td>
<td>100</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Hydrogenated coconut oil — 10</td>
<td>1067 ± 123</td>
<td>88</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

*The linoleic acid concentrate, obtained from Nutritional Biochemicals Corporation, was technical grade material of approximately 60 per cent purity, with the remainder primarily oleic acid. On analysis it was found to have an iodine value of 144.5, and to contain 51.7 per cent linoleic acid, 49.0 per cent oleic acid.

The oleic acid, so designated, was obtained from the Nutritional Biochemicals Corporation, and was a product meeting USP specifications, with a specific gravity of 0.889, acid value of 188-203, iodine value of 85-95. On analysis, its iodine value was found to be 90.1, and its composition was 94.0 per cent oleic acid, 1.7 per cent linoleic acid.

Egg yolk, 37 per cent, incorporated in mash yielded a ration containing 1 per cent cholesterol, 10.8 per cent fat, 5.0 per cent phospholipids, and 24 per cent protein. No pure cholesterol was added to the diet of this group. This ration is higher in protein than the mash fed other groups in this experiment.

The Neofat, 92-04, was obtained from the Armour Laboratories, and was stated to have the following composition: oleic acid, 86 per cent; linoleic acid, 6 per cent; palmitic acid, 5 per cent; myristic, 2 per cent; stearic, 1 per cent. Neither the linoleic, oleic nor Neofat preparations contained more than 5 per cent of a transisomer.

On analysis, 5 samples of rendered chicken fat gave the following mean composition: stearic acid, 8.0 per cent; palmitic + myristic + lauric acids, 17.0 per cent; oleic + vaccenic acids, 52.4 per cent; linoleic acid, 17.0 per cent; lower volatile acids, 0.1 per cent; unsaponifiable matter, 0.3 per cent; glycerine, 4.4 per cent.

†Lesions in the thoracic aorta were graded on an arbitrary scale from 0-4.* Average of the grades for those birds with lesions; chicks graded 0 were excluded in this calculation.

‡Two standard Sudan-stained sections of the heart were examined microscopically, a count was made of all arterioles and arteries visualized, and the percentage of those exhibiting atherosclerotic plaques noted. This is the mean per cent for the group and is an index of severity of atherosclerotic involvement.

§Cholesterol incorporated in the mash, 0.5 or 1.0 per cent.

¶Hydrogenated vegetable shortening.
ever only the difference in plasma cholesterol levels was statistically significant (table 1, S31). Hypercholesterolemia was less marked with fish oils than with cottonseed oil, but no differences in atherogenesis were observed.

_Fats and Oils High in Mono-Unsaturated Oleic Acid_ (10 per cent). Hypercholesterolemia tended to be slightly less marked in cockerels fed various fats and oils (oleic acid, olive oil, Neofat, chicken-fat) high in mono-unsaturated fatty acid, compared with birds fed cottonseed oil at the same level (10 per cent) in cholesterol-supplemented mash (table 1). However, this was not a consistent finding. Thus, in series 49 (1 per cent cholesterol mash fed during weeks 14 to 19), findings with cottonseed oil vs. oleic acid at the 10 per cent level were: plasma cholesterol, 766 and 1,010 mg. per cent; aorta lesions, 100 and 57 per cent, grade 1.0 and 0.9; incidence of coronary lesions, 100 and 71 per cent; extent of coronary lesions, 14 and 8 per cent. In both S49 and S51, the oleic acid group exhibited less atherosclerosis than the cottonseed oil group. The mechanism and significance of this finding remains to be elucidated.\(^{14,15}\) No other significant differences in atherogenesis were observed among the several groups studied in experiment S51 (table 1).

_Saturated Fats and Oils_ (5 and 10 per cent). In groups of cockerels fed saturated fat (lard, Crisco, butter, egg yolk, coconut oil, hydrogenated coconut oil) incorporated in cholesterol-supplemented mashes, levels of hypercholesterolemia and atherogenesis were either similar to or less than those observed in birds receiving cottonseed oil (table 1).

_Effects of Polyunsaturated Oils Incorporated at Different Levels in Cholesterol-Supplemented Mashes_  
Hypercholesterolemia and atherosclerosis were slightly to markedly greater in cockerels receiving 20 per cent corn oil, compared with birds on 5 or 10 per cent oil.

_Effects of Supplementation Egg Yolk Mask with Linoleic Acid Concentrate (5 per cent) ._  
Both groups consumed mash plus egg yolk, 37 per cent, yielding a ration of 1 per cent cholesterol, 10.8 per cent fat, 5 per cent phospholipids and 24 per cent protein. In addition, the second group was given 5 per cent linoleic acid concentrate. No significant differences were noted between control and experimental groups in this case. Thus, the findings in the two groups were: plasma cholesterol, 365 (±24) and 466 (±46) mg. per cent; aorta lesions, 90 and 90 per cent, grade 1.2 (±0.3) and 0.7 (±0.1); incidence of coronary lesions, 13 per cent (±3) and 14 per cent (±3).

_Effects of Substituting Polyunsaturated Oil for Saturated Fat in Cockerels Ingesting Cholesterol-Supplemented Mashes_  
Hypercholesterolemia and atherosclerosis were not significantly influenced by substitution of corn oil (10 per cent) for butter (10 per cent) during a three week period.

**DISCUSSION**  
These experiments have yielded overwhelmingly negative results. More precisely, hypercholesterolemia and atherosclerosis are similar in cholesterol-fed chicks ingesting unsaturated vs. saturated fats. Further, supplementation of a cholesterol-containing mash with large amounts of unsaturated oils rich in essential fatty acids (EFA) failed to suppress hypercholesterolemia and atherogenesis. Similarly transfer from a cholesterol mash of high saturated fat content (butter) to one of high EFA content (corn oil) failed to reduce hypercholesterolemia and atherogenesis. These negative results tend to refute speculation that atherosclerosis is a disease of essential fatty acid deficiency.\(^{11}\) The invalidity of this hypothesis is further indicated by the finding that chicks on diets supplemented with cholesterol and oil—with resultant hypercholesterolemia and atherogenesis—have high levels of polyunsaturated fatty acids in their sera and tissues.\(^{12,13}\)

The negative results of the present experiments in chicks are in apparent contrast to those in man.\(^{1-8}\) However, these seemingly disparate findings may not actually be contradictory. Rather, they may merely be a resultant of the differences in design of the human and animal experiments. Thus, most of the
human subjects were hypercholesterolemic individuals habituated to high-fat, high-saturated-fat, high-cholesterol diets. Their experimental regimens entailed substitution, partial or complete, of unsaturated oils for saturated fats, with an associated reduction in cholesterol intake. Under these circumstances, serum cholesterol levels declined significantly. In contrast, chick studies involved animals accustomed to low-fat, low-cholesterol rations. Prior to institution of the experimental regimes, these birds exhibited low normal serum cholesterol levels. Assessment of the comparative effects of unsaturated vs. saturated fats on cholesterolemia and atherogenesis was made with the birds continuously on a diet containing a constant high level of cholesterol. Under these circumstances, serum cholesterol levels did not decline with unsaturated oil ingestion. The experimental designs of these chick and human investigations are therefore quite different.

Data available from previous studies indicate that removal of cholesterol from experimental rations of chicks is associated with regression of hypercholesterolemia and atherosclerosis, even when the dietary fat intake remains high. These and other observations indicate that in this avian species dietary cholesterol is a key factor in effecting hypercholesterolemia and atherogenesis. In contrast, limited studies suggest that in man intake of saturated fat—not of cholesterol—is of decisive importance in influencing serum cholesterol level. However, the thesis that serum cholesterol level in man is not influenced by dietary cholesterol has a slim basis of experimental support. This problem therefore needs further exploration, especially in view of extensive data demonstrating the opposite in at least 10 animal species.

While differences in experimental design may account for the apparently disparate results of studies with different fats in man vs. chick, it must also be recognized that species differences may actually be basically involved. Further work is needed before accepting this conclusion, particularly in view of the limited data on the interrelated effects on cholesterolemia of fat-cholesterol ingestion in man.

Summary

Groups of cholesterol-fed cockerels ingesting mash supplemented with various unsaturated oils, and with various saturated fats (5 or 10 per cent) exhibited similar patterns of hypercholesterolemia and atherogenesis. Unsaturated oils failed to suppress hypercholesterolemia and atherogenesis. Cholesterol-fed cockerels ingesting mash supplemented with various fats and oils high in oleic acid tended to exhibit slightly (but not markedly) lower serum cholesterol levels. A group given oleic acid per se had less atherosclerosis, otherwise no differences were observed.

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It is a pleasure to acknowledge the contribution of the technical members of the department's atherosclerosis research team, Miss Mildred Michael, Mrs. Eva Miller, Mrs. Charlene Thompson, Mrs. Montrce Vankinseott and Mr. Grady Crowley.

Summary in Interlingua

Gruppos de gallettos recipiente dietas rie in cholesterol exhibiva simile formas de hypercholesterolemia e de atherogenese quando illos ingereva pastas supplementate per 5 o 10 pro cento de (1) varie non-saturate oleos e (2) varie grassias saturate. Le non-saturate oleos non suprimeva le hypercholesterolemia e le atherogenese. Quando gallettos recipiente dietas rie in cholesterol ingereva pastas supplementate con varie grassias e oleos a alte contento de acido oleic—i.e. alte in comparation con oleo de coton—illos tendeva a exhibir levemente (sed non mareatemente) plus basse
nivellos de cholesterol in le sero. Un grupo de gallettos recipiente acido oleic per se ha-beva minus atherosclerosis; alteremente nulle differentia esseva observate.

REFERENCES
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