# Hypercholesterolemic Effect of Menhaden Oil in the Presence of Dietary Cholesterol in Swine.*† (30183)

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Previous studies on the feeding of soybean phosphatides to swine(1) showed some relative reduction in plasma cholesterol in a short-term experiment. Since it was unclear whether this effect was transitory, and whether the result was due primarily to the unsaturated fats present in the phosphatides, a long-term study was initiated using both a soybean phosphatide supplement and a marine oil supplement to supply a source of highly unsaturated fatty acids.

Marine oils have been shown(2,3) to have depressing effects on blood and tissue cholesterol levels in short-term trials with rats, while in swine(4) it had been reported that unsaturated fats with cholesterol actually raised the blood and tissue levels of cholesterol somewhat. To resolve some of these questions a long-term (approximately one year) trial was conducted with miniature swine.

*Materials and methods.* Thirty male miniature swine approximately 8 weeks of age were obtained from The Hormel Foundation herd and were divided into 3 groups on the basis of body weight and litter number. The basal diet shown in Table I was fed so that all groups received the same number of calories per kg of body weight. After 2 weeks, supplements were added to the diets of 2 of the 3 groups. The supplements were added at the 3% level at the expense of the tallow in the diet. The first supplement, an alcohol-insoluble fraction of soybean lecithin,‡ contained approximately 66% inositol phosphatides, 33% chemical cephalin, and a trace of lecithin. The second supplement was fresh menhaden oil.§ All diets were fed at the same caloric level throughout the experiment. The pigs were weighed and blood samples were taken at 4-week intervals. The blood plasma samples were analyzed individually for total cholesterol by the Abel method(5).

TABLE I. Composition of Basal Diet.

| Ingredient | % of diet |
|---|---|
| Corn, ground | 15 |
| Oat groats | 30 |
| Soybean oil meal, solvent-extracted | 20 |
| Skim milk, dried | 10 |
| Fish solubles, dried | 2 |
| Salt | 0.5 |
| Mineral mix* | 1 |
| Steamed bone meal | 1 |
| Vitamin B mix† | 0.2 |
| Vitamins A and $D_2$‡ | 0.3 |
| Tallow, purified | 15 |
| Cerelose | 3 |
| Alphacel | 2 |

* Mineral mix contained 40.55% $CaHPO_4$, 41.9% $CaCO_3$, 13.7% NaCl, 1.4% $FeSO_4 \cdot 7H_2O$, 0.673% $MnSO_4$, 0.508% $FeCO_3$, 0.605% $ZnSO_4$, 0.0306% CuO, 0.0091% KI, and 0.0049% $CoCO_3$.

† Vitamin mix contained 2 g riboflavin, 4 g pantothenic acid, 9 g niacin and 10 g choline per lb.

‡ Supplied 6,740 I.U. of vit. A and 1,088 I.U. of vit. $D_2$ per kg of diet, as A and D feeding oil containing 2,250 I.U. of A and 400 I.U. of $D_2$ per g.

At the end of 16 weeks each of the 3 dietary groups was divided into 2 subgroups and 0.5% free cholesterol was added to the diet of one of the subgroups of each pair. At 48 weeks the experiment was terminated, the pigs were slaughtered, and blood and tissue samples were taken for analysis. Before slaughter, fecal samples were collected for total cholesterol analysis.

*Results.* Growth rates of the swine were essentially the same throughout the experiment. Average body weights at the end of 48 weeks on trial were 104.1 kg (range of 92-118 kg) with no significant differences between groups.

Blood plasma cholesterol values are shown in Fig. 1. Plasma cholesterol values were similar over the first 16 weeks, although the tallow control group was somewhat higher than the other two groups. However, after

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‡ Gliddex I, supplied by Glidden Co., through the courtesy of H. T. Iveson.

§ Marine Chemurgies, Inc., Moorehead City, N. C.

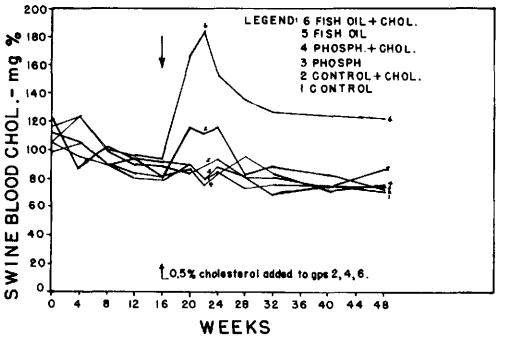


FIG. 1. Average swine plasma cholesterol as affected by dietary beef tallow, soybean phosphatides, and menhaden oil with and without dietary cholesterol.

addition of cholesterol to the diet, several marked differences appeared. All the subgroups that received no added cholesterol showed no essential change in plasma cholesterol. Similarly, the animals that received the 3% crude soybean phosphatides plus added cholesterol showed no change. On the other hand, the control group that received beef tallow and a supplement of cholesterol showed a significant, but not marked, increase in plasma cholesterol which was transitory, disappearing after 12 weeks. The pigs that received 3% menhaden oil with the cholesterol supplement showed a 100% increase. After this initial large increase there was a gradual decline, but average plasma cholesterol leveled off well above the normal value, and the elevated value persisted until the experiment ended, some 8 months after the first addition of cholesterol.

An explanation of these results may be found, in part, by the analyses shown in Table II of tissue and fecal cholesterol. The 3 dietary subgroups that received no supplemental cholesterol exhibited relatively low fecal cholesterol levels, although the value was somewhat higher for the group receiving the soybean phosphatides. The 3 subgroups that received the supplemental cholesterol exhibited higher values. The most important finding, however, was that the average fecal cholesterol in the group which received menhaden oil plus cholesterol was less than one-half that of the other 2 cholesterol-supplemented groups. The cholesterol values of heart and liver lipid showed that the menhaden oil-fed groups, regardless of supplementation with cholesterol, had much less cholesterol in the tissue lipids.

An examination of the hearts and aortas of these swine revealed no visible lesions of atherosclerosis in any of the animals. Fatty acid analyses of the heart and liver lipids showed only minor differences, with the exception that the lipids of the menhaden-fed pigs contained large amounts of highly unsaturated fatty acids (pentaenoic and hexaenoic acids), as would be expected.

*Discussion.* These findings suggest that the highly unsaturated fish oil facilitated absorption of cholesterol and that these unsaturated fatty acids may serve as mobilizers of the cholesterol to prevent a buildup of cholesterol in the heart and liver. Further, it is evident that only small amounts of these highly unsaturated fatty acids are effective, since only a small proportion (3% of a total of 15%) of the fat was of this nature.

These results are in contrast to many literature reports of the hypocholesterolemic action of fish oils, but in the studies reported here only a small portion of the total fat supplement was fish oil. Of the few reports with swine, Oldfield *et al*(6) reported slightly elevated levels of swine plasma cholesterol levels with menhaden oil in a shorter experi-

TABLE II. Total Cholesterol in Swine Tissues and Feces as Affected by Dietary Fats.

| Dietary groups | Heart (mg/100 mg lipid) | Liver (mg/100 mg lipid) | Feces (mg/100 g dry matter)* |
|---|---|---|---|
| 15% Tallow | 10.2 ± 1.30† | 13.7 ± .91 | 187 |
| *Idem* + .5% cholesterol | 12.8 ± 1.64 | 18.9 ± .93 | 1097 |
| 12% Tallow + 3% phosphatide | 12.6 ± 3.35 | 17.7 ± .95 | 340 |
| *Idem* + .5% cholesterol | 7.1 ± 2.14 | 11.2 ±2.54 | 1072 |
| 12% Tallow + 3% menhaden oil | 2.0 ± .69 | 4.7 ± .69 | 156 |
| *Idem* + .5% cholesterol | 3.6 ± .31 | 5.9 ± .38 | 498 |

* Analyses conducted on samples pooled by groups. † Standard error of mean.

ment. It is interesting to note that in numerous studies, egg yolk, which contains appreciable amounts of highly unsaturated fatty acids, and also an appreciable amount of cholesterol, has been found to cause an elevation of plasma cholesterol. One may speculate whether this effect may be related to the effect observed in this experiment, that is, whether absorption of cholesterol is facilitated by the content of highly unsaturated fatty acids.

*Summary.* Thirty male miniature swine were fed a beef tallow basal ration supplemented with crude soybean phosphatides and menhaden oil with and without 0.5% cholesterol for a period of 48 weeks. The animals that received no cholesterol supplement all maintained normal plasma cholesterol values (75-85 mg %). The swine fed soybean phosphatides plus cholesterol showed no change in plasma cholesterol values. The swine fed beef tallow showed on cholesterol supplementation a rise in plasma cholesterol, but returned to normal in 12 weeks. The swine fed menhaden oil showed a 100% increase in plasma cholesterol when supplemented with 0.5% cholesterol and, although this level decreased, it remained substantially above the remaining groups throughout the experiment. Fecal analyses of cholesterol showed much less cholesterol in the feces of fish oil-fed pigs supplemented with cholesterol, indicating that the fish oil (or the highly unsaturated fatty acids in fish oil) may have facilitated the absorption of the dietary cholesterol. The heart and liver analyses showed much lower levels of cholesterol than the other groups, suggesting that the polyunsaturated fatty acids of the fish oil may have aided in transport of the cholesterol to prevent an accumulation in these tissue sites.

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## Use of the Y Chromosome in the Wistar/Furth Rat as a Cellular Marker.* (30184)

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In connection with a long-term program of research on leukemogenesis in the rat and, in particular, in regard to studies presently underway concerning the viral induction of this disease it became essential to determine the normal chromosome constitution in the specific strain of rat under study, namely the Wistar/Furth inbred albino laboratory rat.

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The first detailed descriptions of the normal rat karyotype were reported by Tjio and Levan(1). Fitzgerald(2), using newer techniques, corroborated these earlier findings. These descriptions suggested a uniformity of chromosome constitution in the rat. However, with the recent publication of Hungerford and Nowell(3) concerning sex chromosome polymorphism in 3 strains of laboratory rat it became apparent that differences in chromosome constitution existed between different strains. Their description of variations of Y chromosomes among strains