The Effect of Dietary Fats on the Serum Lipoproteins of Normal Dogs[1] (35422)

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In previous communications (1, 2) this laboratory has demonstrated the remarkable effect of coconut oil on the serum lipids of normal dogs and dogs made hypothyroid by surgery. Total cholesterol, phospholipids, and triglycerides showed large increases in normal dogs while they were on a diet containing 40% of calories as coconut oil. Dogs made hypothyroid had much larger increases in total cholesterol than normal dogs but the increase in phospholipids and triglycerides was not as great, resulting in an excess of cholesterol over phospholipid. The present communication deals with the effect of a broader variety of dietary oils on serum lipoproteins of normal dogs. Density gradient fractionation of the serum was done to identify which of the cholesterol-bearing proteins is responsible for the increase in total serum lipids.

Methods. Adult male dogs were used for this study. They were maintained on a low fat diet for 2-week control periods at the beginning and the end of the dietary experiments. The basic low fat control diet consisted by weight of 10% dry skim milk, 40% meat,[3] 40% cornmeal, 6% sugar, 3% dried yeast, and 1% cod liver oil. This diet contains 4.6% of wet weight as fat, 13.7% as protein, and 39.7% as carbohydrate to conform with previous studies. The special diets were made by mixing 16 parts of oil (coconut, safflower, olive, or menhaden) with 84 parts of the low fat diet. While the protein and carbohydrate are reduced to 11.5 and 33%, respectively in the special diets there is still sufficient protein for the dog in such a diet. The experimental diets derived 40% of calories from fat. Coconut oil consists of 94% saturated fatty acids of which 57% are C_{12} and C_{14} fatty acids. Olive oil consists of 65% monunsaturated fatty acids primarily oleic. Safflower oil consists of 76% polyunsaturated fatty acids primarily linoleic, while menhaden oil consists of 37% saturated, 33% monunsaturated, and 29% polyunsaturated fatty acids.

The dogs were divided into four groups of four animals each and the diets were rotated through these four groups in such a way that during any 2-week period all four diets were being used by one or another of the groups. Dogs were maintained on each diet for 2 weeks. Blood was sampled after an overnight fast by drawing 35 ml from the femoral artery into a tube containing 1 mg of EDTA. Serum lipoproteins were separated by the technique of Havel *et al.* (3) except for some adjustments of density. The following fractions were separated by ultracentrifugation using the Spinco L2 65 B centrifuge:

1. The fraction of density less than 1.006 (very low density lipoprotein). This is the natural density of protein-free plasma.

2. The fraction of densities between 1.006 and 1.063 (low density lipoprotein). The serum was adjusted to density 1.063 to obtain this fraction.

3. The fraction of densities between 1.063 and 1.21 (high density lipoprotein). The serum was adjusted to density 1.21 to obtain this fraction.

The residual cholesterol-containing protein at a density greater than 1.21 was consid-

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[3] From meat manufactured by Federal Foods Inc., Thiensville, Wisconsin 53092.

ered to be the very high density lipoprotein and was not further fractionated. Chylomicrons were present in such low concentrations that they were not separated as such but would appear in the very low density fraction. Centrifugation was carried out for 18 hr at 50,000 rpm and 10° in the Spinco 65 rotor for the very low density and low density lipoproteins. The centrifugation to obtain the high density lipoproteins was done at 65,000 rpm. After completion of centrifugation the top cloudy fractions were passed though G50 Sephadex columns to remove salts from the protein. This was necessary because the salts interfered with the subsequent analysis. Normal saline was the eluent used in column chromatography.

Cholesterol was determined in each fraction by the procedure of Abel *et al.* (4), phospholipids were determined by the method of Youngburg and Youngburg (5), and protein was determined by the procedure of Lowry *et al.* (6) using bovine serum albumin suspended in isotonic saline as the standard. All of the results were calculated as milligrams per 100 ml referred back to the original serum. Disc electrophoresis using the Canalco "quick disc" method was done on a number of fractions to demonstrate the extent of purification of the lipoproteins separated by the centrifugation procedure. No modifications were made of the methods described in the Canalco literature. The standard error of the mean was calculated for each of the groups. The control period at the beginning and the end of the experiment was averaged for comparison with each of the dietary groups.

Results. Lipoprotein centrifugation. The values for protein, cholesterol, and phospholipids of the individual lipoprotein fractions are shown in Table I. As shown, the majority of the serum lipids in the normal dogs are carried in the high density or alpha lipoprotein fractions. The four experimental diets caused no changes in the relative con-

TABLE I. The Effect of Various Oils on Serum Lipoproteins in Normal Dogs.
Units: mg/100 ml ± SEM; $n = 16$.

	VLD[a]	LD	HD	Total serum protein (g/100 ml)	Total body wt
			Protein		
Control[b]	12.3 ± 1.9	45.6 ± 5.5	223 ± 9	7.82 ± 0.24	38.4 ± 2.3
Coconut oil	6.3 ± 0.6	95.5 ± 10.9[e]	246 ± 8	6.84 ± 0.29[e]	39.3 ± 1.4
Olive oil	6.4 ± 1.0	55.8 ± 8.5	220 ± 13	7.06 ± 0.27[f]	40.2 ± 1.4
Safflower oil	7.9 ± 0.7	48.9 ± 7.4	234 ± 15	7.47 ± 0.24	38.5 ± 1.6
Menhaden oil	9.2 ± 1.0	40.4 ± 7.3	209 ± 11	7.49 ± 0.22	36.3 ± 1.4
			Cholesterol		
				VHD	Total
Control	3.2 ± 0.4	27.9 ± 3.0	122 ± 6	20.5 ± 2.9	179 ± 9
Coconut oil	2.3 ± 0.4	81.2 ± 12.3[e]	117 ± 6	16.9 ± 2.9	286 ± 21[e]
Olive oil	2.6 ± 0.2	46.1 ± 7.6[f]	117 ± 7	21.5 ± 2.8	235 ± 14[d]
Safflower oil	3.0 ± 0.5	38.8 ± 6.2	104 ± 8	17.3 ± 1.5	238 ± 19[e]
Menhaden oil	3.9 ± 0.5	41.4 ± 7.1	92 ± 7[f]	19.4 ± 1.9	185 ± 15
			Phospholipid		
Control	6.3 ± 0.7	29.3 ± 2.5	196 ± 10	36.0 ± 1.5	318 ± 12
Coconut oil	6.7 ± 0.5	77.8 ± 10.2[e]	209 ± 7	40.9 ± 1.9	443 ± 21[e]
Olive oil	5.3 ± 0.6	48.1 ± 8.5[f]	186 ± 11	41.5 ± 4.1	392 ± 15[e]
Safflower oil	6.8 ± 0.6	50.3 ± 9.1[f]	202 ± 16	45.0 ± 3.7[f]	406 ± 20[e]
Menhaden oil	7.0 ± 0.6	43.2 ± 6.6	158 ± 11[e]	33.6 ± 2.0	239 ± 16

[a] VLD, LD, HD, VHD = Stand for very low density, low density, high density, and very high density lipoproteins (see text).

[b] Control is the mean of a series before and after experimental diets.

[c] Difference from control is significant, $p < 0.001$; [d] $p < 0.005$; [e] $p < 0.025$; [f] $p < 0.05$.

TABLE II. Cholesterol/Phospholipid Ratios in Various Lipoprotein Fractions.

	VLD[a]	LD	HD	Original
Control before diet	0.55 ± 0.03	0.80 ± 0.08	0.57 ± 0.03	0.57 ± 0.01
Coconut oil	0.36 ± 0.02	1.01 ± 0.04	0.56 ± 0.03	0.64 ± 0.01
Olive oil	0.58 ± 0.02	1.06 ± 0.08	0.61 ± 0.03	0.60 ± 0.02
Safflower oil	0.49 ± 0.03	0.93 ± 0.09	0.51 ± 0.02	0.59 ± 0.03
Menhaden oil	0.62 ± 0.02	1.07 ± 0.10	0.56 ± 0.03	0.54 ± 0.05
Control after diet	0.45 ± 0.01	0.94 ± 0.07	0.54 ± 0.01	0.54 ± 0.05

[a] See Table I for heading identification.

centration of protein, cholesterol, or phospholipid in the high density lipoprotein fraction with the exception of menhaden oil which produced a reduction of the phospholipids in this fraction. The high density protein and cholesterol contents were not significantly lowered with menhaden oil.

In contrast the low density lipoprotein fraction, which is the beta lipoprotein fraction, did demonstrate changes related to the diets. The cholesterol level in low density lipoproteins increased more than threefold with the coconut oil diet. Protein and phospholipid content in the low density fraction during the coconut oil diet increased about twofold. Olive oil caused a small increase in low density cholesterol and phospholipid while safflower oil caused only an increase in low density cholesterol and phospholipid. Menhaden oil had no significant effect in either reducing or increasing the protein or cholesterol in low density lipoproteins.

The very low density lipoprotein fraction which under these circumstances is not quantitatively a large fraction in a dog showed very little change in any of three categories, protein, cholesterol, and phospholipid.

Total serum protein measured by the Lowry technique showed a change in relation to the diets, that is, with the high fat diets, coconut and olive oil, the total serum protein tended to diminish. This change could be artifactual and possibly related to methodology. Total serum cholesterol showed the expected rise with coconut oil and was also somewhat higher than normal with olive oil and safflower oil. There were similar changes in total phospholipid.

The ratios of cholesterol to phospholipid are shown in Table II. This ratio is highest

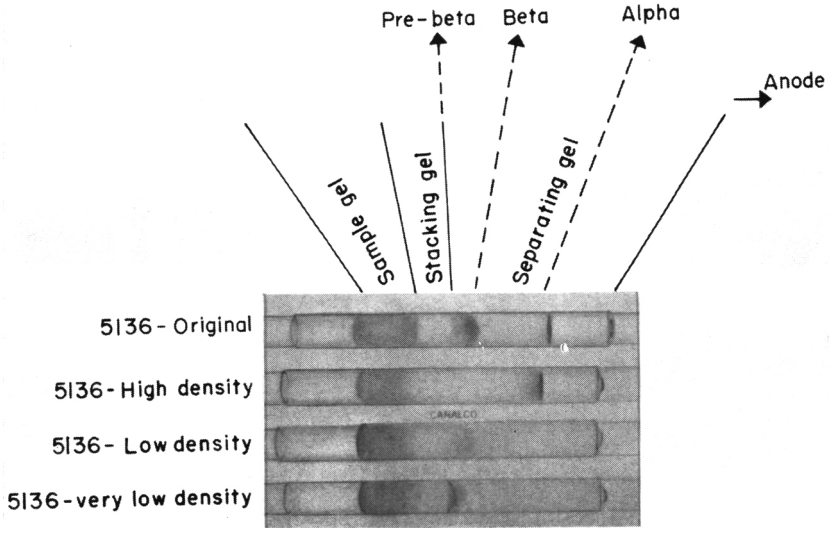

FIG. 1. The identification of various lipoproteins in dog serum after polyacrylamide gel electrophoresis: The top pattern is the original serum. HD refers to high density lipoproteins; LD refers to low density lipoproteins; VLD refers to very low density lipoproteins.

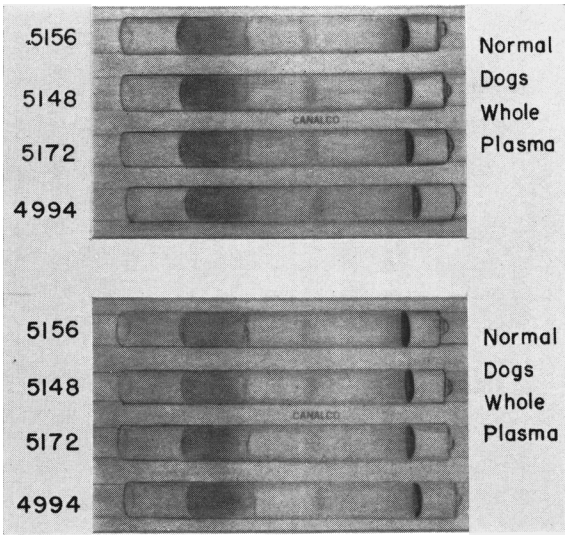

FIG. 2. Serum electrophoretic patterns of eight normal dogs on a low fat diet.

in the low density fraction as is the case in human serum. The very low density and high density fractions have about the same ratios as does the original serum. The diets seem to have very little effect on these ratios in normal dogs if one takes into account both control periods.

Electrophoresis of fractions. The patterns obtained with polyacrylamide gel electrophoresis of the dog lipoprotein fractions demonstrate types of migration similar to human lipoproteins. Figure 1 shows a pattern from a hypothyroid dog simply to demonstrate the completeness of the fractionation methods. Note that in this dog there is a pronounced beta lipoprotein band in the original serum. The high density lipoprotein migrates in the alpha position. The low density lipoprotein migrates in the beta position while the very low density lipoprotein (here the sum of chylomicrons and very low density lipoprotein) appears in the pre-beta[4] and chylomicron position. There is some heterogeneity in the patterns from the isolated fractions suggesting that absolutely discrete protein types are not separated by centrifugation.

Lipoprotein electrophoresis of the unfractionated serum in eight dogs on the control diet is shown in Fig. 2. They demonstrate that the major lipoprotein in the serum of the dog is the alpha lipoprotein. The reproducibility of the polyacrylamide technique is well shown. Figure 3 shows two examples of normal dogs on a coconut oil diet and the beta lipoprotein band seems to be mildly increased as would be expected from the quantitative data in Table I. There seems to be increased heterogeneity of the isolated high density lipoprotein fractions.

Discussion. The data demonstrate that a diet high in saturated fat causes an increase in beta lipoprotein in normal dogs. This is to be expected if the dog were to respond to such diets as man (7). Other diets containing safflower and olive oil caused small increases in total serum cholesterol and phospholipid; however, in the fractionation data after feeding these two oils, only olive oil showed a significant increase in beta lipoprotein cholesterol. Beta lipoprotein phospholipid was increased with both of the latter oils. Since our recovery of cholesterol and phospholipid in the fractions was about 80% compared to whole serum, some loss of sensitivity in detecting dietary changes with fractionation might be expected.

Dog lipoproteins have been studied by Havel *et al.* (3) using somewhat different density regions to obtain their fractions. Their fraction isolated at density greater

[4] Referring to conventional paper electrophoresis; in polyacrylamide gels this fraction is actually post beta.

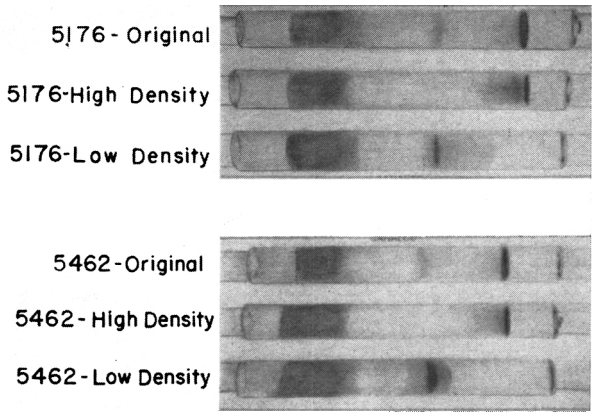

FIG. 3. Electrophoretic patterns of the original serum, high density and low density lipoproteins in two normal dogs on a coconut oil diet for 2 weeks. Enhancement of the beta lipoprotein can be seen.

than 1.063 is equivalent to our high density and very high density fractions combined. They found cholesterol and phospholipids of 127 and 325 mg/100 ml, respectively, in their highest density fraction which compares to 133 and 232 mg/100 ml for our series of control studies (adding the HD and VHD fractions). In their lowest density fractions (sum of the less than 1.019 and the 1.019–1.063 fraction) they found 12 and 29 mg/100 ml of cholesterol and phospholipid, respectively, compared to 31 and 35 mg/100 ml in our control series.

Grande *et al.* (8) studied total serum cholesterol and the percentage of cholesterol in alpha and beta lipoprotein in the dog. They observed on a low fat diet 157 and 40 mg/100 ml of cholesterol in alpha and beta lipoproteins, respectively. These are somewhat higher than our results by centrifugation as was their total serum cholesterol. Their method resulted in no losses since they simply took the percentage distributions of dye on paper electrophoretograms and obtained the absolute cholesterol content in the lipoprotein fractions by multiplication of the total. These investigators found that alcohol caused increases in both alpha and beta lipoprotein cholesterol.

Milch *et al.* (9) showed that the low density serum lipoproteins in dogs were strongly influenced by thyroidectomy. Their normal control dogs correlate well with our normal dogs relating to total cholesterol. They found somewhat less low density and somewhat more high density lipoprotein. Thyroid ablation by ^{131}I caused the low density fraction to increase over 10 times, while high density lipoproteins only increased 2 times.

Cornwall and Kruger (10) have pointed out that dog lipoproteins do not separate as cleanly by centrifugation as do the human lipoproteins which would appear to be the case from our electrophoresis data. Alpha lipoprotein as previously observed (3, 8) was the major cholesterol-bearing entity in dog plasma; however, it does not seem to undergo as pronounced change in response to high fat diets as do the low density or beta lipoprotein fractions.

One other difference between our data and those of Havel *et al.* (3) is the more pronounced variation in cholesterol/phospholipid ratios between density classes. These ratios were 0.50, 0.87, and 0.56 for the very low density, low density, and high density fractions, respectively. This is also more comparable to man than previously appreciated (10). From our data, it is difficult to see evidence of alteration of the cholesterol/phospholipid ratio by high fat diets in normal dogs.

The electrophoretic patterns in the dog show similar proteins to man and only the relative quantities seem to differ. These data are the first carefully controlled dietary experiment showing that in the normal dog, like man, the beta lipoprotein fraction is respon-

sive to saturated fat diets in spite of the fact that the major cholesterol-containing protein is the alpha lipoprotein. The ability to cause changes in the beta lipoprotein fraction may make the dog a useful experimental animal in atherosclerosis research. These results offer a base line for the next study of dietary effects on lipoprotein fractions in thyroidectomized dogs.

Summary. The serum lipoproteins of normal dogs were studied while on different dietary regimens including low fat and various high fat diets containing coconut, olive, safflower, or menhaden oil. The serum low density (beta lipoprotein) fraction was increased nearly threefold by feeding coconut oil in contrast to the other diets which, except for a small increase with olive oil, showed little effect on the lipoproteins. High density lipoproteins, while constituting the major fraction in dogs, did not respond to the diets. Reasonably good separation into alpha and beta lipoprotein by ultracentrifugation was demonstrated by electrophoresis of the fractions on polyacrylamide gels.

The authors are indebted to Mary Ann Strauss and Laura Werner for their technical assistance in this project.

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Received July 21, 1970. P.S.E.B.M., 1971, Vol. 136.