Effects of dietary fat composition on the Ehrlich ascites tumor fluid lipoproteins

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Abstract Mice bearing the Ehrlich ascites tumor were fed diets rich in either coconut oil or sunflower oil. From 20 to 40% less lipid was present in the ascites tumor fluid when the mice were fed the sunflower oil diet. This was associated with a reduction in the amount of very low density lipoproteins (VLDL) and high density lipoproteins (HDL), the main lipoprotein fractions present in the ascites tumor fluid. The VLDL from the mice fed sunflower oil contained more cholesteryl esters and a lower free to esterified cholesterol ratio than those from the mice fed coconut oil. Very little change occurred in the composition of the HDL. All of the lipids contained in both lipoprotein fractions exhibited appreciable differences in fatty acid composition. Much more monoenoic and less polyenoic fatty acid were present in the lipids from the mice fed the coconut oil diet, but no appreciable change in saturated fatty acid content occurred. Similar changes in fatty acid composition were observed in the blood plasma of the tumor-bearing mice. There was no qualitative difference in the apolipoprotein patterns of either the ascites fluid VLDL or HDL. Pyrene fluorescence studies indicated that the fluidity of the VLDL was increased when the mice were fed the sunflower oil diets. No difference in HDL fluidity, however, was observed by this technique. These results indicate that the amount, composition, and physical properties of certain of the lipoproteins contained in the ascites tumor fluid can be modified by changing the composition of the dietary fat fed to mice bearing the Ehrlich ascites tumor.

Supplementary key words cancer · pyrene · fluorescence · very low density lipoproteins · high density lipoproteins · phospholipids · triacylglycerol · cholesterol · apolipoproteins · saturated and polyunsaturated fats

Lipoproteins are contained in the fluid in which Ehrlich ascites tumor cells are suspended in the mouse peritoneal cavity (1). These lipoproteins have been separated into three electrophoretically distinct fractions using a combination of preparative ultracentrifugation and agarose column chromatography (2). The very low density lipoprotein (VLDL) fraction, which accounts for only about 15% of the ascites fluid apolipoprotein content, contains more than half of the ascites lipids. A distinct low density lipoprotein (LDL) fraction also can be isolated from the ascites fluid, but it is present in only very small amounts (2). The high density lipoprotein (HDL) fraction contains about 75% of the ascites apolipoprotein content and 30% of the lipoprotein lipids (2). Although conclusive evidence concerning the origin of the ascites fluid lipoproteins has not as yet been obtained, all of the available data suggest that they are derived from the blood of the tumor-bearing mouse and not from the tumor cells themselves (1, 2).

Recent studies indicate that the fatty acid composition of the Ehrlich ascites tumor cell lipids can be modified extensively by feeding the tumor-bearing mice different dietary fats (3, 4). This is associated with a change in the cholesteryl ester content of the cells (5). As part of a continuing study of the interaction between the ascites lipids and the tumor cells (6), we wished to determine whether these diet-induced cellular changes might be associated with similar changes in the structure and properties of the ascites fluid lipoproteins. The present results indicate that the amount and lipid composition of certain of the ascites tumor fluid lipoproteins can be altered by changing the composition of the fat fed to the tumor-bearing mice. In the case of the VLDL, these changes are associated with differences in fluidity as estimated by pyrene fluorescence measurements (7).

MATERIALS AND METHODS

Diets and tumor growth

Weanling male CBA mice were fed a semisynthetic diet containing 26% casein, 10% corn starch, 43% sucrose, 4% mineral mixture, and 1% vitamin mixture (Teklad, Madison, WI). This diet was supplemented with either 16% sunflower oil (89% unsaturated fatty acids) or 16% coconut oil (6% unsaturated fatty acids). The fatty acid composition of these diets is presented

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Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; I_E, pyrene excimer fluorescence intensity; I_M, pyrene monomer fluorescence intensity.

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in **Table 1.** Mice were fed these experimental diets for 4 weeks prior to intraperitoneal transplantation of 4×10^6 Ehrlich cells. The experimental diet was continued during tumor growth, and the Ehrlich cells were harvested 14 days after transplantation.

TABLE 1. Fatty acid composition of coconut and sunflower oil diets

Fatty Acid[a]	Composition	
	Coconut	Sunflower
	%	
8:0	5.6	
10:0	6.8	
12:0	52.0	
14:0	19.0	0.1
16:0	8.5	7.0
18:0	1.5	4.0
18:1	4.9	17.1
18:2	1.3	58.5
18:3		12.1
20:4		1.3

[a] Abbreviated as chain length: number of double bonds.

Plasma and lipoprotein preparation

The ascites fluid was separated from the cells by centrifugation at 700 *g* for 5 min (2). Chylomicrons were removed from the fluid by flotation at 20,000 *g* for 30 min. In most cases, there was little or no material recovered in this fraction. The remaining lipoproteins were isolated by preparative ultracentrifugation into three fractions: VLDL, d < 1.006 g/ml, LDL, d 1.006–1.063 g/ml, and HDL, d 1.063–1.21 g/ml. Each of the three lipoprotein fractions was washed twice by flotation through a salt solution of appropriate density and then dialyzed against 0.15 M NaCl containing 1 mM EDTA and 1 mM NaN_3, pH 7.5. After dialysis the lipoprotein fractions were concentrated from 15 to about 0.3 ml using Centriflo membrane ultrafilters (Amicon). The membranes then were washed 10 times with 0.1 ml of the NaCl solution to insure complete removal of the lipoproteins from the membranes; the washings were combined and the final volume of the lipoprotein solution was made up to 1.5 ml. The purity of the lipoprotein fractions was established by electrophoresis and agarose column chromatography (2). Protein was measured by the method of Lowry et al. (8) using bovine serum albumin as the standard.

Blood was collected from the tumor-bearing mice on day 14 after tumor transplantation by heart puncture using a heparin-coated syringe. The blood plasma was separated from the cells by centrifugation at 700 *g* for 5 min.

Lipoprotein analysis

Tetramethylurea-soluble apoproteins were estimated according to the method of Kane (9). Sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis was done as described by Laemmli using 15% gels (10). Gels were stained with Brilliant Blue R (Sigma Chemical Co., St. Louis, MO) according to the procedure of Weber and Osborn (11). The gels were destained by 4–5 changes of 7% acetic acid–5% methanol solution at 45°C (2).

Lipids were extracted from the ascites fluid and lipoprotein fractions with 20 volumes of chloroform–methanol 2:1 (v/v) (12). The chloroform phase was isolated after addition of 4 ml of 0.04 N HCl, collected and evaporated under nitrogen. The lipid residue was dissolved in 10 ml of chloroform–methanol 2:1 and aliquots were taken for the various lipid analyses. Triacylglycerol and cholesterol were measured by the Technicon Autoanalyzer II method (13). Phospholipids were assayed by the method of Raheja et al. (14).

Additional aliquots of the lipid extracts from the intact ascites fluid and the isolated VLDL and HDL fractions were taken for fatty acid analysis. The lipids were separated on silica gel thin-layer plates (Anasil G; Analabs, Inc., North Haven, CT) using a solvent system containing hexane–diethyl ether–methanol–acetic acid 180:40:4:6 (v/v) (15). The phospholipids contained in the lipid extracts were separated on different chromatograms using sodium carbonate-containing silica gel plates and a solvent system of chloroform–methanol–acetic acid–water 100:60:16:8 (v/v) (16). Standards obtained from Nu Check Prep (Elysian, MN) were run simultaneously on each chromatogram. Only the standard lane was exposed to iodine vapor in order to visualize the lipids. The segments of the silica gel plates corresponding to the location of the various lipid standards were scraped and eluted with a chloroform–methanol mixture (14). Methyl esters of the fatty acids contained in each of the eluted lipid fractions were prepared using boron trifluoride–methanol reagent (17). The fatty acid methyl esters were separated by gas–liquid chromatography using a 4-mm ID column containing SILAR-10 C on 100/200 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, PA). A 5710 A Hewlett Packard gas chromatograph with temperature programming from 180 to 210°C was employed for the separation. Peak areas were obtained with a Hewlett Packard 3380 A integrator.

Pyrene fluorescence

Pyrene (Aldrich Chemical Co., Milwaukee, WI) was incorporated into the isolated VLDL and HDL frac-

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tions. A stock solution was prepared in ethanol containing 0.5 mg/ml of pyrene. A Hamilton syringe was used to add 0.2 ml of this solution to 10 ml of a solution containing 0.15 M NaCl, 1 mM EDTA, and 1 mM NaN_3, pH 7.5. A Vortex mixer was used to agitate the aqueous solution vigorously during addition of the pyrene. The lipoprotein solutions were adjusted so that their absorbance at 335 nm was approximately 0.8 in a cuvette having a 1-cm light path, and 1.5 ml of the lipoprotein solution was mixed with 1.5 ml of the diluted pyrene solution. The final concentration of the pyrene in these lipoprotein solutions was 5 μg/ml, and the absorbance of these solutions at 335 nm in a cuvette having a 1-cm light path was about 1.0. Fluorescence measurements were made in quartz cuvettes containing 3 ml of this lipoprotein solution. The cuvette was held in a thermostated cell compartment (±0.5°C) of a MPF-2A Hitachi-Perkin-Elmer fluorescence spectrophotometer. Fluorescence spectra were recorded from 0 to 50°C at 2°C intervals. At each temperature, 15 min was allowed for thermal equilibrium of the sample. An excitation wavelength of 335 nm was used for all measurements. The excitation slit width was 2 nm, and the emission slit width was between 2 and 3 nm. Spectra were recorded between 360 and 600 nm. The pyrene excimer fluorescence intensity (I_E) at 475 nm and monomer fluorescence intensity (I_M) at 392 nm were determined by measuring the peak heights at these two wavelengths (7).

RESULTS

Ascites tumor fluid lipid concentrations

Table 2 gives the lipid concentrations of the ascites tumor fluid obtained from mice fed the diet containing either coconut or sunflower oil. In the first experiment, the analysis was made in ten individual animals on each diet on the 14th day after tumor transplantation. The concentrations of cholesterol, triacylglycerol, and phospholipids were 20–40% lower in the mice fed sunflower oil. There was no significant difference in the total volume of the tumor in the mice fed coconut or sunflower oil, 3.54 ± 0.45 ml as compared with 3.12 ± 0.29 ml ($P > 0.1$, n = 11). Likewise, there was no significant difference in the volume of the ascites fluid portion of the tumor, 1.46 ± 0.20 ml as compared with 1.21 ± 0.16 ml ($P > 0.1$, n = 11). Therefore, the differences observed in the ascites fluid lipid concentrations between the two dietary groups were not due to differences in the volumes of the tumors.

For comparison, the concentrations of these lipids in the blood plasma of the tumor-bearing mice also were measured. The same pattern was exhibited in that the values were 17–33% lower in the mice fed sunflower oil. Because of the difficulties in obtaining sufficient amounts of blood from the tumor-bearing mice, it was necessary to pool the blood from all of the mice on each of the diets, and only a single value was obtained. The concentration of each of the lipids was considerably higher in the blood plasma than in the tumor fluid, a finding that is in agreement with our previous observations (1).

The tumor growth already had reached the stationary phase on day 14 when the above differences were observed. We wished to determine whether similar differences would occur in mice fed these diets while the tumor was still in the logarithmic phase of growth. In our subline of the Ehrlich ascites tumor, it is not possible to obtain enough tumor fluid for extensive lipid analysis before day 10. Therefore, we

TABLE 2. Effect of dietary fat on the lipid concentrations in the ascites tumor fluid and blood plasma

Experiment	Sample	Days After Transplantation	n	Ascites Fluid Lipids[a] Cholesterol Coconut[b]	Cholesterol Sunflower[b]	Triacylglycerol Coconut[b]	Triacylglycerol Sunflower[b]	Phospholipids Coconut[b]	Phospholipids Sunflower[b]
				mg/dl					
1	Ascites fluid	14	10	102 ± 4	79 ± 4[c]	241 ± 25	140 ± 20[d]	128 ± 5	95 ± 6[c]
2	Blood plasma	14	—[e]	173	128	795	530	345	286
3	Ascites fluid	10	4	109 ± 10	77 ± 4	402 ± 38	361 ± 48	94 ± 13	46 ± 9
		12	4	116 ± 34	92 ± 5	396 ± 66	263 ± 39	114 ± 13	50 ± 10
		16	5	134 ± 12	99 ± 3	298 ± 25	229 ± 21	129 ± 13	96 ± 16

[a] Each value is the mean ± SE. A statistical analysis was performed only in Experiment 1 where 10 animals on each diet were tested.
[b] Fat present in the diet.
[c] The difference between the mean values for the fluid from the animals fed coconut and sunflower oil is significant at the $P < 0.001$ level.
[d] The difference between the mean values for the fluid from the animals fed coconut and sunflower oil is significant at the $P < 0.01$ level.
[e] The blood plasma values were obtained from a single pooled sample from 11 mice on each of the diets.

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designed a third experiment in which groups of mice were sampled on days 10 and 12, when the tumor is rapidly growing, and on day 16 when it is stationary. Although the actual lipid concentrations varied somewhat, the overall result was the same as in the first experiment. On each day, lower lipid concentrations were present in the ascites tumor fluid of the mice fed sunflower oil as compared with coconut oil.

Ascites tumor plasma lipoproteins

As suggested by the data in Table 2, we routinely recovered more lipoprotein material from the ascites fluid of the mice fed coconut oil as compared with sunflower oil. This was confirmed by measurement of the individual lipoprotein fractions isolated from the ascites fluid. In the VLDL fraction, we recovered 13.7 mg protein/100 ml of original ascites fluid from the mice fed coconut oil but only 5.8 mg protein/100 ml from those fed sunflower oil, a 58% reduction. Likewise, for the HDL fraction, we recovered 60.7 mg protein/100 ml in the mice fed coconut oil and 48.9 mg protein/100 ml from those fed sunflower oil, a 19% reduction. An insufficient amount of LDL was recovered from these mice to accurately determine either the protein or lipid contents of this fraction.

Table 3 shows the lipid composition of the VLDL and HDL fractions obtained from the ascites tumor fluid. The lipid values are expressed relative to the protein content of each lipoprotein fraction. Although differences in the triglyceride content were observed in the VLDL, they were not statistically significant. There was, however, an increase in the cholesteryl ester content of the VLDL in the mice fed sunflower oil, and the free to esterified cholesterol ratio of these VLDL was lower. The lipid composition of the HDL was not appreciably altered by the diets. Although the free to esterified cholesterol ratio was somewhat lower in the HDL from mice fed coconut oil, the total cholesterol content was almost the same in both of the HDL samples.

Fatty acid composition

Considerable differences were noted in the fatty acid composition of ascites fluid lipids when the mice fed the coconut oil diet were compared with those fed the sunflower oil diet. **Table 4** shows the cholesteryl ester fatty acid composition. In the mice fed coconut oil, the cholesteryl esters of the intact ascites fluid contained more monoenoic and less polyenoic fatty acids. These differences were accounted for primarily by changes in the 18:1 and 18:2 contents.[1] Qualitatively similar results were observed for the cholesteryl esters of the isolated VLDL and HDL fractions. With both diets, the HDL cholesteryl esters contained much more polyenoic fatty acid than the cholesteryl esters in VLDL.

Table 5 shows the fatty acid composition of the triacylglycerols. Again, the main difference was an increase in monoenoic and a decrease in polyenoic fatty acids in the ascites fluid VLDL and HDL of the mice fed the coconut oil diet. This difference also was accounted for primarily by changes in the 18:1 and 18:2 contents. As opposed to the cholesteryl esters, the fatty acid composition of the triacylglycerols was quite similar in VLDL and HDL on each of the diets.

As shown in **Table 6,** similar results were obtained for phospholipids. The values for the individual phospholipid classes of the intact ascites tumor fluid are

[1] Fatty acids are abbreviated as chain length: number of double bonds.

TABLE 3. Effect of dietary fat on the composition of the ascites tumor fluid lipoproteins[a]

Lipoprotein Class	Dietary Fat	Lipoprotein Lipids[b]: Phospholipids	Triacylglycerol	Cholesterol: Free	Esterified[c]	Ratio F/E[d]
		μmol P/mg protein	*mg/mg protein*	*mg/mg protein*		
VLDL	Coconut oil	4.75 ± 1.1 (6)	15.8 ± 2.4 (6)	2.67 ± 0.2 (4)	1.11 ± 0.1[e] (4)	2.40
	Sunflower oil	4.70 ± 1.7 (5)	10.6 ± 3.6 (4)	3.40 ± 1.0 (3)	3.04 ± 0.5 (3)	1.10
HDL	Coconut oil	0.59 ± 0.06 (5)	0.09 ± 0.01 (6)	0.12 ± 0.01 (4)	0.35 ± 0.05 (4)	0.34
	Sunflower oil	0.58 ± 0.06 (5)	0.07 ± 0.01 (6)	0.16 ± 0.02 (4)	0.28 ± 0.02 (4)	0.57

[a] The lipoproteins were isolated from ascites fluid obtained 14 days after tumor transplantation.
[b] Each value is the mean ± SE of the number of separate measurements listed in parentheses
[c] Values are expressed as mg of the cholesterol component of the cholesteryl ester.
[d] Ratio in mg/mg of free to esterified cholesterol.
[e] Significantly different from the value for cholesteryl ester content of the VLDL from the mice fed sunflower oil ($P < 0.05$).

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TABLE 4. Fatty acid percentage composition of cholesteryl esters[a]

Fatty Acid	Ascites Fluid		VLDL		HDL	
	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]
			%			
Saturation class						
Saturated	16	14	18	26	13	11
Monoenoic	31	15	46	26	32	8
Polyenoic	53	70	36	49	56	82
Major individual acids						
14:0	2 ± 0.7	3 ± 1.5	3 ± 0.5	4 ± 1.0	2 ± 0.4	2 ± 0.4
16:0	8 ± 0.8	6 ± 0.2	10 ± 0.2	10 ± 1.5	8 ± 1.0	6 ± 0.4
16:1	8 ± 0.5	5 ± 0.9	10 ± 1.3	9 ± 0.6	9 ± 1.0	2 ± 0.2
18:0	4 ± 0.1	4 ± 0.1	3 ± 0.2	9 ± 0.9	2 ± 0.7	2 ± 0.2
18:1	19 ± 3.2	9 ± 0.8	31 ± 0.2	14 ± 0.7	21 ± 1.1	5 ± 0.1
18:2	19 ± 1.9	29 ± 3.5	15 ± 0.8	22 ± 1.5	28 ± 1.0	43 ± 2.4
20:4	18 ± 0.4	22 ± 3.4	16 ± 3.0	15 ± 2.3	23 ± 2.1	28 ± 1.9
22:4	6 ± 0.2	8 ± 0.2	T[c]	T	T	T
22:6	4 ± 1.1	5 ± 0.8	1 ± 0.3	7 ± 1.4	2 ± 0.1	4 ± 0.6

[a] Mean ± SE of four samples.
[b] Dietary fat.
[c] Less than 1% of fatty acids.

shown in **Table 7.** In each case, there were more monoenoic and less polyenoic fatty acids when the mice were fed coconut oil, these changes being most pronounced in the choline phosphoglycerides.

In all of the lipid classes and phospholipid fractions, the coconut oil and sunflower oil diets produced very little difference in saturated fatty acid content. Moreover, medium-chain fatty acids did not accumulate in any of the lipids as a result of feeding the coconut oil diet.

For comparison, the fatty acid compositions of the lipids contained in the blood plasma of the tumor-bearing mice were determined. These results are shown in **Table 8.** Because of the small quantities of blood plasma that could be obtained from the tumor-bearing mice, these values are for single pooled samples from 11 animals in each dietary group. The results are basically similar to those obtained in the tumor fluid (Tables 4–6). The coconut oil diet produced large increases in the monoenoic fatty acid content and decreases in the polyenoic fatty acid content. Some differences in the absolute amounts of several of the acids, particularly 18:2 and 20:4, were noted in comparing the blood plasma and ascites tumor lipids. The most marked differences in this regard were seen in the cholesteryl esters.

TABLE 5. Fatty acid percentage composition of triacylglycerols[a]

Fatty Acid	Ascites Fluid		VLDL		HDL	
	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]
			%			
Saturation class						
Saturated	31	32	31	22	32	35
Monoenoic	61	29	60	28	55	29
Polyenoic	9	39	10	50	14	37
Major individual acids						
14:0	3 ± 2.0	3 ± 2.0	4 ± 0.7	T[c]	2 ± 0.7	2 ± 0.3
16:0	23 ± 1.6	20 ± 0.4	23 ± 0.6	16 ± 1.7	18 ± 0.1	24 ± 0.8
16:1	7 ± 0.3	6 ± 0.8	7 ± 0.5	3 ± 0.2	8 ± 0.3	6 ± 0.6
18:0	3 ± 0.1	8 ± 1.8	2 ± 0.2	4 ± 0.2	8 ± 0.7	8 ± 0.8
18:1	49 ± 2.1	22 ± 4.0	47 ± 2.2	21 ± 2.5	38 ± 2.7	22 ± 1.3
18:2	6 ± 0.5	34 ± 4.0	7 ± 0.7	36 ± 1.6	7 ± 0.7	25 ± 1.8
20:4	2 ± 0.4	T	2 ± 0.3	2 ± 0.2	2 ± 0.3	
22:4	T	T	1 ± 0.2	3 ± 1.0		3 ± 1.5
22:6	T	T	T	3 ± 1.3	2 ± 0.5	2 ± 0.7

[a] Mean ± SE of four samples.
[b] Dietary fat.
[c] Less than 1% of fatty acids.

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TABLE 6. Fatty acid percentage composition of total phospholipids[a]

Fatty Acid	Ascites Fluid		VLDL		HDL	
	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]
			%			
Saturation class						
Saturated	46	48	48	47	46	49
Monoenoic	29	14	29	15	31	12
Polyenoic	25	38	23	38	23	39
Major individual acids						
16:0	22 ± 1.4	21 ± 2.8	24 ± 0.4	19 ± 1.4	21 ± 1.8	20 ± 1.5
16:1	3 ± 0.2	T[c]	2 ± 0.2	2 ± 0.4	2 ± 0.2	2 ± 0.1
18:0	18 ± 1.7	21 ± 2.2	20 ± 1.1	24 ± 1.1	21 ± 1.8	25 ± 1.1
18:1	23 ± 2.0	13 ± 1.4	25 ± 1.5	12 ± 1.0	26 ± 1.2	9 ± 0.5
18:2	11 ± 0.6	17 ± 1.0	11 ± 0.3	22 ± 1.1	11 ± 0.9	20 ± 0.3
20:4	7 ± 1.0	9 ± 0.8	7 ± 0.5	8 ± 1.6	7 ± 7.5	9 ± 1.2
22:6	4 ± 0.2	8 ± 0.8	3 ± 0.2	5 ± 1.1	3 ± 0.8	6 ± 0.9

[a] Mean ± SE of four samples.
[b] Dietary fat.
[c] Less than 1% of fatty acids.

Apolipoprotein composition

As shown in **Fig. 1,** there were no qualitative differences in the sodium dodecylsulfate–acrylamide gel electrophoretic patterns of either the ascites tumor fluid VLDL or HDL fractions obtained from mice fed the coconut oil or sunflower oil diets. All of the nine major electrophoretic bands seen previously in these lipoprotein fractions when the tumor-bearing mice were fed regular mouse chow (2) were observed in the present samples. Although the possibility of quantitative differences is suggested by variations in the intensity of staining of several of the bands, techniques are not available at present to adequately explore this possibility for mouse ascites tumor plasma apolipoproteins. Additional studies with the VLDL indicated that 61 ± 5% of their apoprotein content was soluble in tetramethylurea (9) when the mice were fed coconut oil and 51 ± 6% when they were fed sunflower oil (mean ± SE of four determinations).

Pyrene fluorescence

The foregoing data indicate that the coconut and sunflower oil diets produced considerable differences in the fatty acid composition of the lipoprotein lipids and some changes in the lipid composition of the VLDL. We wished to determine whether these changes might be associated with alterations in the physical properties of the ascites tumor fluid lipopro-

TABLE 7. Fatty acid percentage composition of the various phospholipids in the intact ascites fluid[a]

Fatty Acids	Ethanolamine Phosphoglycerides		Serine and Inositol Phosphoglycerides		Choline Phosphoglycerides		Sphingomyelin	
	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]
				%				
Saturation class								
Saturated	43	38	66	57	46	47	60	57
Monoenoic	30	17	21	13	37	8	33	25
Polyenoic	27	45	12	30	17	45	8	18
Major individual acids								
14:0	3 ± 0.2	2 ± 0.4	4 ± 0.9	3 ± 0.9	T[c]	T	4 ± 0.7	4 ± 0.9
16:0	19 ± 1.8	16 ± 0.4	21 ± 1.5	15 ± 1.5	24 ± 0.2	22 ± 0.5	28 ± 1.2	31 ± 0.2
16:1	4 ± 0.1	3 ± 0.2	4 ± 0.6	3 ± 0.8	2 ± 0.05	1 ± 0.1	8 ± 0.9	6 ± 2.0
18:0	17 ± 0.3	17 ± 0.7	34 ± 4.0	36 ± 1.7	18 ± 0.2	24 ± 0.4	23 ± 2.4	16 ± 1.5
18:1	23 ± 0.7	12 ± 1.0	15 ± 0.9	9 ± 0.9	31 ± 0.6	7 ± 0.3	22 ± 0.4	16 ± 3.0
18:2	5 ± 0.03	12 ± 0.8	2 ± 0.2	7 ± 0.4	10 ± 0.4	28 ± 1.1	5 ± 0.6	14 ± 2.3
20:4	14 ± 0.4	14 ± 1.2	9 ± 0.2	18 ± 2.9	4 ± 0.1	9 ± 0.8	2 ± 0.2	2 ± 0.6
22:6	4 ± 0.2	11 ± 1.5	T	3 ± 0.9	1 ± 0.1	7 ± 0.8	T	T

[a] Mean ± SE of four determinations.
[b] Dietary fat.
[c] Less than 1% of fatty acids.

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TABLE 8. Fatty acid percentage composition of blood plasma lipids of tumor-bearing mice[a]

Fatty Acids	Cholesteryl Esters		Triacylglycerol		Phospholipids	
	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]	Coconut[b]	Sunflower[b]
			%			
Saturation class						
Saturated	14	12	23	22	43	44
Monoenoic	58	14	57	23	37	6
Polyenoic	28	73	20	56	20	50
Major individual acids						
14:0	1.3	1.2	2.2	0.9	0.4	0.3
16:0	9.9	6.7	18.6	18.4	25.2	24.2
16:1	10.3	2.0	6.9	3.4	3.5	
18:0	1.9	4.0	1.2	2.4	16.8	18.6
18:1	43.9	11.7	46.6	19.6	32.0	5.5
18:2	8.9	38.9	14.2	46.7	9.6	28.9
20:4	17.5	26.7	4.6	2.8	10.4	11.4
22:4			0.2	0.1		0.1
22:6		5.3		1.4		8.8

[a] The values were obtained on single samples of blood plasma pooled from 11 mice in each of the dietary groups.
[b] Dietary fat.

teins. The pyrene fluorescence method was employed in an attempt to assess the fluidity of these lipoproteins (7). This technique is based on the assumption that the translational diffusion rate of pyrene is dependent on the viscosity of the system. **Fig. 2** shows typical emission spectra of pyrene intercalated into the ascites VLDL and HDL obtained from mice fed either the coconut oil or the sunflower oil diet. A prominent peak at 470 nm was observed in both of the VLDL samples but not in either of the HDL samples. Both samples, however, exhibited peaks at 390 nm.

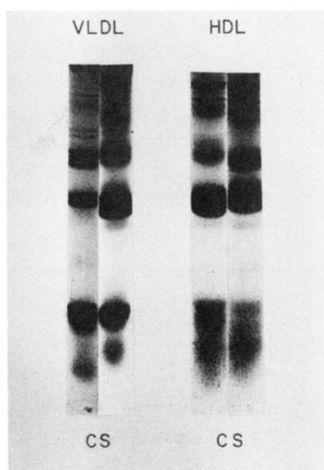

Fig. 1. Sodium dodecylsulfate–acrylamide gel electrophoretic patterns of the VLDL and HDL apolipoproteins. The samples were run in 15% gels and were stained with Brilliant Blue R (2). *C* refers to samples obtained from the mice fed coconut oil, *S* to samples from the mice fed sunflower oil.

Fig. 3 shows the excimer (I_E) to monomer (I_M) pyrene fluorescence intensity ratio for the VLDL plotted as a function of temperature. The slopes of these curves provide information about the microviscosity of the system. The greater the slope of I_E/I_M as a

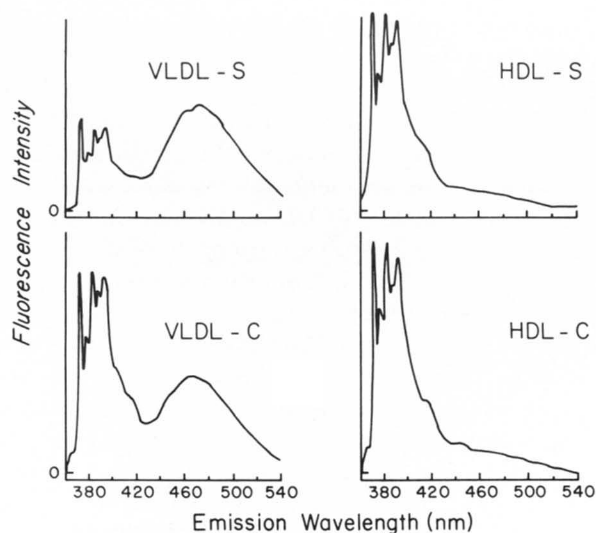

Fig. 2. Pyrene fluorescence emission spectra of the ascites tumor fluid VLDL and HDL obtained from mice fed the coconut oil (*C*) and sunflower oil (*S*) diets. Each of the isolated VLDL and HDL samples was diluted with a solution containing 0.15 M NaCl, 1 mM EDTA, and 1 mM NaN_3, pH 7.5, so that the absorbance at 335 nm was 0.8 in a cuvette having a 1-cm light path. A 1.5-ml aliquot of the lipoprotein solution was mixed with 1.5 ml of a pyrene solution so that the final concentration of pyrene was 5 μg/ml and the ratio of pyrene to lipoprotein was approximately equal in each case. The samples were excited at 335 nm, and the fluorescence intensity was recorded between 360 and 540 nm. These spectra were obtained at 22°C.

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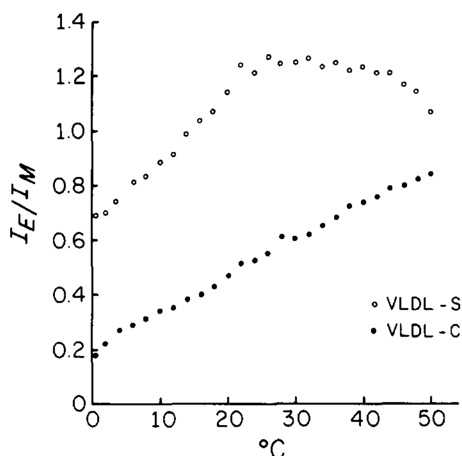

Fig. 3. Excimer (I_E) to monomer (I_M) fluorescence intensity ratio of pyrene in VLDL as a function of temperature. The VLDL were obtained from mice fed the coconut oil (*C*) or the sunflower oil (*S*) diets. The experimental conditions were the same as described in Fig. 2, except that the fluorescence spectra were measured at 2°C intervals between 0° and 50°C.

function of temperature, the larger the increase in fluidity (7). Differences were noted in this plot when the VLDL obtained from the mice fed coconut oil were compared with those from the mice fed sunflower oil. These VLDL were isolated from pooled samples of ascites plasma obtained from six mice bearing the Ehrlich ascites tumor. With the VLDL from the mice fed coconut oil, an approximately linear plot was obtained over the entire temperature range, 0–50°C. By contrast, a biphasic plot was obtained with the VLDL from the mice fed sunflower oil. A linear increase was observed between 0°C and about 24°C. The slope of this segment, 0.023, was considerably greater than that for VLDL obtained from the mice fed coconut oil, 0.014. The ratio of these slopes, 1.65, indicates that the VLDL from the mice fed sunflower oil have a greater fluidity. Above 24°C, the I_E/I_M ratio for the VLDL obtained from the mice fed sunflower oil became horizontal, and it decreased as the temperature was raised above 40°C. At each temperature, the absolute magnitude of I_E/I_M was much larger for the VLDL from the mice fed sunflower oil. The absolute value of I_E/I_M depends on the microscopic pyrene concentration in a particular lipid domain. Therefore, the difference in the I_E/I_M intercept values for the two VLDL preparations does not, of itself, necessarily imply different fluidities for these lipoproteins (18). Similar results were obtained in a second experiment in which the VLDL also were prepared from pooled ascites plasma obtained from six mice. The slope of I_E/I_M between 0°C and 24°C was 0.045 for the VLDL isolated from the mice fed sunflower oil and 0.020 for the VLDL isolated from the mice fed coconut oil. The ratio of these slopes, 2.20, also indicates that the VLDL obtained from the mice fed sunflower oil have a greater fluidity.

Unlike VLDL, the HDL isolated from the mice fed the sunflower and coconut oil diets appeared to be similar by this technique (**Fig. 4**). The absolute values of the I_E/I_M pyrene fluorescence intensity ratio were almost identical for both samples, and the plots of these values as a function of temperature were essentially superimposible. In both cases, the portion of the curve between 0°C and 28°C had a slightly smaller slope than that between 30°C and 50°C. There was no difference, however, in the values of the slopes for the HDL obtained from the mice fed coconut oil as compared with sunflower oil. These data were obtained using HDL prepared from pooled ascites plasma samples taken from six mice. Although the absolute values differed slightly, the same result was obtained in a second experiment with HDL.

DISCUSSION

Our findings indicate that the ascites tumor fluid lipoproteins can be modified by changing the type of fat fed to the tumor-bearing mice. The lipoproteins appear to be formed by the liver or intestine of the mouse, not by the tumor cells (1, 2). The mechanism whereby the diets influenced lipoprotein produced in the tumor-bearing mice is unknown at this time. An obvious possibility is the differences in the saturation of the coconut and sunflower oil fatty acids (Table 1). However, the differences in the chain lengths of their fatty acids also must be considered. The longer

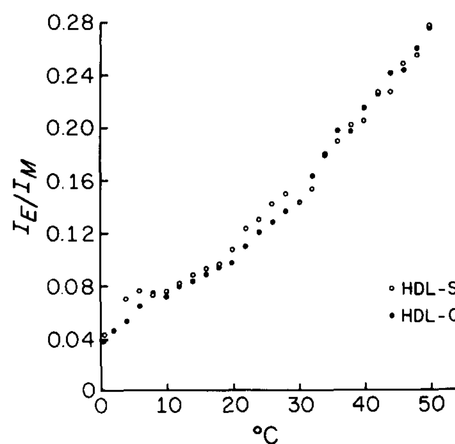

Fig. 4. Excimer (I_E) to monomer (I_M) fluorescence intensity ratio of pyrene in HDL as a function of temperature. The HDL were obtained from mice fed the coconut oil (*C*) or sunflower oil (*S*) diets. The experimental conditions were the same as described in Fig. 2, except that the fluorescence spectra were measured at 2°C intervals between 0° and 50°C.

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fatty acids of the sunflower oil would be absorbed in chylomicrons, whereas the medium chain fatty acids of the coconut oil would be expected to pass directly to the liver in the portal circulation. This difference in absorption might influence the kinds and amounts of fatty acids available for incorporation into the lipoproteins circulated into the ascites tumor fluid. These lipoproteins have been shown to supply lipids to the tumor cells (19, 20). Therefore, the fact that the surrounding fluid contains somewhat different amounts and types of lipids might have an effect on the properties or composition of the tumor cells. In this context, the cells grown in mice fed the coconut oil diet have a higher cholesterol content (5). Of particular interest in this regard is the increase in the VLDL content of the ascites fluid that occurs when the coconut oil diet is fed, for these lipoproteins transfer cholesterol to the cells (20). To what extent this might be involved in the elevated cellular cholesterol content, however, remains to be elucidated.

Although the fatty acid compositional changes varied somewhat in each lipid class, the basic difference was similar in every case. The coconut oil diet raised the monoenoic fatty acid content, whereas the sunflower oil diet raised the polyenoic fatty acid content. These differences were accounted for primarily by changes in 18:1 and 18:2. The saturated fatty acid content was not affected appreciably by the two diets. Moreover, the coconut oil diet did not appreciably increase the medium chain fatty acid content even though 8:0, 10:0 and 12:0 accounted for 65% of the fatty acids in this diet. The observed differences in lipoprotein fatty acid composition are similar to those produced in human serum by feeding diets rich in either saturated or polyunsaturated fat (21, 22).

The reduction in cholesterol concentration produced by the sunflower oil diet was due to a decrease in the lipoprotein content of the ascites tumor fluid. The cholesterol content relative to protein or phospholipid was unchanged in the HDL fraction and actually increased in the VLDL fraction. Based on their human studies, Spritz and Mishkel (23) suggested that the cholesterol-lowering effect of polyunsaturated fat might be explained by differences in lipoprotein composition. According to their hypothesis, LDL carry less cholesterol when they are enriched in polyenoic fatty acids because these acids occupy more space than either monoenoic or saturated acids. This clearly is not the explanation of the cholesterol-lowering effect of the sunflower oil diet in the ascites tumor fluid. It must be remembered, however, that the ascites tumor fluid contains mostly VLDL and HDL, and the structural relationships in these lipoproteins probably are different from those in LDL. In this context, Spritz and Mishkel also found no differences in HDL lipid composition even though the HDL, like LDL, were enriched in polyenoic fatty acids (23).

Changes in the apolipoprotein content have been reported in human and animal lipoproteins in response to dietary modification (24–27). Since the VLDL lipid composition was altered, we wondered whether the diet-induced modifications also might be associated with apolipoprotein changes. No qualitative differences were observed in either the VLDL or HDL fraction by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Fig. 1). Some variation in staining intensity was noted in several of the electrophoretic bands, and a small difference in the amount of tetramethyl urea soluble protein was observed in the VLDL. Therefore, some minor quantitative changes may have occurred. This is tenuous, however, for methods are not as yet available to actually measure apolipoproteins in this mouse system.

The pyrene fluorescence results indicate that the lipid modifications produced in the VLDL by these diets were sufficient to alter the physical properties of the lipoproteins. Studies with bacterial membranes have shown that the I_E/I_M pyrene fluorescence ratio is a measure of membrane fluidity (18). Using this technique, we find that the ascites tumor fluid VLDL from the mice fed sunflower oil have greater fluidity than those from the mice fed coconut oil (Fig. 3). This could be due either to their higher content of polyenoic fatty acids or the differences in their lipid compositions. In this context, Na^+, K^+-ATPase studies have suggested that these diets also alter the fluidity of the tumor cell membranes (28). Greater VLDL fluidity also has been oberved with 12-doxyl stearate as the probe in a human study when a diet rich in polyunsaturated fat was substituted for one rich in saturated fat (29). By contrast, the pyrene data indicate that HDL fluidity was not affected (Fig. 4). This suggests that the microviscosity of the HDL is dominated by the large percentage of protein that they contain rather than by differences in fatty acid composition. Alternatively, the failure to detect a change in HDL may be due to limitations of the pyrene method, for changes in HDL fluidity were detected in the human dietary study with 12-doxyl stearate under conditions where the pyrene fluorescence method gave negative results for HDL (29).

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polyunsaturated and saturated fat diets on the chemical composition and thermotropic properties of human plasma lipoproteins" presented at a symposium, *The Chemistry and Biochemistry of Polyunsaturated Fatty Acids*, sponsored by the American Oil Chemists' Society, September 28–October 1, 1975. We also wish to thank Dr. H. J. Pownall of this group for his advice concerning the pyrene fluorescence method.

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