Influence of dietary *trans*-fatty acids on swine lipoprotein composition and structure

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Abstract Four groups of 20 weanling swine each were fed either (*a*) basal diet, (*b*) basal plus hydrogenated fat (13% *trans*), (*c*) basal plus hydrogenated fat (13% *trans*) and 0.4% cholesterol, or (*d*) basal plus beef tallow (all *cis*). After six months of feeding, the animals were killed and the blood and aortas were removed. Very low density, low density, and high density lipoproteins were then isolated from the plasma by ultracentrifugal flotation. Although the fatty acid composition of the basal diet was different from the diets supplemented with either hydrogenated fat containing *trans*-fatty acid or beef tallow containing all *cis*, the lipid and fatty acid compositions of each of the isolated lipoprotein classes for the four groups of animals were remarkably similar. Elaidate was clearly incorporated into the lipoproteins of animals fed hydrogenated fat, but the level of incorporation was generally less than 5%. In a direct comparison of the structure of the lipoproteins from the different groups, we did not find any significant differences in their physical properties as determined by pyrene fluorescence and electron paramagnetic resonance methods. Grossly visible fatty streaks and fibrous plaques were not found in any of the swine aorta. However, light and electron microscopy indicated the presence of atherosclerotic lesions in the distal abdominal aorta and bifurcation. These studies demonstrate that a diet containing a substantial amount of *trans*-fatty acid leads to a small but definite incorporation into the swine lipoproteins. However, such changes had relatively little effect on lipoprotein structure or the presence of atherosclerotic lesions in these 6-month-old swine.

Supplementary key words plasma lipoproteins · dietary fat · *trans*-fatty acids · cholesterol

A diet containing both a high percentage of saturated fat and cholesterol has commonly been used to produce atherosclerosis in various animal models (1–6). In many of these studies, the saturated fats were obtained from commercial hydrogenation of vegetable shortenings and oils. Because of the occurrence of *trans*-fatty acids (elaidic acid) in these fats, the animal studies have raised an important question of the atherogenicity to man of certain hydrogenated vegetable fats. Although it is well documented that dietary *trans*-fatty acids are incorporated into serum lipids and tissues (7–10), fragmentary information is available on their effect on the chemical and physical properties of the isolated lipoprotein fractions. The present study was designed to determine these effects using two different types of dietary saturated fat. In addition, the degree of atherosclerosis was determined by gross inspection after Sudan staining and with the aid of light and electron microscopy. Groups of 20 (each) weanling swine were fed for 6 months either hydrogenated fat containing *trans*-fat or beef tallow containing only *cis*-fat. Since high cholesterol feeding (1–2%) is known (2, 3) to induce both hyperlipidemia and atherosclerosis in pigs, we avoided this complicating factor to more precisely delineate the effect of a diet rich in *trans*-fatty acids. This baseline information concerning the effects of a high *trans*-fatty acid diet as distinct from a hypercholesterolemic diet on the plasma lipids and lipoproteins may be of value to others who attempt to assess the dietary contributors to atherogenicity using the pig as a model system.

MATERIALS AND METHODS

Animals and diets

Eighty purebred Yorkshire barrow piglets were maintained in an air-conditioned facility equipped

Abbreviations: VLDL, very low density lipoproteins (d < 1.006); LDL_1, low density lipoproteins (d 1.020–1.060); HDL, high density lipoproteins (d 1.090–1.210); GLC, gas–liquid chromatography; TLC, thin-layer chromatography; EPR, electron paramagnetic resonance; FA, fatty acid; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl.

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with a slotted concrete floor and self-feeding and self-watering devices. The swine were divided into four groups and were fed the diets shown in **Table 1.** The total fatty acid composition of each diet is given in **Table 2.** The overall P/S ratios of the basal diet, basal plus hydrogenated fat, and basal plus tallow were 2.90, 0.55, and 0.34, respectively. The basal diet that contained 3% fat and furnished the equivalent of 14.3% protein was composed of 1,745 pounds ground corn, 200 pounds defatted soybean meal, and 55 pounds of a lysine, multiple vitamin, and mineral premix/ton of basal ration (11). The cholesterol content of all diets, with the exception of group 1, was less than 0.01%. All piglets were fed the indicated diets from approximately 8 weeks of age until they were slaughtered at 6 months. The average initial weight was 42 pounds and the final weight was 230 pounds. When the animals reached approximately 230 pounds, they were fasted for 12 hr and slaughtered in groups of 20 each. The blood from each animal was collected in EDTA and sodium azide to give final concentrations of 0.01% and 0.001%, respectively; the cells were removed by low-speed centrifugation and the plasma was stored at 4°C for subsequent lipoprotein isolation.

Preparation of tissue

The aorta was removed as soon as possible and processed for Sudan staining or for light and electron microscopy. For Sudan staining, the aorta was opened longitudinally along the anterior wall, flattened and fixed in formalin, stained for lipid with Sudan IV, stored in sealed plastic bags (13), and shipped to New Orleans for grading. For light and electron microscopy, the aorta was fixed by immersion in 4% glutaraldehyde in 0.1 M Sorensen's phosphate buffer pH 7.4. For light microscopy, six evenly spaced cross sections were cut from the abdominal aorta from the diaphragm through the trifurcation and were stained with hematoxylin–eosin, periodic acid–Schiff, Weigert elastica, or Sudan III. Specimens for electron microscopy were obtained from the distal abdominal aorta. They were post-fixed in buffered 1% osenic acid for 2 hr, dehydrated with alcohol, embedded in epoxy resin, sectioned with a Porter Blum MT-1, and double stained with uranyl acetate and lead citrate. The frequency of degenerated smooth muscle cells in 3–6 embedded blocks was counted at 5,000 or 9,000× magnification and the results were compared (14).

TABLE 1. Composition of swine diets

Group	Stock[a]	Used Fat[b]	Hydrogenated Fat[c]	Beef Tallow[d]	Cholesterol[e]
	lb	*lb*	*lb*	*lb*	*g*
1	100	10	3		200
2	100	10	3		0
3	100			13	0
4	100				0

[a] Stock diet contained 1,745 lb of ground yellow corn, 200 lb defatted soybean meal and 55 lb of a lysine, multiple vitamin, and mineral mix. The mix consisted of 5% lysine, 20% calcium, 9% phosphorus, 15% sodium and potassium chloride, 0.004% calcium iodate, 0.018% zinc, 0.18% iron, 0.14% manganese as oxides or carbonates, and the following vitamins per pound: 40 mg riboflavin, 100 mg *d*-pantothenic acid, 300 mg niacin, 2,000 mg choline, 0.32 μg vitamin B_{12}, a minimum of 60,000 USP units of vitamin A palmitate, 60,000 IU of vitamin D_3, and 165 IU of vitamin E (11, 12).
[b] Discarded hydrogenated soybean oil obtained from a local deep fat frying operation.
[c] Fan Fry (Courtesy of Swift and Company, Chicago, IL.)
[d] Courtesy of Oscar Meyer and Co., Madison, WI.
[e] The crystalline cholesterol was dissolved in the used fat.

TABLE 2. Fatty acid composition of diets[a]

Fatty Acid[b]	Basal	Basal + Used + Hydrogenated Fat	Basal + Beef Tallow
12:0		0.4	
14:0	0.2	1.5	3.1
14:1			1.0
16:0	16.1	19.1	24.5
16:1	0.9	0.9	3.1
17:0	0.9	0.2	0.8
16:2	1.7		1.2
18:0	2.3	8.6	15.1
18:1 *cis*	23.1	39.8	37.7
18:1 *trans*	<0.5	13.0	<0.5
18:2	53.0	15.8	12.3
18:3	1.8	0.7	0.4
20:1			0.8

[a] Wt percent of total fatty acids. Each value is mean of at least two individual determinations.
[b] Number of carbon atoms:number of double bonds.

Isolation of lipoproteins

Each of the lipoprotein classes was isolated from a pool of the plasma within each group of animals. The lipoproteins were isolated by the ultracentrifugal flotation procedure described previously (15, 16). A Beckman Model L2-65 centrifuge (Spinco Div., Palo Alto, CA) equipped with a 60 Ti rotor was operated at 8°C and 55,000 rpm. The density ranges for the isolation of the lipoproteins were as follows: VLDL, d < 1.006; LDL_1, d 1.020–1.060; and HDL, d 1.090–1.210. The density fraction between 1.060–1.090 was not studied since it was shown previously that it contained both LDL_2 and HDL (16). In general, this density range (LDL_2) accounted for less than 10% of the plasma lipid. Each of the lipoproteins was refloated twice at its highest density. By immunochemical techniques, VLDL and LDL reacted with antisera prepared to pig apoB, but not to the major

HDL protein, apoA-I; HDL gave precipitin lines only to anti-apoA-I. None of the lipoproteins reacted with anti-pig albumin. The isolated lipoproteins were extensively dialyzed against 0.9% NaCl, 0.01% EDTA, 0.001% sodium azide pH 7.4. In some of the studies, the samples were concentrated to about 30 mg/ml in a collodion bag (Schleicher and Schuell, Keene, NH).

Lipid determination

Plasma cholesterol and triglyceride were determined by automated methods (17). Total lipids from each lipoprotein class were extracted with chloroform–methanol 2:1 by the method of Folch, Lees, and Sloane Stanley (18). Lipid extracts were diluted to known volumes and aliquots from each sample were taken for determination of the total weight. Separation of lipid classes was performed by preparative thin-layer chromatography (TLC) on silica gel G as described previously (19, 20). Free and esterified cholesterol were determined on appropriate aliquots of the isolated lipids by the method of Sobel and Fernandez (21), phospholipid phosphorus by the method of Bartlett (22), and triglycerides by gravimetric methods (20). The fatty acid composition of the methyl esters of the appropriate lipid class was determined by gas–liquid chromatography (GLC) using a Barber-Coleman (Rockford, IL) Model 5000 gas chromatograph as described previously (19, 20). The instrument was equipped with a 1.8 m U-shaped glass column, 3.2 mm ID, containing ethylene glycol succinate polyester, 15% on Chromosorb W AW, 80–100 mesh.

The percentages of methyl oleate (C 18:1 *cis*) and methyl elaidate (C 18:1 *trans*) were determined by capillary GLC (23) (hexadeconate was used as an internal standard) or by infrared spectrophotometry with the aid of a Beckman IR-7 spectrometer equipped with a beam condenser and sodium chloride ultra-microcavity liquid cells. A 10% concentration of samples of methyl esters and standard mixtures of methyl oleate and methyl elaidate from 5, 10, 20, 30, and 40% in CS_2 were read and quantified by triangulation and read from a standard curve (24).

Pyrene-fluorescence studies

Lipoproteins were labeled by a modification of a previously described method (25). Typically, 0.025 ml of an ethanolic solution of pyrene (20 mg/ml) was thoroughly mixed (vortex mixer) with 5 ml of 0.05 M Tris-HCl pH 7.4. One ml of the resulting fine mixture was then added to 1 ml of lipoprotein (2 mg protein/ml) and gently agitated. The excimer/monomer fluorescence ratios were determined using an Aminco Bowman spectrofluorimeter (American Instrument Co., Silver Spring, MD) that was equipped with a thermostated cell compartment. The instrumental parameters and methods for data calculation were identical to those described by Soutar et al. (26).

Electron paramagnetic resonance (EPR) studies

To determine the effect of diet on the fluidity of the lipoprotein particle, we have used the paramagnetic compound 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and have measured its thermotropic properties by EPR (27, 28). TEMPO was prepared by the method of Rozantzev (29). To each lipoprotein (30 mg/ml) was added 5 μl of an aqueous solution of 2.33 mM TEMPO. The mixtures were then transferred to Corning micropipettes and the EPR spectra were recorded on a Varian E-12 spectrometer (Varian Assoc., Palo Alto, CA) operated at a microwave frequency of 9.15 GHz. The temperature of the microwave cavity was regulated by a Varian variable temperature controller and was measured by a Tri-R electronic thermometer (Tri-R Instruments, Rockville Center, NY) to an accuracy of ±0.5°C. Samples were measured at 3°C intervals from 0–69°C after equilibration of the sample for 5 min. By this procedure, none of the lipoproteins studied appeared to undergo irreversible denaturation in the temperature range studied.

The fluidity parameter f defined by the equation

$$f = \frac{H}{H + P}$$

was determined from the relative amplitudes of the polar (P) and hydrophobic (H) components of the high-field resonance line (Fig. 2) (27, 28). The value of f at each temperature was computed by digitization of the polar and hydrophobic spectral component amplitudes with a Hewlett-Packard (Palo Alto, CA) calculator model 91000-B equipped with a 9107-A digitizer. An XY plotter was used to plot these values against temperature.

RESULTS

Plasma lipid composition

Plasma lipid levels in the four groups of animals are presented in **Table 3.** At the low concentration of cholesterol used in the diet (0.4%), there were only small differences in the plasma cholesterol concentration in the animals fed hydrogenated fat plus or minus cholesterol (compare groups 1 and 2). The

same general trend was true for the plasma triglyceride levels. Lipoprotein electrophoresis patterns of each from the four groups failed to reveal any differences; each showed characteristic α- and β-migrating bands. In addition, the ultracentrifugal flotation distributions of each plasma were identical. Based on these two techniques, there was no indication of a B-VLDL or HDL_c, which are present in swine fed 1.5% cholesterol (30).

Lipid and fatty acid composition of lipoprotein fractions

The lipid compositions of isolated VLDL, LDL_1, and HDL for each diet group are given in **Table 4.** With the exception of minor differences, the lipid compositions of each lipoprotein class were all within reasonable agreement. To determine if the different diets were associated with changes in the FA spectra, the lipid fractions were methylated and the methyl esters were analyzed by GLC. As shown in **Table 5,** approximately 50% of the VLDL triglyceride FA was 18:0 and 18:1. The major saturated FA was 16:0. In general, there were no discernable differences in the FA composition of VLDL triglycerides in the four diet groups. The FA spectra of LDL cholesteryl esters and phospholipids were also similar for each diet. Approximately 75% of all the LDL_1 cholesteryl esters were oleic acid and linoleic acid. In contrast, the major fatty acids (50%) of the LDL_1 phospholipids were palmitic and stearic acids. The FA compositions of the HDL cholesteryl esters and phospholipids were nearly identical to those found in LDL_1 and, again, the variations between each diet group were only minor.

No detectable amounts of C 18:1 *trans*-FA (elaidate) were found in any of the lipoproteins from the basal diet (group 4) or the *cis* beef tallow diet (group 3). Elaidate was clearly incorporated into the lipoproteins of animals fed used fat and hydrogenated soybean oil (groups 1 and 2). However, the amount of elaidate was usually less than 5% of the total (except HDL cholesteryl esters) and considerably less than the *trans*-fatty acid composition of the diet; the HDL cholesteryl esters contained 10% elaidate.

TABLE 3. Plasma-lipid composition[a]

Group[b]	Plasma Triglyceride	Plasma Cholesterol	VLDL Cholesterol	LDL_1 Cholesterol	HDL Cholesterol
1	70	149	17	91	41
2	41	124	11	66	47
3	25	119	5	71	43
4	46	99	5	64	36

[a] Lipid values are expressed as mg/100 ml and are the analyses of pooled blood from 20 swine per group.
[b] Diets described in Table 1.

TABLE 4. Percent distribution of lipids in isolated lipoprotein fractions

Lipoprotein Fraction	Group	Triglycerides	Phospholipids	Cholesterol	Cholesteryl Esters
VLDL d < 1.006	1	83.5	4.4	6.4	5.7
	2	84.2	6.5	4.5	4.8
	3	86.1	6.5	4.3	3.1
	4	83.1	9.2	3.9	3.6
LDL_1 d 1.020–1.060	1	6.0	22.5	13.9	57.6
	2	8.0	19.2	14.4	58.3
	3	11.2	25.9	14.9	48.0
	4	10.4	16.9	14.0	58.7
HDL d 1.090–1.210	1	1.7	25.4	6.5	66.3
	2	1.5	26.8	5.6	66.1
	3	1.3	25.0	4.8	68.9
	4	2.5	25.7	6.7	65.1

Pyrene fluorescence studies

As shown previously (25, 26, 31), the pyrene excimer/monomer (E/M) ratio provides a sensitive measure of changes in the viscosity of the hydrocarbon core of lipoproteins. Therefore, the E/M ratios of pyrene-labeled LDL_1 and HDL from groups 1–4 were measured as a function of temperature (**Fig. 1**). In each instance, an increase in temperature caused an increase in the E/M value except at higher temperatures, where thermal dissociation of the excimer form is competitive with excimer fluorescence. Regardless of the diet, there was little change in the E/M versus temperature for each lipoprotein class.

EPR studies

TEMPO distributes between the bulk aqueous phase and the fluid-lipid phase of a lipoprotein particle (**Fig. 2**). The EPR spectrum of TEMPO in such a system normally exhibits three sharp, equally spaced, hyperfine lines. The high-field hyperfine line is split into two components, the lower field of which originates from TEMPO in the hydrophobic environment and the higher field, which is due to TEMPO in the polar aqueous environment. As the temperature of this system is increased, more lipoprotein lipid becomes fluid, resulting in a greater distribution of TEMPO into the hydrophobic phase and producing an increase in the amplitude of the hydrophobic component and a decrease in the amplitude of the polar component. The ratio of the hydrophobic spectral component to the sum of the hydrophobic

TABLE 5. Fatty acid composition of very low density lipoprotein (VLDL) triglycerides, low density lipoprotein (LDL) phospholipids and cholesteryl esters, and high density lipoprotein (HDL) phospholipids and cholesteryl esters[a]

	Fatty Acids				
			18:1 (n − 9)		
	16:0	18:0	*cis*	*trans*	18:2 (n − 6)
Very low density lipoprotein triglycerides					
Group 1	28.6	6.6	38.3	3.0	14.4
Group 2	31.2	6.7	37.5	3.5	13.4
Group 3	32.5	8.1	34.5		18.2
Group 4	33.3	6.4	31.8		32.2
Low density lipoprotein phospholipids					
Group 1	24.8	23.1	17.9	2.8	18.8
Group 2	22.1	24.0	17.9	3.7	19.5
Group 3	20.8	26.2	20.0		17.7
Group 4	24.3	26.0	18.5		17.0
cholesteryl esters					
Group 1	10.3	2.4	28.3[b]	*c*	48.0
Group 2	10.8	1.9	28.0[b]	*c*	48.0
Group 3	11.5	2.1	28.0		47.1
Group 4	10.5	3.3	27.5		45.7
High density lipoprotein phospholipids					
Group 1	17.8	24.0	24.1	5.5	20.2
Group 2	17.8	24.9	24.1	*c*	17.7
Group 3	17.2	27.2	19.3		16.6
Group 4	16.3	24.6	18.7		14.6
cholesteryl esters					
Group 1	11.5	1.1	27.7[b]	*c*	51.5
Group 2	12.2	0.8	19.2	10.0	47.1
Group 3	19.6	0.9	29.1		41.2
Group 4	13.0	0.9	31.1		40.5

[a] Expressed as percent of total. For clarity, the minor FA constituents were not listed.
[b] *Cis* plus *trans*.
[c] Insufficient material.

plus polar components can be used to calculate a relative fluidity parameter, f, for lipoprotein lipids.

Plots of f versus temperature for groups 1–3 are shown in **Fig. 3** for VLDL, LDL_1, and HDL. For VLDL, the curve is initially a straight line and begins to reach a limiting value of about 0.3 at 33°C. For LDL_1, the curve is somewhat sigmoidal, exhibiting a sharp increase in fluidity in the range 27–33°C. After this sharp increase, the fluidity of LDL continues to increase linearly up to 69°C. This behavior is unlike that of VLDL in which the fluidity plateaus at about 33–36°C. Transition curves for HDL are also sigmoidal in shape. However, the change in fluidity in the range 24–36°C is not nearly so abrupt as that in LDL_1. This difference may be a reflection of the significantly greater amount of protein present in HDL that can serve to more effectively immobilize the lipids. Arrhenius plots of the data shown in Fig. 3 are presented in **Fig. 4.** For VLDL, two abrupt changes in slope are observed. For the lower transition, T_1, this occurs at 27.5–32.0°C and for the higher transition, T_2, it occurs at 41–44°C. For LDL_1, three abrupt changes in slope of the Arrhenius plots are observed. The lowest of these occurs at 26–27°C, the central transition at 31–33°C, and the upper transition (T_3) at 58.5–61.0°C. For HDL, only two abrupt changes in slope are observed, the lower of these (T_1) occurring at 16.0–26.5°C and the higher (T_2) at 41–49°C. Tabulation of the transition temperature

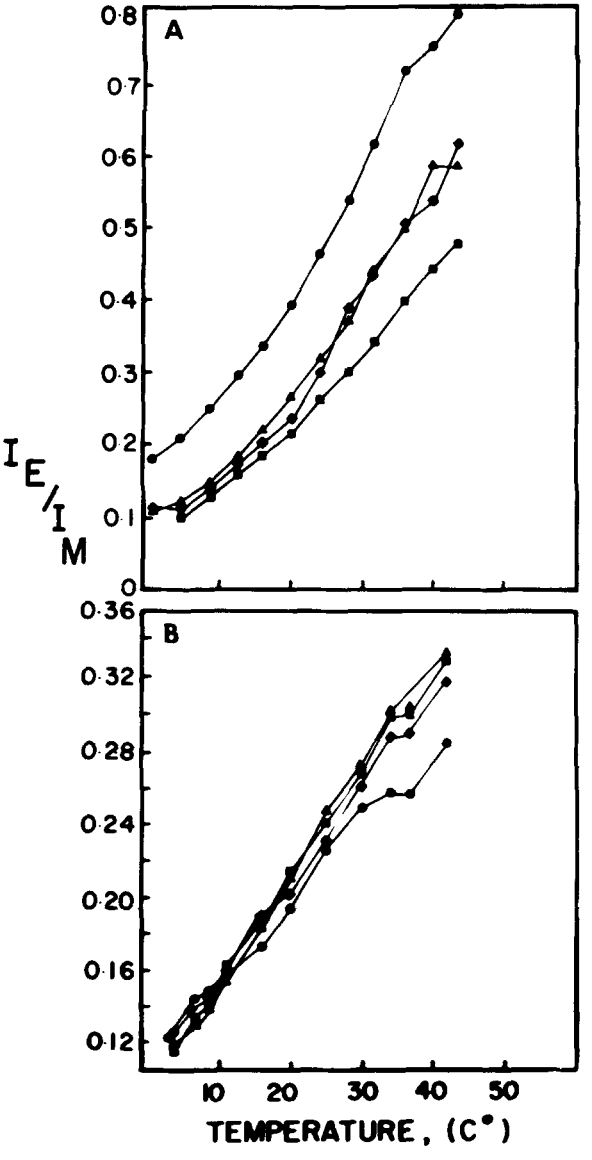

Fig. 1. Plots of pyrene excimer-monomer fluorescence intensity ratios (I_E/I_M) vs. temperature for HDL (A) and LDL_1 (B). Group 1, circles; group 2, triangles; group 3, diamonds; and group 4, rectangles.

data for each of the lipoprotein classes from each diet group is given in **Table 6.** The close similarity in the transition temperature of each diet indicates that each lipoprotein class has similar thermotropic properties consistent with its lipid composition.

Changes in the aorta

Grossly visible, whitish, slightly elevated lesions, which were mostly in the distal abdominal aorta and trifurcation area, were infrequently observed in the freshly excised aorta from all groups at 6 months of age. No gross lesions were present in the proximal portions of the aorta from all groups. The abdominal aorta contained slightly more superficial Sudan IV-stained areas than the thoracic aorta (**Table 7**). However, no statistically significant differences were noted in the visible Sudan IV-stained areas between the aortas from swine fed animal fat, vegetable fat, or cholesterol. No advanced atherosclerotic lesions were observed in any group at 6 months of age. Under light microscopy, elevated lesions had collections of cells and extracellular materials. Superficial lipid deposits and foam cells were sparse. In these areas, the internal elastica appeared broken or absent and, when stained with Sudan III, contained sudanophilic granules in the vicinity of the internal elastica and in the inner media. Portions of the aorta that appeared normal by light microscopy had, on electron microscopy, scattered degenerated smooth muscle cells and a small quantity of cell debris in the inner media. Grossly normal areas of the aortas from swine fed additional fat and cholesterol did not have a significantly higher frequency of degenerated smooth muscle cells than the grossly normal areas of the aorta from swine fed only the corn–soybean basal ration of 5.5 ± 0.18% and 5.1 ± 0.35%, respectively.

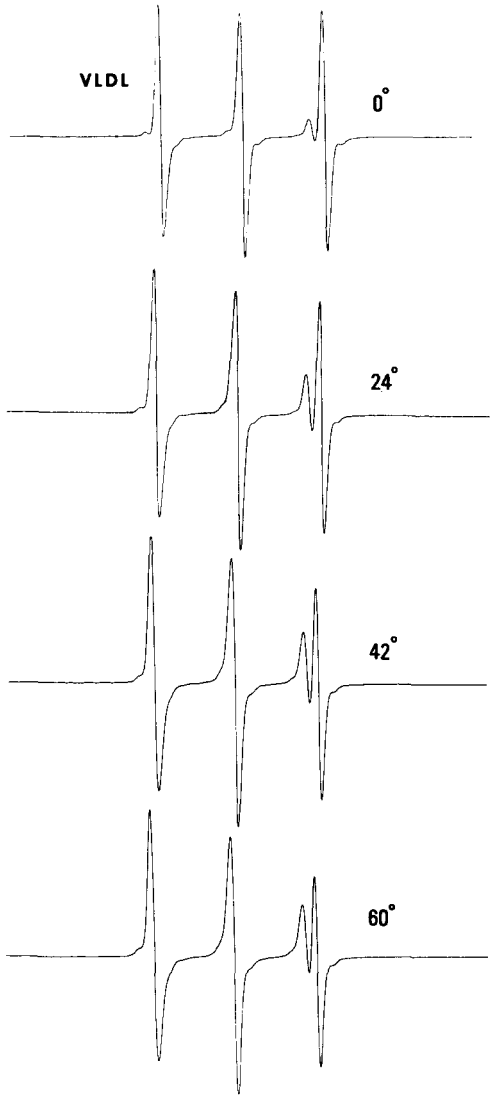

Fig. 2. Electron paramagnetic resonance spectrum of TEMPO in the presence of a 30 mg/ml solution of VLDL isolated from group 1. As the hydrophobic lipid region of the lipoprotein becomes more fluid, more TEMPO distributes into that fluid phase, resulting in an increase in the amplitude of the low-field portion of the high-field resonance line.

DISCUSSION

The plasma cholesterol concentrations of animals fed saturated fat and cholesterol were only slightly elevated compared to those of animals receiving the basal diet alone or basal plus saturated fat. Since the dietary sterol concentration was only 0.4%, we expected the plasma cholesterol values to be only slightly elevated. The major fatty acid of the basal diet was linoleic acid (53%). Compared to the basal diet lipoprotein FA, supplementing the diet with either hydrogenated vegetable fat or animal fat did not significantly alter the fatty acid compositions. Elaidic acid was present in the lipids from animals fed hydrogenated fat. With the exception of the cholesteryl esters of the HDL, however, they accounted for less than 5% of the total FA; the elaidate content of the HDL cholesteryl esters from Group 2 was 10%. The small incorporation of *trans*-FA did not lead to detectable differences in the thermotropic properties of each lipoprotein as determined by fluorescence and EPR methods.

In other studies (31) from this laboratory, it has been shown that these physical techniques can measure changes in the structure of lipoproteins. Morrisett et al. (31) altered the FA composition of diets that were highly unsaturated (P/S = 4) or highly saturated (P/S = 0.25). In contrast to the present swine study,

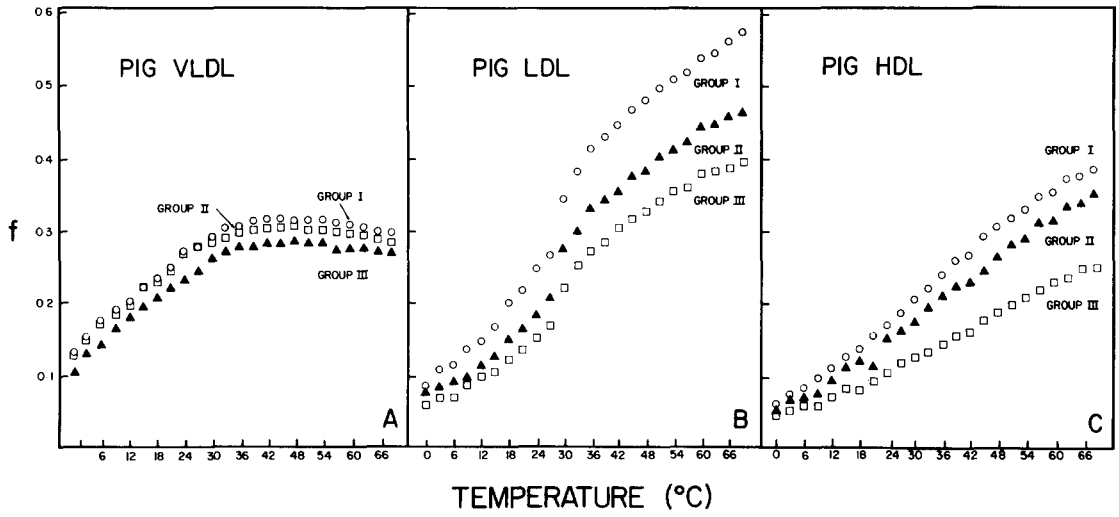

Fig. 3. Typical plots of the fluidity parameter, f, vs. temperature as determined with TEMPO for pig VLDL, LDL_1, and HDL. Values of f were calculated from the hydrophobic and polar components of the high-field resonance line (illustrated in Fig. 2).

the FA compositions of the human lipoproteins were markedly changed and resembled the dietary FA composition; the unsaturated diet lipoproteins were more fluid than the saturated ones.

The inability to show differences in the overall composition and structure of swine lipoproteins on the various diets may be due to the high content of linoleic acids, which may have swamped the effects of the *trans*-fatty acids. However, swine require a high level of linoleic acid in their diet, so it was not possible to vary this factor (32). Mattson et al. (33) have shown that the isomeric form of fatty acids in a hydrogenated fat that contained 34% 18:1 *trans* did not alter serum cholesterol or triglyceride in human subjects on a liquid formula diet, when fed with 20% linoleic acid. On the other hand, Vergroesen (34) found that, at an equivalent level of 34% elaidic acid, the serum cholesterol level was increased when either 10 or 24% linoleic acid was fed in a liquid formula diet to human subjects.

To date, the presence of Sudan IV-stainable fatty streaks and raised plaques has served as a convenient

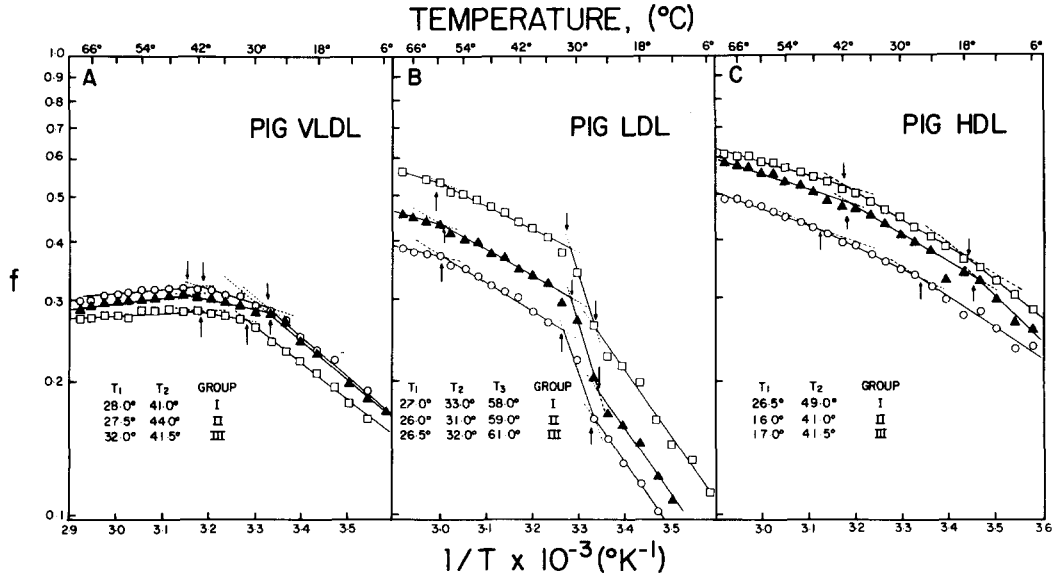

Fig. 4. Arrhenius plots of the fluidity parameter, f, for pig VLDL, LDL_1, and HDL isolated from the various groups. The temperatures at which these slope changes occur are a reflection of thermotropic changes of the constituent lipids.

TABLE 6. Transition temperatures for lipoprotein classes (Determined with TEMPO)

Lipoprotein Class	Group	Transition Temperature T_1	T_2	T_3
VLDL	1	17	34	
	2	28	44	
	3	32	42	
	4	26	42	
LDL_1	1	27	33	59
	2	26	31	59
	3	26	32	67
	4	24	31	57
HDL	1	27	49	
	2	16	41	
	3	17	42	
	4	28	52	

means of judging the extent of atherosclerosis (13). The absence of Sudan IV-stainable fatty streaks at 6 months of age may be explained on the basis that atherosclerosis does not develop readily unless excessive amounts of fat and cholesterol are present in the diet (35) and fed over an extended period of time (6). In older swine, such visible signs of atherosclerosis were more apparent (32). Higher levels of dietary fat and dietary cholesterol would probably have caused a more rapid accumulation of lipid and cell debris in the abdominal aorta. The diet of man contains a higher percentage of fat but a lower percentage of cholesterol than used in the present study. The 6-month-old swine on the fat and cholesterol supplemented diet consumed approximately 5 pounds of feed/day which, at a level of 0.4% cholesterol, is equivalent to the consumption of the amount of cholesterol in 40 eggs/day.

The subtle change in the aortic smooth muscle cell of the 6-month-old swine had no direct correlation to the composition and structure of serum lipoproteins. However, the presence of degenerated smooth muscle cells may reflect one of the first steps in the initiation of atherosclerosis. Time is required for the full expression of lesions. If this assumption is true, then inhibition of the degeneration of arterial smooth muscle cells would seem important for the control of the disease process. The present study indicated that the origin of dietary saturated fat, whether primarily of animal or vegetable origin, did not influence lipoprotein composition or structure when accompanied by a minimum level of dietary cholesterol.

TABLE 7. Involvement of aorta[a]

Group	Thoracic Aorta (Mean ± SEM)	Abdominal Aorta (Mean ± SEM)
1	0.42 ± 0.12	2.17 ± 0.98
2	0.18 ± 0.03	1.32 ± 0.67
3	1.24 ± 0.69	3.22 ± 0.67
4	0.44 ± 0.12	1.26 ± 0.78

[a] Percentage involved to normal areas. No statistically significant differences between groups.

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