Influence of an atherogenic diet on the structure of swine low density lipoproteins

Henry J. Pownall, Richard L. Jackson, Robert I. Roth, Antonio M. Gotto, Josef R. Patsch, and Fred A. Kummerow

Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030, The Burnsides Research Laboratory, University of Illinois, Urbana, IL 61801, The Harlan E. Moore Research Foundation, Champaign, IL 61820, and the Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267[1]

Abstract Five groups of three swine each were fed a basal diet supplemented with 15% tallow and either 0.0, 1.0, 1.5, 2.0%, or 2.5% cholesterol. The animals were studied over a period of 9 weeks to observe changes in plasma lipids and low density lipoproteins (LDL). At the end of the study period, LDL was analyzed by rate zonal ultracentrifugation, characterized chemically, and examined by differential scanning calorimetry. Within 3 weeks of initiation of the cholesterol-supplemented diets, there was an increase in the plasma levels of cholesterol and total LDL. LDL from swine fed the basal diet and the basal diet plus 1.0% cholesterol appeared in two LDL populations (LDL_1 and LDL_2) when analyzed by rate zonal ultracentrifugation. After diets containing 1.5, 2.0, and 2.5% dietary cholesterol, there was an increase in the mean flotation rate of total LDL which shifted to a lower density. LDL_1 from the high cholesterol diets had a decreased triglyceride content when compared to those of the low cholesterol diets. When examined by differential scanning calorimetry, the LDL_1 from the animals fed at least 1.5% cholesterol had phase transitions above body temperature, whereas the LDL from those fed 0 and 1.0% cholesterol had phase transitions below 37°C. In contrast, the thermal behavior and fatty acid compositions of the extracted cholesteryl esters of the LDL obtained after the five different diets were not remarkably different. Since LDL triglyceride and cholesteryl esters are predicted to coexist in a common phase in the LDL core, the different thermal behavior of the LDL obtained after diets with different cholesterol contents is due to differences in triglyceride content which are a secondary effect of cholesterol-feeding. From these data we conclude that dietary cholesterol increases plasma LDL content, decreases LDL triglyceride content, and alters the particle structure. These changes in lipoprotein structure may contribute to the known development of atherosclerosis in cholesterol-fed swine.—**Pownall, H. J., R. L. Jackson, R. I. Roth, A. M. Gotto, J. R. Patsch, and F. A. Kummerow.** Influence of an atherogenic diet on the structure of swine low density lipoproteins. *J. Lipid Res.* 1980. **21:** 1108–1115.

Supplementary key words cholesterol feeding · rate zonal ultracentrifugation · differential scanning calorimetry

Human plasma lipoproteins are composed of structurally distinct particles having characteristic sizes and compositions. They are operationally defined according to their density as the high, low, and very low density lipoproteins, HDL, LDL, and VLDL, respectively (1–3). Recent studies using differential scanning calorimetry have greatly contributed to our understanding of the structure of LDL in man (4, 5) and in various animal models (6–8).

The swine is one of the species that is susceptible to atherosclerosis and upon cholesterol feeding develops hypercholesterolemia which appears to be related to an acceleration of the disease (9–13). In the miniature swine, cholesterol feeding induces the formation of a new lipoprotein, designated HDL_c, which contains apoE (arginine-rich protein) as its major protein (9). The compositions of human LDL and HDL_c from the miniature swine can be correlated with their thermal properties. The present study was undertaken to establish a correlation between the composition, structure, and thermal behavior of swine LDL on various diets containing increasing amounts of cholesterol. Our studies show that all of the changes in the LDL induced by cholesterol feeding over a period of 4 weeks are maximal at 1.5% or greater dietary cholesterol.

MATERIALS AND METHODS

Animals and diets

Twenty-four Yorkshire weanling swine were fed till 4 months of age on a basal diet containing 3% fat and 14.3% protein as furnished by 1,745 lb of ground yellow corn, 200 lb of defatted soybean meal, and 55 lb of a premix of multiple vitamins and minerals per

Abbreviations: VLDL, LDL, and HDL, very low, low, and high density lipoproteins, respectively; DSC, differential scanning calorimetry.

[1] R. L. Jackson.

TABLE 1. Composition of swine diets

Group[a]	Basal[b]	Whole Egg Powder	Beef Tallow[c]	Used Fat[d]	Cholesterol[e]
I	75.0	0	5.0	10	0
II	74.5	10	5.0	10	1.0
III	74.0	10	5.0	10	1.5
IV	73.5	10	5.0	10	2.0
V	73.0	10	5.0	10	2.5

[a] Each group of pigs consisted of three Yorkshire swine which had been fed a basal diet to 4 months of age and subsequently started on the indicated diets. The numerical values are lb.
[b] Basal diet contained 1,745 lb of ground yellow corn, 200 lb of defatted soybean meal, and 55 lb of a vitamin-mineral mixture as indicated in the text.
[c] Courtesy of Oscar Mayer and Co., Madison, WI.
[d] Courtesy of Kentucky Fried Chicken.
[e] The crystalline cholesterol was dissolved in the beef tallow before mixing with the basal diet. The 0.5% contributed by the egg powder is included in this value.

ton of feed. The mix consisted of 5% lysine, 20% calcium, 9% phosphorus, 15% sodium and potassium chloride, 0.004% calcium iodate, 0.015% 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, 2000 mg choline, 0.32 g 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. The cholesterol content of the basal diet was less than 0.1%. At 4 months of age the pigs were divided into five groups and fed the diets shown in **Table 1.** The swine were maintained in an air-conditioned facility with a slotted concrete floor. Food and water were available ad libitum. At 3-week intervals, blood was obtained by ear puncture after an overnight fast. After 3 months on the diets, the swine were killed and blood was collected in ethylenediaminetetraacetic acid (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 isolation of lipoproteins.

Plasma lipoprotein isolation and characterization

Swine lipoproteins were initially isolated by sequential ultracentrifugal flotation in KBr at 59,000 rpm for 24 hr in a Beckman 60 Ti rotor. After centrifugation at plasma density, the infranatant was collected and adjusted to d = 1.090 g/ml by addition of KBr and the lipoproteins of d < 1.090 g/ml were floated. The d = 1.006 to 1.090 g/ml fraction was subjected to rate zonal ultracentrifugation in a Beckman Ti 14 rotor employing a linear gradient extending from d = 1.00 to 1.300 g/ml as described previously (14). The zonally isolated LDL or LDL subfractions were dialyzed against 0.9% NaCl, 0.01% EDTA, 0.001% sodium azide, pH 7.4, and concentrated by pressure filtration using an Amicon XM-100 membrane for compositional and electrophoretic analysis. Portions of each sample were further concentrated by centrifugation in an Amicon Centriflo CF-50 at 2000 rpm and used for calorimetry.

Analytical methods

Cholesterol and triglyceride determinations were obtained from the Core Laboratory of the Baylor Lipid Research Clinic using an Autoanalyzer procedure (15). The electrophoretic behavior of LDL was measured in agarose using a barbital buffer, pH 8.2, with an ionic strength of 0.045. Each sample was run 2 volts/cm for 50 min after which the lipoprotein bands were visualized in Oil Red 0. Proteins were quantified by the method of Lowry et al. (16) using bovine serum albumin as a standard, and phospholipid was determined as phosphorus by the method of Bartlett (17). LDL lipids were obtained by extraction into chloroform–methanol 2:1. After evaporation and solubilization in hexane, the cholesteryl esters and triglycerides were separated by chromatography on a column of silica gel using hexane–ether 6:1 as the eluant. A trace of [^{14}C]cholesteryl linoleate and [^{3}H]triolein were added to the crude lipid mixture to facilitate the location of the cholesteryl esters and triglycerides by scintillation counting. Fatty acid analyses were obtained by gas–liquid chromatography of the methyl esters of the isolated lipids (18).

Differential scanning calorimetry (DSC)

The thermal properties of LDL were investigated by DSC using a Perkin Elmer DSC-II. A 50-μl aliquot of concentrated LDL (150–250 mg/ml) was transferred to a 75-μl stainless steel pan which was hermetically sealed. The sample was placed in one compartment of the calorimeter; into the other compartment was placed a similar pan containing 50 μl of buffered water. Each sample was analyzed

TABLE 2. Plasma triglyceride of pig plasma[a]

Group	Weeks of Feeding			
	0	3	6	9
			mg/dl	
I	44	52	51	51
II	33	28	63	64
III	27	35	56	47
IV	24	37	44	50
V	24	29	43	56

[a] The plasma triglyceride values are the average values of three animals fed the diets shown in Table 1. Average deviations were less than 10%.

using heating/cooling rate of 5°C/min and a sensitivity of 0.2 and 0.5 mcal/sec for LDL and isolated LDL cholesteryl esters, respectively. In a typical experiment the LDL was initially cooled to 0°C until thermal equilibrium was achieved after which the sample was heated to 60°C. The thermal transitions of the extracted cholesteryl esters of LDL were studied similarly except that samples weighing 15–20 mg were used and the reference pan was empty. All reported transition temperatures are those of the endothermal maxima.

RESULTS

Plasma triglyceride and cholesterol levels in the five groups of animals fed the different diets are shown in **Table 2** and **Fig. 1,** respectively. Feeding the swine a diet high in fat supplemented with increasing amounts of cholesterol had little effect on plasma triglyceride levels but resulted in a progressive increase of plasma cholesterol from a normal value of approximately 90 mg/dl to 380 mg/dl within 3 weeks after initiation of the diets. The time required for maximal plasma cholesterol was dependent on the percent cholesterol in the diet (Fig. 1). After 9 weeks of feeding, the plasma cholesterol values of the animals fed 2.5, 2.0, and 1.5% cholesterol were approximately the same (275 mg/dl); feeding 1.0% cholesterol had little effect on plasma cholesterol levels when compared to tallow alone.

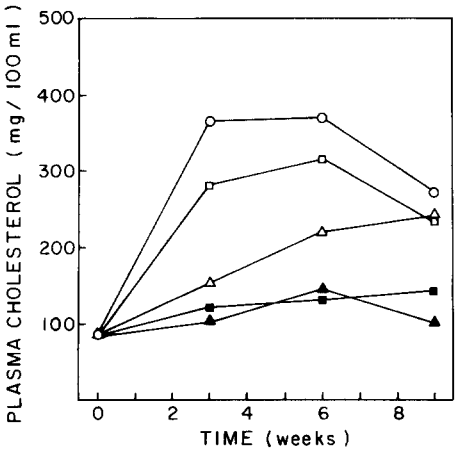

Fig. 1. Changes in plasma cholesterol in pigs fed a control diet and atherogenic diets. The numbers represent the average plasma cholesterol values of three animals (cf. Table 2) fed a basal diet with 15% fat (▲); or basal with 15% fat plus cholesterol, 1.0% (■), 1.5% (△), 2.0% (□), and 2.5% (○). Values are ±10% or less.

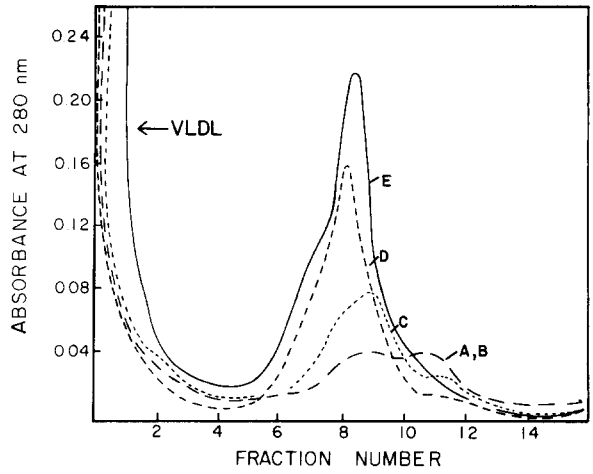

Fig. 2. Rate zonal ultracentrifugation of plasma lipoproteins of 10 ml of plasma from swine fed basal diet with 15% fat, A; basal diet plus 15% fat and 1.0% cholesterol, B; basal diet plus 15% fat and 1.5% cholesterol, C; or basal diet plus 15% fat and 20% cholesterol, D; and 2.5% cholesterol, E.

The rate zonal ultracentrifugal profiles of the d = 1.006 to 1.090 g/ml fraction are shown in **Fig. 2.** The zonal ultracentrifugation removed lipoprotein contaminants from LDL, and demonstrated changes in LDL flotation rate as a function of dietary regimen. It is evident in Fig. 2 that zonal ultracentrifugation also removed significant amounts of VLDL from the LDL isolated in an angle-head rotor. The absence of triglyceride-rich lipoproteins proved to be an important factor in light of the correlation of the thermal data with triglyceride content. Groups I and II gave rate zonal patterns which were nearly identical and displayed two distinct populations referred to as LDL_1 and LDL_2. The ratio of LDL_1 and LDL_2 was nearly identical in the animals on diets of 1% or less cholesterol. With 1.5, 2.0, or 2.5% cholesterol feeding there were marked changes in the flotation behavior (Fig. 2). The hypercholesterolemia produced in Groups III–V was characterized by LDL having higher flotation rates characteristic of increased size and/or decreased hydrated density. Hill, Silbernick, and Lindgren (19) have reported

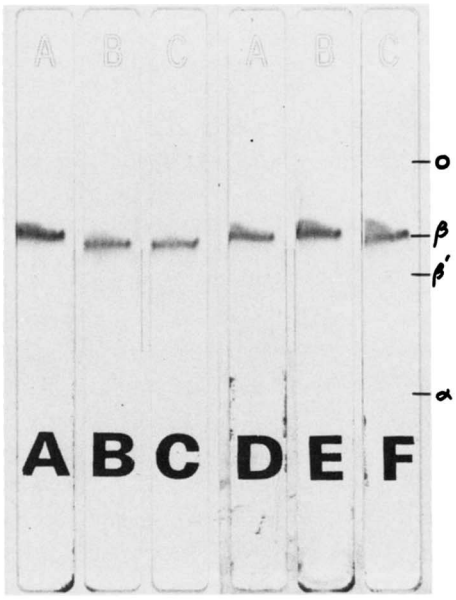

Fig. 3. Agarose gel electrophoresis of LDL, isolated from plasma of swine fed a basal diet and atherogenic diets for 9 weeks. LDL was isolated by rate zonal ultracentrifugation. Groups I–VI are as shown in Table 1. A–E, respectively, are the electrophoretograms of the LDL from the various swine fed diets containing 0, 1.0, 1.5, 2.0, and 2.5% cholesterol. F is LDL isolated from animals fed no cholesterol or lard. In the margin the respective positions labeled O, β, β' and α are those of the origin, LDL, VLDL and HDL.

that the cholesterol-fed swine has LDL that is significantly less dense than that of control animals. In this procedure, intermediate density lipoproteins, if present, would appear in fractions 3–6; the zonal patterns indicate that there was little intermediate density lipoprotein formed when compared to the quantity of LDL. Agarose gel electrophoresis of LDL from each of the test diets is shown in **Fig. 3.** Each LDL fraction produced a single migrating lipoprotein band. LDL isolated by zonal ultracentrifugation gave immunoprecipitin lines to pig anti-apo B but no immunoprecipitin lines developed against pig anti-apo A–I. Polyacrylamide gel electrophoresis of the various LDL fractions in 0.1% SDS gave a single protein staining band at the top of the gel, a finding which is characteristic of apo B. Although apo E is found in the density range of LDL in the cholesterol-fed miniature swine (9), we found no band in zonally isolated LDL characteristic of this apoprotein in any of the animals in this study. This finding leads one to suggest that none of the subpopulations producing shoulders in the LDL zonal bands is due to HDL_c, since the major component of the latter is apo E. Our studies do not exhaustively exclude the presence of a particle similar to HDL_c in the plasma, since its density is a function of amount and duration of cholesterol feeding and its presence in other density regions cannot be rigorously excluded.

For purposes of comparison of the values for composition and calorimetric data we analyzed LDL eluting at fractions 7 to 10 (Fig. 2) in the zonal runs; those fractions corresponded to LDL_1 of the basal diet. The chemical composition of LDL_1 from the various test diets is shown in **Table 3.** With cholesterol feeding, there was a relative increase in total cholesterol and a decrease of triglyceride content. In **Table 4,** the fatty acid composition of the LDL cholesteryl esters is given. These data show that there are no large differences in the cholesteryl ester composition of the swine LDL after the different diets. In all cases, the major fatty acids were oleic and linoleic acids. This pattern is similar to that of normal human subjects fed saturated and polyunsaturated fat diets, except that the ratios of oleic to linoleic acid are much lower in man (18).

The melting behavior of the isolated LDL obtained from the animals different diets was studied by differential scanning calorimetry (**Fig. 4, Table 5**). Without dietary cholesterol, the average transition temperature was 24°C. Upon increasing the dietary cholesterol, the transition temperature increased to a plateau of about 38°C between 1.0 and 1.5% cholesterol.

The melting temperature of the extracted cholesteryl esters of the LDL was also determined (**Fig. 5A, C, E, Table 6**). During the first heating of the ester from 0 to 60°C, we observed a single transition

TABLE 3. Composition of swine LDL after feeding different amounts of cholesterol for 9 weeks[a]

Group	(% Cholesterol in Diet)	Protein	Total Cholesterol	Triglyceride	Phospholipid
I	(0.0)	25	60	3.5	12
II	(1.0)	24	60	2.4	13
III	(1.5)	22	65	1.2	12
IV	(2.0)	22	66	1.2	11
V	(2.5)	23	64	1.3	12

[a] Percent of each component by weight; average deviations within each group were 12% or less.

TABLE 4. Fatty acid composition pig low density lipoprotein cholesterol esters

Diet	Dietary Cholesterol			
	0% (Basal)[a]	1.5%	2.0%	2.5%
14:0	2.3 ± 0.03	2.5 ± 1.0	1.4 ± 0.48	0.78 ± 0.3
16:0	8.6 ± 0.02	8.1 ± 0.1	7.5 ± 0.12	7.3 ± 0.2
16:1	4.7 ± 0.6	5.9 ± 2.3	4.8 ± 1.5	4.9 ± 1.5
18:0	3.3 ± 0.9	3.5 ± 0.9	3.4 ± 0.5	6.0 ± 1.6
18:1	34.6 ± 2.0	31.7 ± 1.3	37.4 ± 1.5	38.1 ± 1.6
18:2	36.8 ± 3.2	36.5 ± 4.0	38.7 ± 0.7	34.6 ± 1.7
>18:2	9.7 ± 1.0	11.8 ± 2.3	7.0 ± 0.8	10.8 ± 1.0

[a] On the basal diet there was no significant difference between the fatty acid compositions of the cholesteryl esters of LDL_1 and LDL_2; the values given are percentages and represent the averages of both density classes.

TABLE 5. Summary of DSC data of pig LDL[a]

% Dietary Cholesterol	T_c Heating	T_c Cooling	T_c Average
0% Cholesterol	25°	23°	24°
1.0%	33°	31°	32°
1.5%	40°	36°	38°
2.0%	39°	35°	37°
2.5%	38°	36°	37°

[a] Average of three samples from three different animals. Average deviation was 1°C or less. Denaturation (not shown) was observed at 77°C after which a second heating gave a transition which is narrower but at the same peak temperature. On the basal diet the melting temperatures of LDL_1 and LDL_2 were identical.

with an enthalpy of 6.5 to 6.9 kcal and a melting peak that was always between 43 and 46°C. Upon cooling to 27°C, a pair of weaker exotherms (Fig. 5B, D, F) were observed; these were seen again upon immediate reheating to 60°C (not shown). The enthalpies of the weak endotherms are unusually high although those observed during the first heating of the samples are within the reported range (4). The strong endotherm observed upon the first heating has been assigned to a crystal to isotropic liquid transition. The two endotherms observed upon reheating are due to smectic → cholesteric and cholesteric → isotropic liquid transitions, respectively (20). This behavior is similar to that observed with human LDL except that the melting points of the isolated cholesteryl esters of the swine LDL are higher than those of man; this reflects the higher saturation of the fatty acids of the former (4).

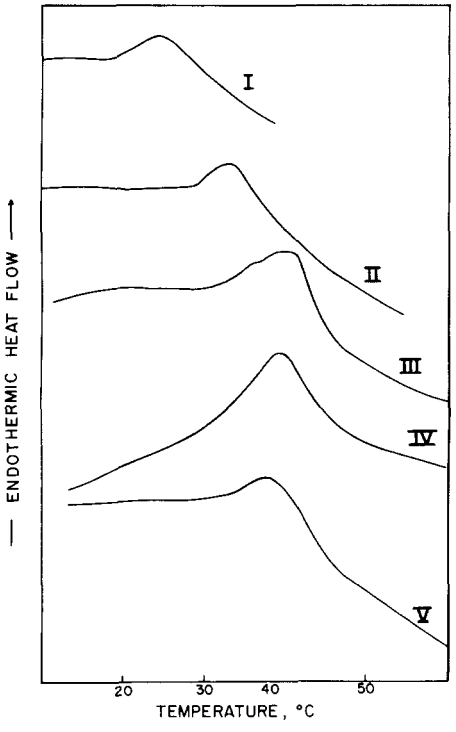

Fig. 4. Differential scanning calorimetric heating curves of swine LDL)50 μl, 100–150 mg/ml), isolated from plasma of swine fed a basal diet or atherogenic diets. The experiments were performed at a sensitivity of 0.2 mcal/sec and a heating rate of 5°C/min. From top to bottom are the DSC of the LDL from swine after 9 weeks on a diet containing 0%, 1.0%, 1.5%, 2.0%, and 2.5% cholesterol.

DISCUSSION

This study was undertaken to evaluate the effects of dietary cholesterol on the composition and thermal properties of the LDL in the swine. Human plasma LDL exhibits a broad reversible transition between 20 and 40°C having a peak temperature that has a negative correlation with the triglyceride/cholesteryl ester ratio; the degree of unsaturation of the cholesteryl esters produces a smaller effect on the melting

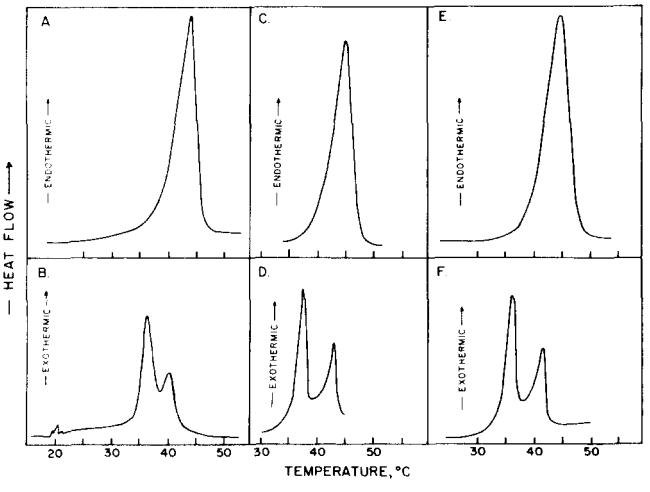

Fig. 5. DSC of extracted cholesteryl esters of LDL after diets having different amounts of cholesterol. 0% cholesterol: A, 1st heating; B, second heating. 1.5% cholesterol: C, 1st heating; D, second heating. 2.5% cholesterol: E, first heating; F, second heating.

TABLE 6. Transition temperatures and enthalpies of the extracted cholesteryl esters of pig LDL as a function of dietary cholesterol content[a]

	0% Cholesterol		1.5% Cholesterol		2.0% Cholesterol		2.5% Cholesterol	
	T_c	ΔH	T_c	ΔH	T_c	ΔH	T_c	ΔH
First heating[b]	44 ± 0.4	6.6 ± 0.3	45.1 ± 0.4	6.5 ± 0.2	44.4 ± 2	6.5 ± 0.3	45.2 ± 0.1	6.9 ± 0.3
First cooling	37.2 ± 0.6 40.5 ± 0.5	2.1 ± 0.4	37.1 ± 0.7 42.4 ± 0.6	2.8 ± 0.9	37.3 ± 0.2 42.5 ± 0.1	1.8 ± 0.2	35.0 ± 0.9 40.2 ± 1.0	1.8 ± 0.4
Second heating	40.0 ± 0.5 44.5 ± 0.6		40.7 ± 0.8 45.8 ± 0.4		40.9 ± 0.2 46.0 ± 0.1		40.0 ± 0.1 45.5 ± 0.5	

[a] All values are the average of at least three animals, except for the basal diet in which 12 animals were studied.
[b] There were some additional shoulders in the first heating at times, but the liquid crystal to isotropic transition was very reproducible.

point (4). In the miniature swine, cholesterol feeding results in hypercholesterolemia with alterations in the type and distribution of the plasma lipoproteins (9). These include a β-migrating lipoprotein, an increase in intermediate density lipoprotein (d = 1.006 − 1.02 g/ml), the occurrence of an α-migrating lipoprotein designated HDL_c, and an increased amount of LDL. The effects of cholesterol feeding on the thermal behavior of cholesteryl ester-rich lipoproteins has been evaluated in several animals. Differential scanning calorimetry of the LDL from the hypercholesterolemic miniature swine revealed a reversible transition of its cholesteryl esters from a smectic to a more disordered state between 30° and 50°C (6). The elevation of the melting temperature of hypercholesterolemic miniature swine LDL was assigned to changes in LDL triglyceride content and the fatty acid composition of the cholesteryl esters (6). The effect of the differences in the cholesteryl ester composition on the melting points was more dramatic in the miniature swine than in man since the triglyceride content of human LDL is so high that its effects on cholesteryl ester melting dominate the more subtle effects of differing fatty acid composition.

In contrast, studies of the composition and thermal properties of LDL from the hyperlipidemic rhesus monkey showed that the effect of triglyceride on the cholesteryl ester transition is minor compared to the changes in the fatty acid composition (8). When fed a diet containing peanut oil or coconut oil plus 2% cholesterol, the LDL of the rhesus monkey has a lower triglyceride content and higher percent of the cholesteryl esters of saturated or monounsaturated fatty acids. Compared to the control group, the LDL in monkeys fed the atherogenic diets had significantly higher melting points. Similarly, the LDL of the *Macaca fascicularis* fed a diet of saturated fat and cholesterol, has an increased content of saturated and monounsaturated cholesteryl esters when compared to control (7). This finding correlates with an elevation of the LDL transition temperature and supports the view that changes in the cholesteryl ester fatty acid composition of LDL are reflected in their thermal behavior. It is significant to note that many of the LDLs isolated after cholesterol feeding have thermal transitions above body temperature; thus the dietary cholesterol results in a structurally different species at physiological temperatures which may be related to its atherogenicity.

In **Fig. 6** we show how our thermal and compositional data correlate with respect to the dietary cholesterol content. It is most notable that after 9 weeks on these diets the major changes in the triglyceride content and melting temperature of LDL occur during diets containing 1.0–2.5% cholesterol. In contrast, there was no comparable change in the melting behavior of the extracted cholesterol esters. Therefore, the decrease in LDL triglyceride rather than the altered LDL cholesteryl ester composition correlates

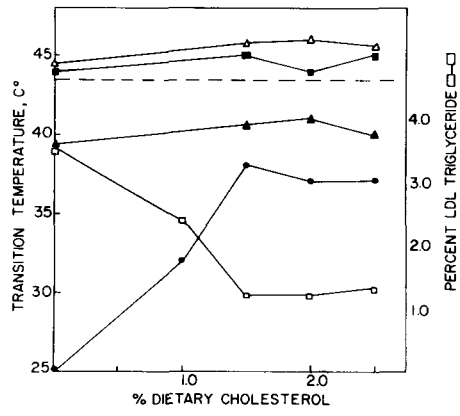

Fig. 6. Correlation of LDL thermal behavior and triglyceride content with the dietary cholesterol intake after 9 weeks. The transition temperatures are the average of three swine and correspond to intact LDL (●), crystal to liquid transition of isolated esters (△), smectic to cholesteric transition of the isolated esters (▲), and cholesteric to isotropic liquid transition of the isolated esters (■). The dashed line obtained from Fig. 7 corresponds to the calculated melting temperature obtained by extrapolation of melting points of LDL to zero triglyceride content (based only on the melting points).

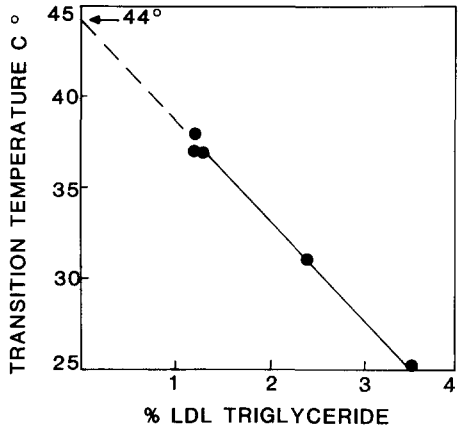

Fig. 7. Correlation of LDL melting temperature and triglyceride content. Average temperature maxima from DSC traces from various diets are given as a function of triglyceride content.

better with the increasing melting temperature of the LDL that was obtained upon feeding the swine increasing amounts of cholesterol.

Fig. 7 shows the relationship between the melting temperature of swine LDL and their respective triglyceride contents. The plot is linear and extrapolation of the line to zero triglyceride content gives an intercept of 44°C. Hypothetically, this temperature should be that of the transition temperature of swine LDL containing no triglyceride. This temperature does not coincide with those of any of the thermal transitions of the isolated LDL cholesteryl esters. It lies between that of the smectic → cholesteric and cholesteric → isotropic liquid transitions. The lack of good correspondence between the extrapolated value and those documented for the melting of the pure cholesteryl esters is difficult to rationalize. It may be due to the reported (4) depression of the LDL transition temperature by cholesterol, if the observed transition in LDL is due to a cholesteric → isotropic liquid transition or a crystal liquid → isotropic transition. However, spectroscopic data on similar systems suggest (21, 22) that the core of LDL is not crystalline so that the latter assignment may be ruled out. Alternatively, we speculate that there is no clear definition between smectic and cholesteric phases and that the broad transition in swine LDL may be due to superposition of smectic → cholesteric and cholesteric → isotropic liquid transitions. A combination of this process and the effects of cholesterol cannot be ruled out and warrant further study for an unambiguous assignment.

The changes in the composition, properties, and structure of LDL from the cholesterol-fed swine were produced by feeding a minimum of 1.5% cholesterol and over the 9 weeks of feeding in our study; diets with a higher dietary cholesterol content were no more efficacious in producing these changes. At the end of the 9-week period, the major changes in the LDL were *1*) increased mean flotation rate as a result of increased flotation rates of both LDL subspecies; *2*) decreased triglyceride content; and *3*) increased thermal transition temperature.

There were no differences in the fatty acid composition of the LDL cholesteryl esters nor in the thermal behavior of the isolated anhydrous cholesteryl esters. Therefore, unlike the rhesus monkey (8) or the *Macaca fascicularis* (7), the cholesteryl ester composition does not have a significant effect on the different melting temperatures of the swine LDL. The changes in the melting point appear to correlate with the triglyceride content of the LDL. Therefore, the behavior of swine LDL is similar to that of the miniature swine (6) and to that of human LDL (4); that is, an increase in triglyceride content produced a significant lowering of the LDL thermal transition. This observation suggests that the cholesteryl esters and triglyceride of swine LDL are in a single homogeneous domain. The denaturation temperature of swine LDL (78°C ± 3) is similar to that of human LDL ~80°C (4) and a parallel study shows that these animals developed arterial lesions during the course of cholesterol feeding.[2] Since a relatively short time interval (9 weeks) of cholesterol-feeding (1.5%) produced the maximal hypercholesterolemia in the swine, our studies provide further support for using the swine as an animal model for atherosclerosis in man.

This work was supported in part by grants from the American Heart Association (RLJ), the American Egg Board (RLJ, JRP) and the National Institutes of Health, through the National Heart and Blood Vessel Research and Demonstration Center (17269). HJP is an Established Investigator of the American Heart Association. We acknowledge the technical assistance of Mrs. Quein Pao, the artistic skills of Ms. Kaye Shewmaker, and the preparation of the typed manuscript by Mrs. Sharon Bonnot.

Manuscript received 12 October 1979 and in revised form 23 May 1980.

REFERENCES

1. Smith, L. C., H. J. Pownall, and A. M. Gotto. 1978. The plasma lipoproteins: structure and metabolism. *Annu. Rev. Biochem.* **47:** 751–777.
2. Morrisett, J. D., R. L. Jackson, and A. M. Gotto. 1977. Lipid-protein interactions in plasma lipoproteins. *Biochim. Biophys. Acta.* **472:** 93–133.
3. The Biochemistry of Atherosclerosis. 1979. A. M.

[2] Pownall, H. J., R. L. Jackson, R. I. Roth, A. M. Gotto, J. R. Patsch, and F. A. Kummerow. Manuscript in preparation.

Scanu, R. W. Wissler, and G. S. Getz, editors. Marcel Dekker, Inc., New York.
4. Deckelbaum, R. J., G. G. Shipley, and D. M. Small. 1977. Structure and interaction of lipids in human plasma low density lipoproteins. *J. Biol. Chem.* **252:** 744–754.
5. Mateu, L., T. Kirchhausen, and G. Camejo. 1977. A low temperature structural transition in human serum low density lipoproteins. *Biochim. Biophys. Acta.* **487:** 243–245.
6. Tall, A. R., D. Atkinson, D. M. Small, and R. W. Mahley. 1977. Characterization of the lipoproteins of atherosclerotic swine. *J. Biol. Chem.* **252:** 7288–7293.
7. Tall, A. R., D. M. Small, D. Atkinson, and L. L. Rudel. 1978. Studies on the structure of low density lipoprotein isolated from *Macca fascicularis* fed an atherogenic diet. *J. Clin. Invest.* **62:** 1354–1363.
8. Kirchhausen, T., S. H. Untracht, G. M. Fless, and A. M. Scanu. 1979. Atherogenic diets and neutral-lipid organization in plasma low density lipoproteins. *Atherosclerosis.* **33:** 59–70.
9. Mahley, R. W., K. H. Weisgraber, T. Innerarity, H. B. Brewer, and G. Assmann. 1975. Swine lipoproteins and atherosclerosis changes in the plasma lipoproteins and apoproteins induced by cholesterol feeding. *Biochemistry.* **14:** 2817–2823.
10. Janado, M., W. G. Martin, and W. H. Cook. 1966. Separation and properties of pig-serum lipoproteins. *Can. J. Biochem.* **44:** 1201–1209.
11. Calvert, G. D., and P. S. Scott. 1975. Properties of two pig low density lipoproteins prepared by zonal ultracentrifugation. *Atherosclerosis.* **22:** 583–599.
12. Jackson, R. L., O. D. Taunton, R. Segura, J. G. Gallagher, H. F. Hoff, and A. M. Gotto. 1976. Comparative studies on plasma low density lipoproteins from pig and men. *Comp. Biochem. Physiol.* **53B;** 245–253.
13. Scott, R. F., A. S. Daoud, and R. A. Florentin. 1971. The Pathogenesis of Atherosclerosis. R. W. Wissler and J. C. Geer, editors. Williams and Wilkins, Baltimore, MD. 120.
14. Patsch, J. R., S. Sailer, G. Kostner F. Sandhofer, A. Holasek, and H. Braunsteiner. 1974. Separation of the main lipoprotein density classes from human plasma by rate zonal ultracentrifugation. *J. Lipid Res.* **15:** 356–366.
15. Manual of Laboratory Operations. Lipid Research Clinics Program. Vol. I, Lipid and Lipoprotein Analysis. National Heart and Lung Institute, NIH.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
17. Bartlett, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234:** 466.
18. Morrisett, J. D., H. J. Pownall, R. L. Jackson, R. Segura, A. M. Gotto, and O. D. Taunton. Polyunsaturated Fatty Acids. American Oil Chemists Society Monograph No. 4. R. T. Holman and W. H. Kunana, editors. A.O.C.S. Publishers, Champaign, IL. 139–161.
19. Hill, E. G., C. L. Silbernick, and F. T. Lindgren, 1975. Development of hyperbetalipoproteinemia in pigs fed atherogenic diet. *Lipids.* **10:** 41–43.
20. Small, D. M. 1972. The physical state of lipids of biological importance: cholesterol esters, cholesterol and triglyceride. *Adv. Exp. Biol. Med.* **26:** 55.
21. Jonas, A. 1977. Microviscosity of lipid domains in human serum lipoproteins. *Biochim. Biophys. Acta.* **486:** 10–22.
22. Sears, B., R. J. Deckelbaum, M. J. Janiak, G. G. Shipley, and D. M. Small. 1976. Temperature-dependent ^{13}C nuclear magnetic resonance studies of human serum low density lipoproteins. *Biochemistry* **15(19):** 4151–4157.