Swine Lipoproteins and Atherosclerosis. Changes in the Plasma Lipoproteins and Apoproteins Induced by Cholesterol Feeding†

Robert W. Mahley,* Karl H. Weisgraber, Thomas Innerarity, H. Bryan Brewer, Jr., and Gerd Assmann

ABSTRACT: Cholesterol feeding in miniature swine resulted in a hypercholesterolemia with a distinctive hyperlipoproteinemia and the subsequent development of atherosclerosis. Alterations in the type and distribution of plasma lipoproteins induced by cholesterol feeding were as follows: (a) the occurrence of β-migrating lipoproteins (B-VLDL) as well as very low density lipoproteins in the $d < 1.006$ ultracentrifugal fraction; (b) an increased prominence of the intermediate lipoproteins ($d = 1.006$–1.02); (c) an increased prominence of low density lipoproteins; and (d) the occurrence of a distinctive lipoprotein with α mobility which was referred to as HDL_c (cholesterol induced). Characterization of the various plasma lipoproteins included chemical composition, size by electron microscopy, and apoprotein content. The B-VLDL resembled the β-migrating lipoproteins of human Type III hyperlipoproteinemia and contained a prominent protein equivalent to the arginine-rich apoprotein in addition to the B apoprotein, apo-A-I, and the fast-migrating apoproteins (apo-C). The HDL_c were rich in cholesterol, ranged in size from 100 to 240 Å in diameter, and contained the arginine-rich apoprotein and apo-A-I but lacked the B apoprotein. The arginine-rich apoproteins isolated from B-VLDL and HDL_c by gel chromatography were similar in amino acid analyses, with glutamic acid as their amino-terminal residue. The occurrence of a spectrum of cholesterol-rich lipoproteins which contained the arginine-rich apoprotein with the occurrence of accelerated atherosclerosis suggested an interesting, although speculative, association.

Earlier work has shown that the spontaneous atherosclerosis of miniature swine can be accelerated by an increase in dietary cholesterol (for review see Scott, 1971). When kept on a control, low-cholesterol diet, miniature swine have plasma lipoproteins which resemble human VLDL, LDL, and HDL[1] (Mahley and Weisgraber, 1974a). However, addition of cholesterol to the diet results in the development of a hypercholesterolemia characterized by marked changes in the type of plasma lipoproteins and in their apoprotein content. The present work characterizes the hyperlipoproteinemia associated with the accelerated atherosclerosis in the miniature swine.

Experimental Procedure

Miniature swine 1–2 yr of age were obtained from Sinclair Research Farm, University of Missouri, Columbia, Mo. Control swine were fed a commercial low-fat hog chow (Zeigler Brothers, Gardners, Pa.). The experimental swine were fed the same chow to which was added 15% lard and 1.5% cholesterol by weight. All animals studied had been on diet 3–8 months prior to lipoprotein analysis. Plasma was obtained by venopuncture after an overnight fast.

Preparation of Plasma Lipoproteins. Plasma was fractionated by ultracentrifugation using established procedures (Havel et al., 1955). VLDL, LDL, and HDL from control plasma were isolated at $d < 1.006$, 1.006–1.063, and 1.10–1.21, respectively, as previously described (Mahley and Weisgraber, 1974a). A combination of ultracentrifugation and Geon-Pevikon block electrophoresis was required for the purification of the lipoproteins induced by cholesterol feeding. The $d < 1.006$ density fraction contained two lipoproteins, referred to as B-VLDL and VLDL, which were separated by the Geon-Pevikon electrophoretic procedure. LDL and HDL_c were isolated by electrophoresis from the density fraction 1.02–1.063 or from multiple density fractions 1.02–1.04, 1.04–1.06, and 1.06–1.087. HDL_2 from the cholesterol-fed swine were isolated at $d = 1.10$–1.21. A detailed description of the Geon-Pevikon electrophoretic method has been reported (Mahley and Weisgraber, 1974a). Recovery of triglyceride and total cholesterol in the ultracentrifugal density fractions compared with the total plasma values was approximately 80%. Recentrifugation of the $d < 1.006$ fraction resulted in lipoproteins with β mobility in the infranatant. Purity of the lipoprotein classes fractionated by the Geon-Pevikon procedure was determined by paper or agarose electrophoresis, immunoelectrophoresis, Ouchterlony immunodiffusion, and electron microscopy by negative staining as previously reported (Mahley and Weisgraber, 1974a,b).

Characterization of the Lipoproteins. Chemical analyses of the plasma and purified lipoproteins included triglyceride (Fletcher, 1968), total cholesterol (Abell et al., 1952), cholesteryl esters (Sperry and Webb, 1950), phospholipid (Zilversmit and Davis, 1950), and protein (Lowry et al., 1951) determinations. The lipoproteins for polyacrylamide gel electrophoresis were delipidated and the apoproteins solubilized as previously described (Mahley and Weisgraber, 1974b). The apoprotein content of the purified lipoproteins was analyzed by gel electrophoresis on 10% polyacrylamide

† From the Section on Experimental Atherosclerosis (R.W.M. and K.H.W.) and Molecular Disease Branch (H.B.B. and G.A.), National Heart and Lung Institute, Bethesda, Maryland 20014, and Meloy Laboratories, Springfield, Virginia 22141 (T.I.). *Received January 14, 1975.* Portions of this work were performed under a National Heart and Lung Institute contract with Meloy Laboratories.

[1] Abbreviations used are: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; HDL_c, cholesterol-induced lipoproteins.

Table I: Plasma Lipids in Control and Cholesterol-Fed Swine.[a]

	Triglyceride	Total Cholesterol	Esterified Cholesterol	Phospholipid
		Controls[b]		
Mean	50	89	69	121
SD	21	23	15	29
		Cholesterol Fed[c]		
Mean	58	395	282	298
SD	41	95	72	63

[a] Lipid values expressed in mg/100 ml. [b] Values obtained from seven swine. [c] Values obtained from seven swine after 6–7 months on diet.

in a Tris buffer system (pH 8.9) in 8 *M* urea (Reisfeld and Small, 1966) and on 10% acrylamide in 0.1% sodium dodecyl sulfate (Weber and Osborn, 1969).

Chromatographic Isolation and Characterization of Apoproteins. The apoproteins of the B-VLDL or $d < 1.006$ fraction from hypercholesterolemic swine and the HDL of control swine were solubilized in 0.2 *M* Tris-Cl (pH 8.2) containing 4 *M* guanidine-HCl. Gel chromatography was performed at room temperature on G-200 Sephadex (Pharmacia) equilibrated with 0.2 *M* Tris-Cl (pH 8.2) containing 4 *M* guanidine-HCl on 2.5 × 200 cm columns; 20–40 mg of protein was chromatographed. Column fractions were dialyzed against 5 m*M* NH_4HCO_3 (pH 8.2) and lyophilized.

The apoproteins of HDL_c from hypercholesterolemic swine were solubilized in 1% sodium dodecyl sulfate in 0.01 *M* NH_4HCO_3 (pH 8.1) at room temperature for 8 hr. Gel chromatography was performed at room temperature on G-200 Sephadex equilibrated with 1% sodium dodecyl sulfate in 0.01 *M* NH_4HCO_3 (pH 8.1) on 2.5 × 200 cm columns. Column fractions were exhaustively dialyzed against 5 m*M* NH_4HCO_3 (pH 8.1) and lyophilized.

Purified apoproteins as judged by sodium dodecyl sulfate or Tris-urea acrylamide gel electrophoresis were subjected to amino acid analysis following hydrolysis on a Beckman 121 automatic amino acid analyzer (Benson and Patterson, 1965). Acid hydrolyses were performed at 110° for 24 hr in constant boiling HCl in the presence of 2-mercaptoethanol (1:2000 v/v) (Keufmann and Potts, 1969). The amino terminal residues were determined by the automated Edman technique with identification of amino acid phenylthiohydantoin by mass spectrometry (Fales et al., 1971; Fairwell and Brewer, 1973) and gas chromatography (Pisano et al., 1972).

Results

Plasma lipid levels in control miniature swine and miniature swine fed 1.5% cholesterol and 15% lard are presented in Table I. Within the first 3–4 months after initiating the high-cholesterol diet the plasma cholesterol ranged from 500 to 800 mg/100 ml and then generally decreased to a mean of approximately 400 mg/100 ml by 6–8 months. Lipoprotein electrophoresis of control plasma on paper and agarose revealed two bands which corresponded to the LDL (β) and HDL (α). Lipoprotein electrophoresis of the plasma from cholesterol-fed swine revealed a characteristic pattern. In addition to the β and α bands a second α-migrating band was evident (labeled HDL_c in Figure 1). The lipoproteins which trail from the origin may represent large or unstable particles.

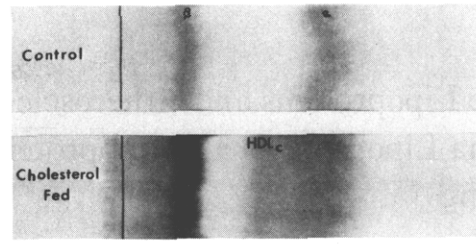

FIGURE 1: Paper electrophoretograms of the plasma lipoproteins from control and cholesterol-fed swine. Abbreviations defined in text.

Lipoprotein Distribution in Ultracentrifugal Fractions. The ultracentrifugal distribution of the plasma lipoproteins of control swine has previously been reported (Mahley and Weisgraber, 1974a). VLDL which resembled the human lipoprotein equivalent were present in the $d < 1.006$ fraction. LDL and HDL with chemical and physical properties similar to their human equivalents extended from densities of approximately 1.02 to 1.08 and 1.09 to 1.21, respectively. Lipoprotein electrophoresis patterns of several density fractions are shown in Figure 2.

Following cholesterol feeding, the type and distribution of the plasma lipoproteins were markedly altered (Figure 2). The $d < 1.006$ fraction in addition to the pre-β migrating (VLDL) lipoproteins also contained β-migrating lipoproteins referred to as β-VLDL (B-VLDL). In contrast to the control swine, the 1.006–1.02 fraction contained a prominent β-migrating lipoprotein. Two bands were observed in the density fractions between 1.02 and 1.087, one with β mobility which will be referred to as LDL and a second with α mobility which will be referred to as HDL_c. In the density fraction 1.087–1.21 there was a single α band migrating slightly faster than HDL_c which will be referred to as HDL_2.

A comparison of the distribution of the total plasma lipids and lipoprotein protein in the multiple density fractions of a control and two cholesterol-fed swine confirmed the qualitative changes suggested by the electrophoretograms (Table II). Lipoproteins of lower densities became more abundant, as reflected by a comparison of the distribution of total plasma cholesterol in the hypercholesterolemic swine with that of control swine. For example, in a representative control swine 64% of the total plasma cholesterol was transported by lipoproteins of $d < 1.06$, whereas following cholesterol feeding 80–90% of the plasma cholesterol was transported by lipoproteins of $d < 1.06$. This shift in prominence of lipoproteins to lower densities was also evident in the comparison of phospholipids and lipoprotein protein (Table II).

Isolation and Characterization of Lipoproteins Associated with Hypercholesterolemia. To characterize further the changes produced by cholesterol feeding, lipoproteins were isolated by the Geon-Pevion electrophoretic procedure from the several density fractions. The purified lipoproteins were subjected to chemical analysis, electron microscopy by negative staining, immunoelectrophoresis, and polyacrylamide gel electrophoresis. The purified lipoproteins from five hypercholesterolemic swine were analyzed with comparable results. The data from one experiment are presented.

Geon-Pevikon electrophoresis of the $d < 1.006$ fraction resulted in a broad band composed of two zones which were removed from the block separately. Approximately 85% of the total lipoprotein protein applied to the block was recovered in these two zones. The zone which was similar in mo-

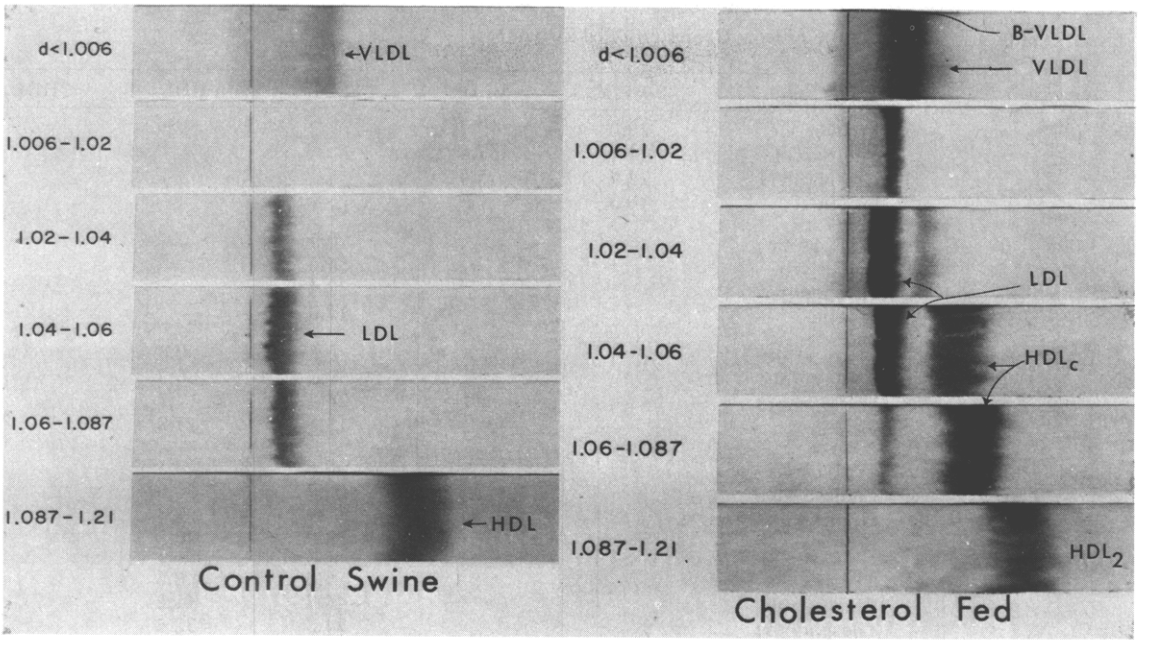

FIGURE 2: Paper electrophoretograms of sequential ultracentrifugal density fractions from a control swine and a cholesterol-fed swine.

bility to control and hypercholesterolemic LDL (approximately 7 cm from the origin) had β mobility on paper or agarose electrophoresis and was referred to as B-VLDL. The faster migrating zone (11 cm from the origin) had pre-β mobility and was referred to as VLDL. The chemical composition revealed that B-VLDL were composed of 29% triglyceride and 45% cholesterol of which 75% was esterified (Table III). By comparison VLDL were more triglyceride rich (50%) and contained 37% cholesterol of which 70% was esterified. Electron microscopy by negative staining demonstrated that the B-VLDL were smaller (200–350 Å) than the VLDL (300–700 Å in diameter; mean 550 Å) (Table IV). By comparison, VLDL from control swine were composed of 55–60% triglyceride and 10% cholesterol and ranged from 270 to 750 Å in diameter (Mahley and Weisgraber, 1974a).

The ultracentrifugal fractions of density 1.02–1.04, 1.04–1.06, and 1.06–1.087 subjected to Geon-Pevikon electrophoresis revealed two well-resolved zones which were removed separately for characterization. The zone which had β mobility on paper and agarose electrophoresis was referred to as LDL and the zone which had α mobility was referred to as HDL_c. The chemical composition and size by negative staining are shown in Tables III and IV, respectively. With increasing density the HDL_c were composed of less cholesterol (66% decreasing to 42%) and more phospholipid and protein. Also with increasing density the HDL_c particles were progressively smaller in size. Similar changes in composition and size occurred with the LDL (Tables III and IV). LDL from control swine were composed of approximately 45% cholesterol and were 180–220 Å in diameter (Mahley and Weisgraber, 1974a).

The ultracentrifugal fraction 1.087–1.21 was a single α band which migrated somewhat faster than the HDL_c on Geon-Pevikon electrophoresis. This fraction was referred to as HDL_2. The chemical composition (Table III) and size by electron microscopy (Table IV) revealed that this lipoprotein was protein rich, approximately 90 Å in diameter, and essentially identical with the main HDL of control swine plasma as previously reported (Mahley and Weisgraber, 1974a).

Table II: Percent Distribution of Lipid and Protein in Ultracentrifugal Fractions.

	Triglyceride	Total Cholesterol	Phospholipid	Protein
	Control Swine			
Plasma[a]	37	132	125	
$d < 1.006$	64.2	2.8	8.5	6.4
1.006–1.02	3.6	0.3	1.3	1.6
1.02–1.04	7.2	11.0	6.4	4.7
1.04–1.06	14.3	49.9	35.3	20.7
1.06–1.09	8.6	23.0	19.2	16.2
1.09–1.21	2.0	13.0	29.2	50.4
	Cholesterol Fed			
Plasma[a]	61	631	442	
$d < 1.006$	86.5	8.2	1.1	6.8
1.006–1.02	1.5	15.6	13.5	7.8
1.02–1.04	2.9	46.5	39.5	11.4
1.04–1.06	7.1	10.2	9.2	9.1
1.06–1.09	1.0	7.8	13.7	34.1
1.09–1.21	1.0	11.8	23.4	30.9
	Cholesterol Fed			
Plasma[a]	80	721	500	
$d < 1.006$	93.8	9.2	6.6	3.5
1.006–1.02	2.8	31.2	26.7	18.0
1.02–1.04	2.5	43.1	39.6	36.1
1.04–1.06	0.4	7.1	8.2	8.6
1.06–1.09	0.1	2.5	5.7	8.2
1.09–1.21	0.5	6.8	13.3	25.5

[a] mg/100 ml.

There were striking changes in the apoprotein patterns by polyacrylamide gel electrophoresis of the various lipoproteins isolated from the plasma of the hypercholesterolemic swine. A prominent apoprotein now appeared in the B-VLDL, VLDL, LDL at $d = 1.02$ and 1.04, and HDL_c. This peptide (labeled b in Figure 3) has been identified (characterization to follow) as equivalent to the arginine-rich apo-

Table III: Percent Composition of Lipoproteins from Cholesterol-Fed Swine.

d		B-VLDL	VLDL	LDL	HDL_c	HDL_2
<1.006	Triglyceride	29.0	49.7			
	Cholesterol	44.7	37.4			
	Phospholipid	14.9	5.4			
	Protein	10.3	7.5			
1.006–1.02	Triglyceride			0.2		
	Cholesterol			55.6		
	Phospholipid			32.4		
	Protein			12.0		
1.02–1.04	Triglyceride			0.5	0.5	
	Cholesterol			59.3	66.2	
	Phospholipid			24.7	17.5	
	Protein			15.6	15.8	
1.04–1.06	Triglyceride			0.6	0.5	
	Cholesterol			60.7	50.0	
	Phospholipid			20.7	29.3	
	Protein			18.0	20.3	
1.06–1.09	Triglyceride			6.0	0.6	
	Cholesterol			46.7	41.2	
	Phospholipid			24.3	32.5	
	Protein			23.0	25.7	
1.09–1.21	Triglyceride					0.5
	Cholesterol					24.2
	Phospholipid					34.3
	Protein					41.0

Table IV: Particle Size (Å) by Negative Staining.[a]

d	B-VLDL	VLDL	LDL	HDL_c	HDL_2
<1.006[b]	200–350	300–700			
1.006–1.02			200–320		
1.02–1.04			180–240	180–240	
1.04–1.06			160–240	140–220	
1.06–1.09				100–160	
1.09–1.21					70–110

[a] The diameters of approximately 200 particles for each lipoprotein class were measured. Each range represents more than 95% of the particles measured excluding the occasional particles at either end of the spectrum of sizes. [b] The unfractionated $d < 1.006$ fraction contained a variable number of 800–1100-Å particles. Approximately 85% of the total lipoprotein protein subjected to the Geon-Pevikon electrophoretic procedure was recovered as B-VLDL and VLDL. The large particles were not recovered and may account for a portion of the loss.

protein, previously identified in human VLDL (Shore and Shore, 1970; Shelburne and Quarfordt, 1974) and in the B-VLDL of Type III hyperlipoproteinemia (Havel and Kane, 1973). This apoprotein was in low concentration in control VLDL and possibly absent in control HDL.

The B apoprotein appeared to be a major component of the VLDL and β-migrating lipoproteins (LDL) isolated at densities of 1.006–1.02, 1.02–1.04, and 1.04–1.06. It was not present in the cholesterol-induced HDL_c or HDL_2. The absence of the B apoprotein was confirmed immunochemically. HDL_c and HDL_2 did not react with antiserum produced against control swine LDL. However, VLDL and the β-migrating lipoproteins from the hypercholesterolemic swine (density 1.006–1.06) reacted with identity against the LDL antiserum.

In addition to the arginine-rich apoprotein, the HDL_c contained a peptide with migration identical with that of the A-I apoprotein of HDL (band c) and an abundance of the fast-migrating peptides (zone d). The HDL_2 contained a major apoprotein equivalent to the A-I apoprotein present in control HDL (characterization to follow). The A-I apoprotein isolated from human HDL coelectrophoresed with the band identified as the A-I apoprotein of HDL_c and HDL_2.

Polyacrylamide gel electrophoresis in the sodium dodecyl sulfate system (Figure 4) confirmed the above impressions. The B-VLDL induced by cholesterol feeding revealed four bands. Band a is identified as the B apoprotein, b as the "arginine-rich" apoprotein, and c as the A-I apoprotein. Band d may represent the C apoproteins. The VLDL and β-migrating lipoprotein at $d = 1.006$–1.02 contained an abundance of the arginine-rich apoprotein. The major apoproteins in HDL_c had migrations identical with those of the "arginine-rich" equivalent, A-I, and the C apoproteins. In addition, the HDL_c (1.06–1.09) contained a slower migrating band which has not yet been characterized. The apoprotein patterns of the control HDL and the hypercholesterolemic HDL_2 were identical and their main apoprotein was identified as the A-I apoprotein.

Apoprotein Isolation and Characterization. Sephadex gel filtration with guanidine of B-VLDL or the $d < 1.006$ fraction resolved three fractions (Figure 5). Fraction I occurred near the void volume of the column and on polyacrylamide gel electrophoresis the protein did not enter the

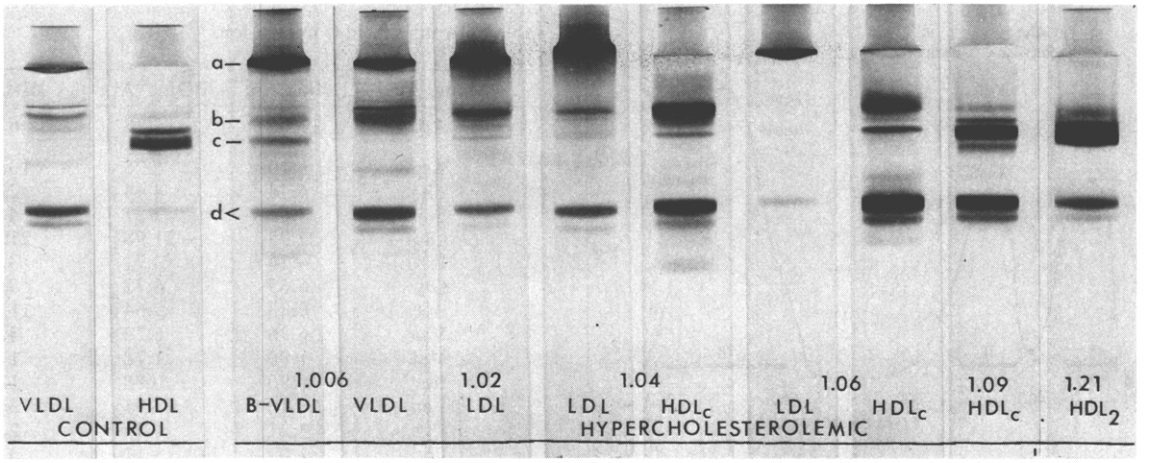

FIGURE 3: Polyacrylamide gel electrophoresis of the apolipoproteins from control and hypercholesterolemic swine on 10% acrylamide (pH 8.9). The sample applied contained 50 μg of lipoprotein apoprotein. The bands labeled a–d corresponded to the B apoprotein, the arginine-rich apoprotein, apo-A-I, and the several fast-migrating bands (probably apo-C), respectively.

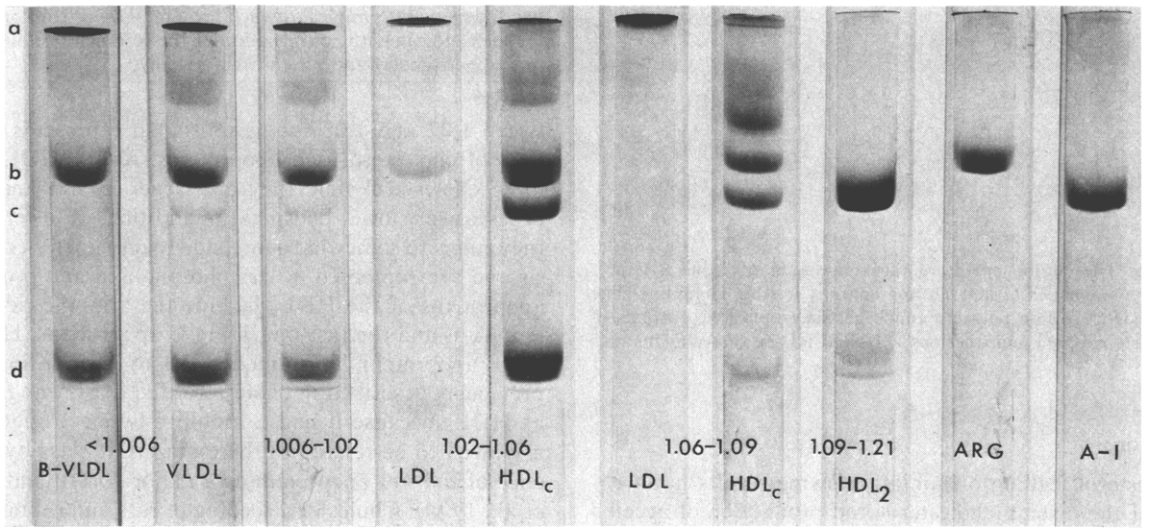

FIGURE 4: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the apolipoproteins from hypercholesterolemic swine and the purified A-I and "arginine-rich" apoproteins. The sample applied contained 8–12 μg of lipoprotein apoprotein. The bands labeled a–d corresponded to the B apoprotein, the arginine-rich apoprotein, apo-A-I, and the low molecular weight apoproteins, respectively.

running gel. Fraction II was obtained from the first portion of the second peak as indicated in Figure 5 and contained a single apoprotein, by sodium dodecyl sulfate and Tris-urea acrylamide gel electrophoresis, which was referred to as the "arginine-rich" equivalent. An apoprotein which coelectrophoresed with the A-I apoprotein of swine HDL contaminated the latter portion of the second peak. The third fraction contained several proteins with an elution volume consistent with proteins of molecular weight less than 20,000.

Sephadex gel filtration with sodium dodecyl sulfate of HDL_c resulted in an elution profile similar to that of the guanidine column with three peaks. The first peak contained an uncharacterized high molecular weight protein or proteins as evidenced by sodium dodecyl sulfate gel electrophoresis. The first half of the second peak contained a single protein, which coelectrophoresed with the B-VLDL protein, referred to as the "arginine-rich" apoprotein. The second half of this peak contained both the "arginine-rich" and A-I apoproteins. The third peak contained several proteins of molecular weight less than 20,000.

The "arginine-rich" apoprotein isolated from B-VLDL or HDL_c had the same migration on acrylamide gel electrophoresis as the major apoprotein present in the several lipoproteins induced by cholesterol feeding (Figures 3 and 4) and had an apparent molecular weight of approximately 34,000. Amino acid analysis revealed that the "arginine-rich" apoproteins of B-VLDL and HDL_c were essentially identical in composition and contained approximately 12 mol % arginine. Glutamic acid was identified as the amino terminal residue (Table V).

The major apoprotein of control swine HDL isolated by Sephadex gel filtration with guanidine coelectrophoresed with the human A-I apoprotein and appeared as a homogeneous protein by acrylamide gel electrophoresis. In addition, the A-I apoprotein isolated from HDL coelectrophoresed with a main band in HDL_2 and HDL_c. The amino acid analysis of swine A-I is compared to that of the "arginine-rich" apoprotein in Table V. The amino terminal amino acid was aspartic acid and the terminal sequence was H_2N-Asp-Asp-Pro-Gln-Ser-Pro-Trp-Asp-Arg-Val-. There was striking homology between this sequence and that reported from human A-I (Baker et al., 1974).

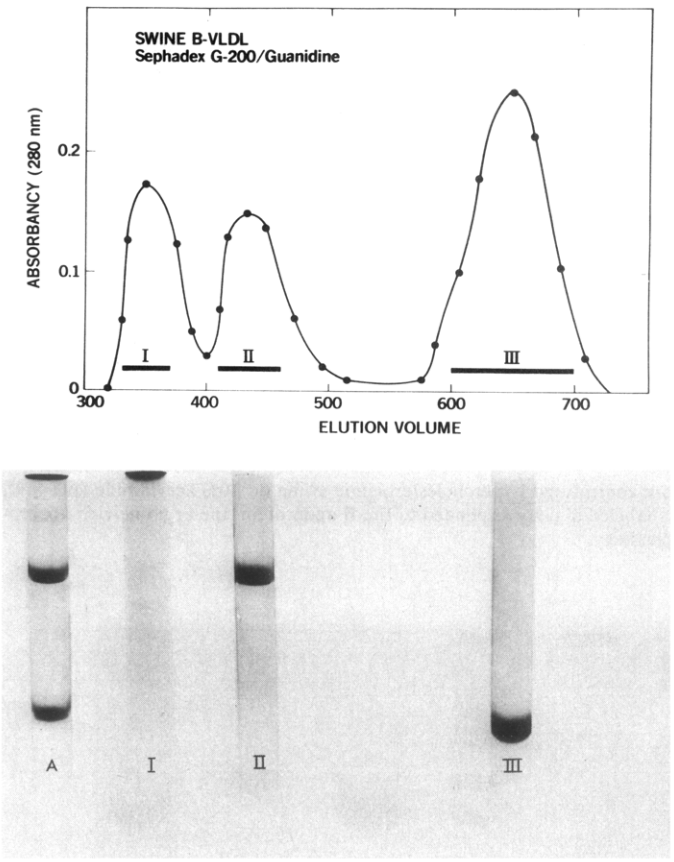

FIGURE 5: The elution profile of the apoproteins of swine B-VLDL obtained by Sephadex G-200 chromatography in 4 *M* guanidine. The sodium dodecyl sulfate polyacrylamide gel electrophoretic patterns of B-VLDL (labeled A) and fractions I, II, and III are shown at the bottom.

Table V: Amino Acid Composition.

Amino Acid[a]	1.006 "Arg"	HDL_c "Arg"	HDL A-I
Arg	11.90	11.78	6.79
Asp	6.19	6.25	10.36
Thr	4.44	4.44	2.55
Ser	7.51	7.37	4.73
Glu	22.95	21.98	21.00
Pro	4.24	3.71	3.54
Gly	6.65	6.72	5.01
Ala	9.83	9.64	11.91
Val	6.26	6.23	4.81
Met	1.90	1.78	1.54
Ile	0.90	0.98	1.11
Leu	13.10	12.95	13.98
Tyr	1.21	1.25	2.49
Phe	1.00	1.11	3.23
Lys	4.23	4.61	9.21
His	1.09	1.00	0.76
NH_2 terminus[b]	Glu	Glu	Asp

[a] Data presented as the average amino acid analysis (mol %) from two independent samples for each protein. [b] Data from one sample for each protein with confirmation of the terminal residue by both mass spectrometry and gas chromatography.

Discussion

Cholesterol feeding of miniature swine results in an elevation of the plasma cholesterol and production of accelerated atherosclerosis (Scott et al., 1971; Flaherty et al., 1972). The atherosclerosis, which begins as a proliferative intimal disease associated with lipid deposition and progresses to a complicated humanoid atheroma, is associated with marked alterations in the distribution and type of plasma lipoproteins as presented in this paper. The $d < 1.006$ lipoprotein fraction becomes a prominent fraction and contains both B-VLDL and VLDL. The B-VLDL of the swine are similar in composition to the B-VLDL reported to occur in human Type III hyperlipoproteinemia in that they are cholesterol rich and contain less than 30% triglyceride (Havel and Kane, 1973). The major apoproteins of the B-VLDL include the B apoprotein, the arginine-rich equivalent, and the fast-migrating apoproteins similar to the C apoproteins. The apoprotein pattern is similar to the pattern reported for Type III B-VLDL (Havel and Kane, 1973). The presence of B-VLDL with similar characteristics including the preponderance of the arginine-rich apoprotein has been reported following cholesterol feeding in rabbits (Shore et al., 1974), dogs (Mahley et al., 1974), the Patas monkey, and rats (unpublished observations).

An α-migrating lipoprotein referred to as HDL_c (cholesterol induced) becomes a prominent lipoprotein in the $d = 1.02$–1.087 density range following cholesterol feeding. A comparison of the physical and chemical characteristics of this α-migrating lipoprotein at various density ranges between 1.02 and 1.087 suggests that it represents a continuum of cholesterol-rich lipoproteins. As the HDL_c become more cholesterol rich, they are larger in size and float at progressively lower densities. In addition, at lower densities they migrate somewhat more slowly on paper electrophoresis and are composed of less phospholipid and protein. The apoproteins of the HDL_c include the arginine-rich equivalent, A-I, and the fast-migrating C apoproteins. HDL_c lack the B apoprotein. This unique class of plasma lipoproteins, previously described in cholesterol-fed dogs, was referred to as HDL_c because it had α mobility by electrophoresis and appeared to be related to the main HDL class by the presence of the A-I apoprotein as a major constituent (Mahley et al., 1974). Cholesterol feeding of rats and rabbits also results in the production of HDL_c (unpublished observations). In addition to the occurrence of B-VLDL and HDL_c, cholesterol feeding also results in an increase in LDL and the prominence of a β-migrating lipoprotein in the intermediate fraction ($d = 1.006$–1.02). The LDL and intermediate lipoproteins are cholesterol rich and apparently contain, in addition to the B apoprotein, the arginine-rich equivalent and fast-migrating C apoproteins in varying amounts.

The apoprotein identified as the "arginine-rich" equivalent in swine B-VLDL and HDL_c coelectrophoreses with the major apoprotein present in human B-VLDL from Type III patients, previously described as the arginine-rich apoprotein (Havel and Kane, 1973). This swine apoprotein has an amino acid composition and an arginine content similar to reported values for the human "arginine-rich" apoprotein (Shore and Shore, 1970; Havel and Kane, 1973). One of the unusual properties of this protein appears to be a strong interaction with the A-I apoprotein which apparently was not dissociated in 6 *M* urea on DEAE or 4 *M* guanidine on Sephadex chromatography. The arginine-rich apoprotein from HDL_c was purified from A-I only after sodium dodecyl sulfate Sephadex chromatography. Precise homology of the swine arginine-rich equivalent with the arginine-rich apoprotein of other species awaits further data.

It is reasonable to speculate on the origin of the B-VLDL and HDL_c in cholesterol-fed swine, dogs, rats, and rabbits.

As suggested for the human Type III hyperlipoproteinemia, the B-VLDL may represent remnants of chylomicrons and/or VLDL catabolism (Havel and Kane, 1973). Alternatively B-VLDL may be synthesized by either the liver (Shore et al., 1974) or the intestine, specifically to transport the increased cholesterol in the diet. On the other hand, the HDL_c may represent HDL_2 which become overloaded with cholesterol and float at progressively lower densities. The production of HDL_c may result from a transfer of cholesterol, phospholipid, and the arginine-rich apoprotein from the B-VLDL to the HDL_2. The interrelationships of the plasma lipoproteins induced by cholesterol feeding and their role in the production of accelerated atherosclerosis are open to speculation.

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