Studies on the Structure of Low Density Lipoproteins Isolated from *Macaca Fascicularis* Fed an Atherogenic Diet

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ABSTRACT Cynomolgus monkeys, *Macaca fascicularis*, fed cholesterol-containing saturated-fat diets develop increased levels of high molecular weight plasma low density lipoproteins (LDL), associated with accelerated atherosclerosis. To study the composition and structure of these abnormal particles, LDL from monkeys, fed atherogenic and control diets, were characterized chemically and examined by differential scanning calorimetry and low-angle X-ray scattering. LDL from animals on the experimental diet showed an increase in molecular weight (4.0 to 7.0 $\times$ 10^6, experimental diet compared with 3.0 to 3.7 $\times$ 10^6, control diet) associated with a large increase in cholesterol ester content and concomitant smaller increases in protein, phospholipid, and free cholesterol. There was a strong positive correlation between molecular weight and the number of saturated and monounsaturated cholesterol esters in the particle. In contrast, particle content of polyunsaturated cholesterol esters remained constant despite large changes in total particle cholesterol esters.

When examined by calorimetry and X-ray scattering, LDL from monkeys on both diets diplayed a reversible transition of cholesterol esters from an ordered smectic-like (layered) structure to a more disordered state. For all animals on the experimental diet, the peak temperature of the cholesterol-ester transition (42–48°C) was above body temperature (39°C), but below body temperature on the control diet (34–38.5°C). In the experimental group, the transition temperature was correlated with the LDL molecular weight. However, after thermal disruption of LDL, liquid-crystalline transitions of LDL cholesterol esters were observed in the same temperature range as in the intact lipoprotein, which shows that changes in particle size had little effect on the cholesterol-ester transition temperature. Rather, the transition temperature was determined by the degree of saturation of the LDL cholesterol ester fatty acids and the LDL cholesterol ester: triglyceride ratio, both of which correlated with increased LDL molecular weight.

The existence of smectic-like cholesterol ester in LDL at body temperature was clearly a discriminating feature between monkeys on control and experimental diets. Diet-induced changes in the lipid composition of precursor lipoproteins of LDL appeared to lead to the existence of smectic-like cholesterol ester in LDL above body temperature. The altered composition and structure of the core lipids of high molecular weight LDL probably account, in part, for the previously documented correlation between increased LDL molecular weight and atherosclerosis in this species.

INTRODUCTION

In an attempt to understand their role in the pathogenesis of atherosclerosis, the plasma lipoproteins have been studied in a number of non-human primate species receiving atherogenic diets (1–7). The hyperlipoproteinemia induced in *Macaca fascicularis* is characterized by increased concentrations of low density lipoprotein (LDL)[1] and decreased concentrations of high density lipoprotein, a profile resembling the atherogenic pattern of humans. In addition,

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[1] *Abbreviations used in this paper*: HDL, high density lipoprotein; HDL_c, HDL isolated from cholesterol-fed swine; LDL, low density lipoprotein.

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studies of LDL chemical composition and size in *M. fascicularis* and in Rhesus monkeys have indicated that these animals respond to atherogenic diets by developing cholesterol ester-enriched LDL particles of increased molecular weight (4–6). The LDL molecular weight of the more atherosclerosis-prone adult male *M. fascicularis* greatly exceeded that of the adult females of this species (7).

In a study of 40 *M. fascicularis* monkeys fed atherogenic and control diets, it was found that the average percent stenosis of the coronary arteries correlated more strongly with LDL molecular weight than with other variables (8), including total plasma cholesterol, LDL cholesterol, LDL cholesterol ester:protein ratio, and molar concentration of LDL. Factors that might explain the relationship between LDL atherogenicity and molecular weight probably include abnormalities of protein or lipid structure associated with increased size of LDL.

Recent studies, with techniques such as differential scanning calorimetry and low-angle X-ray scattering, have helped to elucidate lipid structure and lipid/protein interactions in the plasma lipoproteins (9–15). Human plasma LDL displays a broad, reversible thermal transition which encompasses body temperature associated with the transition of its core-located cholesterol esters from a smectic-like (layered) to a more disordered state (11, 15). In studies of normal human lipoproteins it was found that the peak temperature of this transition varied from 28 to 37°C and was related to the LDL cholesterol ester:triglyceride ratio and, to a lesser extent, the degree of saturation of the LDL cholesterol ester fatty acids (11). Because the peak temperature of the cholesterol-ester transition corresponds with the temperature at which about one-half of the cholesterol esters are ordered in a smectic-like state, and because the transition occurs over a broad temperature range, those individuals who have a peak temperature near body temperature probably have some ordering of their LDL cholesterol esters. Although the importance of the lipid organization of human LDL is unknown, studies of LDL from miniature swine fed atherogenic diets showed that their LDL cholesterol ester was more ordered at body temperature than that of normal human LDL, which suggests that the physical state of the LDL cholesterol esters might have a role in determining atherogenicity of LDL (13).

To investigate the interrelations between chemical composition, LDL molecular weight, and the physical state of LDL cholesterol esters, we have undertaken a detailed chemical and physical study of LDL from groups of *M. fascicularis* monkeys fed atherogenic and control diets. Our specific aims were (*a*) to examine the relationship between chemical factors known to influence the structure of LDL core lipids (cholesterol ester:triglyceride ratio and cholesterol ester fatty acid composition) and LDL molecular weight; and (*b*) to determine the effect of increased size of LDL on the structural organization of LDL core lipids.

METHODS

A group of 40 adult male *M. fascicularis* were conditioned to their new laboratory environment throughout a 90-day quarantine period. At the end of this period, a test diet containing 40% of calories as fat with a cholesterol-level of 0.70 mg/kcal (0.3% of diet) was fed for 60 days. At the end of this feeding period, the animals fasted for 24 h, a 30-ml blood sample was taken from each of the animals, and the group was then placed on a monkey chow diet (Ralston Purina Co., St. Louis, Mo.). After plasma cholesterol concentrations had returned to prechallenge levels, eight of the animals were fed a control diet for 60 days of the same composition as the test diet, except that the cholesterol level was 0.03 mg/kcal (0.015%). Blood samples were collected from these animals after a 24-h fasting period. All animals were immobilized with ketamine-HCl, 10 mg/kg, before blood sample collection. Blood collections and subsequent handling were carried out as described previously (16).

Plasma lipoproteins were isolated by centrifugation and separated by agarose column chromatography. Before examination by calorimetry or X-ray diffraction, samples were concentrated by vacuum dialysis. Lipoprotein cholesterol and protein distribution were determined after chromatographic separation (17). Measurement of cholesterol content was carried out by the method of Rudel and Morris (18) and protein was assayed with the procedure of Lowry et al. (19), with bovine serum albumin, fraction V (Sigma Chemical Co., St. Louis, Mo.) as the standard. Measurement of the molecular weight of the LDL was routinely carried out during chromatography with a standard [^{125}I]LDL marker as described previously (5). In addition, eight selected samples were subjected to molecular weight determination by the analytical ultracentrifuge method of Nelson et al. (20). The agreement between the two methods was excellent (Analytical ultracentrifugation, 5.24 ± 0.30 [$\times 10^6$] vs. Column method 5.2 ± 0.31 [$\times 10^6$], mean±SEM). LDL, isolated as peak III of the column elution profile, were then subjected to detailed chemical analysis, as described previously (5). Briefly, lipids were extracted, individual lipid classes separated by thin-layer chromatography, and determinations of the amounts of triacylglycerol (21) and free and esterified cholesterol (18) were performed. Phospholipid phosphorus (22), total cholesterol, and protein content was determined directly in the lipoprotein solution.

The cholesterol-ester fatty acid compositions were determined by gas-liquid chromatography, essentially according to Kuksis (23). The region of the thin-layer chromatography plate that contained the cholesterol esters was identified by staining an adjacent spot with rhodamine. The spots were scraped, extracted, and the esters were saponified. The fatty acids were then methylated with boron trifluoride in methanol (24). The methyl esters were then extracted into hexane, and separation of the fatty-acid methyl esters were carried out isothermally in a Bendix model 2500 Chromatograph (Bendix Corp., Environmental & Process Instruments Div., Baltimore, Md.) in 6-foot × ⅛-inch internal diameter glass columns packed with 10% EGSS-X on Gas-Chrom. P., 100–200 mesh (Applied Science Labs, Inc., State College, Pa.). Data were calculated and expressed as molar percentage values; standard National Institutes of Health fatty acid mixtures (Applied Science

TABLE I

Influence of Dietary Cholesterol on Distribution of Lipoprotein Cholesterol and Protein among Size Populations of Plasma Lipoproteins

Diet	n	Lipoprotein elution region on agarose column				Whole plasma*
		I	II	III	IV	
				mg/dl		
Cholesterol						
Control	8	2.1±1.21‡	13.9±1.81	79.7±10.6	68.1±5.00	163.8±13.24
Test	36	15.8±2.42	105.2±7.92	653.0±33.32	22.1±1.71	823.3±40.75
				mg/dl		
Protein						
Control	8	2.0±0.93	10.2±1.14	51.38±3.85	224.4±15.40	285.4±14.20
Test	36	4.7±0.67	49.3±3.78	340.2±17.60	81.3±7.02	475.5±24.16

* Determined on the samples used for lipoprotein isolation.

‡ Mean±SEM.

Labs, Inc.) were used to establish the relative weight responses of individual fatty acids.

Physical methods. Differential scanning calorimetry was performed on a Perkin-Elmer DSC-2 instrument (Perkin-Elmer Corp., Norwalk, Conn.), calibrated with cyclohexane, indium, and dimyristoyl lecithin, as previously described (9–12). 75-μl samples were sealed hermetically in sample pans and scanned at heating and cooling rates of 2.5 or 5°C/min. Most experiments were performed at a sensitivity of 0.1 or 0.2 mcal/s.

X-ray scattering measurements were made on samples sealed in Lindeman glass tubes (Lindeman Corp., Indianapolis, Ind.) with CuKα radiation from an Elliott GX-6 rotating anode X-ray generator (Marconi-Elliott Avionics, Ltd., Borehamwood, England), with Franks or toroidal mirror optics (15).

Hot-stage polarizing light microscopy was performed with a Zeiss NL polarized light microscope (Carl Zeiss, Inc., New York) as previously described (25). Samples were examined under glass coverslips at heating and cooling rates of 1–2°C/min. The smectic phase of cholesterol esters was identified by its positive sign of birefringence, and the cholesteric phase by its negative sign (25).

Statistical analysis were performed as described in Snedecor and Cochran (26), and differences are at the $P < 0.01$ level, unless otherwise indicated.

RESULTS

The agarose column elution profiles of the plasma lipoproteins resembled that described previously.[2] The control samples contained more material absorbing at 280 nm in the region IV high density lipoproteins (HDL) than in the other lipoprotein fractions. The hyperlipoproteinemia induced by the test diet was characterized by a shift in lipoprotein distribution so that LDL of region III contained by far the most 280-nm-absorbing material, while the amount of material in the HDL region was decreased compared with the control samples. The average size of the test-diet plasma LDL was larger, as demonstrated by the shift to a lower elution volume at the center of peak III. To quantitate the shift in lipoprotein distribution induced by dietary cholesterol, the total cholesterol and protein content in each of the regions of the column elution profile was measured (see Table I). Significant increases in the amount of protein and cholesterol in regions I (very low density lipoprotein), II (intermediate density lipoprotein), and III (LDL) occurred, and significant decreases in the region IV (HDL) were present.

Samples from nine animals that encompassed the range of LDL molecular weight variation observed among those that received the test diet, were selected for detailed studies. Samples from eight monkeys on the control diet were also studied in detail (Tables II–V, and Figs. 1–5). Four of the nine animals examined on the atherogenic diet were also examined on the control diet; that is, they acted as their own controls. There was a significant increase in LDL molecular weight induced by dietary cholesterol; i.e., control $3.3\pm0.10 \times 10^6$ vs. atherogenic $5.3\pm0.33 \times 10^6$ (Table II). Also, there was a higher percentage of protein ($P < 0.05$) and phospholipid, and lower percentage of cholesterol ester in the low molecular weight LDL of the control diet group (Table II). The mean composition within the particle was calculated from the percentage composition and the molecular weight data as shown in Table III. Free cholesterol and phospholipid increased in proportion to molecular weight, while the phospholipid:free-cholesterol ratio decreased. The most striking increase was in the number of cholesterol ester molecules per particle. Because there was a small decrease in the tryglyceride content of LDL on the experimental diet compared with the control, there was an increase in the cholesterol ester:triglyceride ratio in the experimental group (mean±SEM = 206±52),

[2] Rudel, L. L., R. Shah, and D. G. Greene. 1978. Study of the atherogenic dyslipoproteinemia induced by dietary cholesterol in Rhesus monkeys (*Macaca mulatta*). Manuscript submitted for publication.

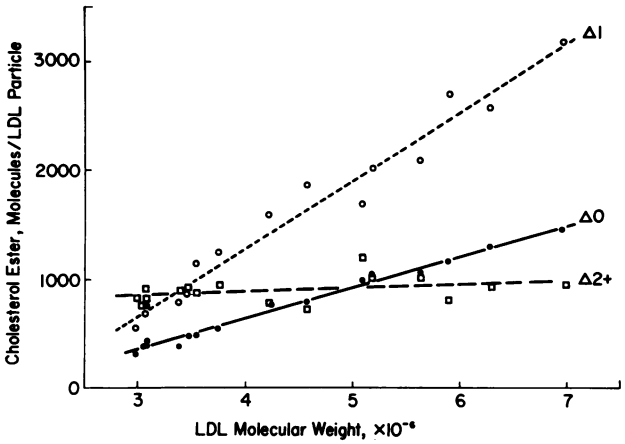

FIGURE 1 Relationship of the content of specific cholesterol esters of LDL to the molecular weight. Plasma LDL samples from eight animals on the control diet (molecular weight $< 4 \times 10^6$) and eight animals on the test diet (molecular weight $> 4 \times 10^6$) were isolated by agarose column chromatography, and the LDL molecular weights were determined. Chemical compositions of each sample were then determined (see Table III), and the cholesterol ester fatty-acid composition was measured by GLC (see Table V). The content of specific cholesterol esters of each LDL sample was then calculated and plotted versus the molecular weight of that sample. Δ0 refers to cholesterol esters with saturated fatty acids; Δ1 refers to cholesterol esters with monounsaturated fatty acids; and Δ2+ refers to cholesterol esters with polyunsaturated fatty acids. The lines drawn are the best fit regression lines.

compared with the controls (41±9.9, $P < 0.05$). On the test diet there was an increase in the protein content of higher molecular weight LDL.

The average diameter for each particle was calculated, with the formula for the volume of a sphere to relate volume and diameter, and with the partial specific volume of each component to calculate volume. These calculations assume that high molecular weight LDL has similar structure to normal LDL, i.e., with a core of apolar lipid and a surface coat of phospholipid, protein, and free cholesterol (15). The diameters increase in proportion to molecular weight from a value of 207 Å at a molecular weight of 2.98×10^6 to a value of 277 Å at 6.97×10^6 (Table IV). The volume of the core of the LDL particle was estimated by adding that of cholesterol ester and triglyceride which occupy the core. When the core diameter was subtracted from that of the whole particle and the difference divided by 2, the thickness of the cholesterol-phospholipid-protein coat was obtained. This value (22 Å) was constant throughout the range of LDL molecular weights. Thus, all of the size difference in the LDL particles resulted from an increase in core diameter. The LDL cholesterol ester fatty-acid composition is shown in Table V. There was a trend in cholesterol ester fatty-acid composition with increasing molecular weight: the higher the molecular weight, the higher the percentage of cholesteryl oleate (18:1) and stearate (18:0), and the lower the percentage of cholesteryl linoleate (18:2) and arachidonate (20:4) (see Table V). The relation between LDL molecular weight and particle content of cholesterol esters of different saturation is shown in Fig. 1. In LDL-particles of increasing molecular weights there was a fixed amount per LDL particle of cholesterol ester which contained polyunsaturated fatty acids (Δ 2 + fatty acids) such as cholesteryl linoleate and arachidonate. However, there was an increased content of saturated and monounsaturated cholesterol esters proportional to LDL molecular weight. The correlation coefficient (r) between LDL molecular weight and the amount of monounsaturated cholesterol esters was $r = 0.98$, while that for saturated cholesterol esters was $r = 0.99$.

Physical data. In the scanning calorimeter, both control and experimental LDL displayed a broad, reversible transition of enthalpy 0.77±0.08 cal/g cholesterol ester (mean±SD), which resembled that previously described for human LDL (10) (Fig. 2). There was a progressive decrease in the peak temperature of this transition with decreasing molecular weight (atherogenic LDL of molecular weight 6.97×10^6 is shown in Fig. 2*a* and *b*, 5.18×10^6 in Fig. 2*c* and *d*, and control-diet LDL of molecular weight 3.08×10^6 in Fig. 2*e* and *f*). On the test diet, the peak temperature of the cholesterol-ester transition ranged from 42 to 48°C and correlated with LDL molecular weight

TABLE II

Comparison of LDL Mass Composition among Normal and Hyperlipoproteinemic M. fascicularis

Diet	n	MW* (×10⁻⁶)	Percentage (*wt/wt*)				
			FC	CE	TG	PL	Pro
Control	8	3.29±0.10‡	8.5±0.27	43.4±1.27	2.0±0.39	23.3±0.55	22.8±1.04
Test	9	5.31±0.33	8.8±0.15	51.1±0.60	0.4±0.13	18.9±0.43	20.7±0.55

* Abbreviations: MW, average molecular weight of LDL particle; FC, free cholesterol; CE, cholesterol ester; TG, triacylglycerol; PL, phospholipid; Pro, protein.
‡ Mean±SEM.

TABLE III

*Relationship of LDL Mean Number of Molecules within Particle to Molecular Weight**

Diet	MW‡	FC	CE	TG	PL	Pro
		molecules/LDL particle				
Control	2.98§	556	1,697	132	945	31
	3.06§	664	1,909	41	880	32
	3.07§	674	1,993	86	891	28
	3.08	677	1,996	51	861	31
	3.37	809	2,057	104	1,126	29
	3.46	687	2,271	84	1,112	30
	3.54	868	2,510	28	1,018	29
	3.74§	840	2,765	41	1,081	28
Test	3.98§	853	2,910	8	1,049	35
	4.22	972	3,138	18	1,130	35
	4.57§	979	3,405	41	1,100	41
	5.09§	1,132	3,846	9	1,179	47
	5.18	1,150	4,108	33	1,274	40
	5.64	1,269	4,230	10	1,460	48
	5.92	1,437	4,714	84	1,288	46
	6.29§	1,432	4,856	41	1,498	52
	6.97	1,710	5,633	66	1,655	50

* Molecular weights used to calculate these data were: FC, 387; CE, 667; PL, 775; TG, 900; Pro, 25,000.

‡ Abbreviations are the same as in Table II.

§ Indicates the four monkeys who served as their own controls.

($r = 0.77$, Fig. 3). Although there were only small amounts of LDL available from monkeys on the control diet, analysis of five samples of LDL, (three individual samples and two pooled on the basis of similarity in molecular weight) also indicated a correlation between transition temperature and molecular weight ($r = 0.83$, Fig. 3). There was a notable hiatus between transition temperatures obtained for LDL from animals receiving control and test diets which occurred at the body temperature of the monkey (39°C).

When heated to higher temperature, LDL displayed a small irreversible endotherm of enthalpy which was ≅1 cal/g protein (e.g., Fig. 2*a*). This value is similar to the lipoprotein denaturation endotherm observed previously for human LDL (10). The peak temperature of the lipoprotein denaturation endotherm ranged from 79 to 87°C and correlated with particle molecular weight ($r = 0.78$). After lipoprotein denaturation a double-peaked endotherm was observed in the same temperature range as the cholesterol-ester transition of the intact lipoprotein. However, this enthalpy, 1.1 ±0.05 cal/g cholesterol ester (mean±1 SD), had a value significantly greater than the corresponding enthalpy in the intact lipoprotein ($P < 0.01$). Despite variation in size, the enthalpy of the cholesterol-ester endotherm of the intact lipoprotein was consistently ≅70% of the enthalpy of the corresponding endotherm in the denatured lipoprotein, which indicates that the LDL cholesterol-ester transition did not vary in enthalpy with molecular weight (Fig. 3). Polarized light microscopy of

TABLE IV

LDL Particle Diameter and Molecular Weight Relationships

Diet	Molecular weight	Particle diameter	Diameter of core	Thickness of coat
		Å	Å	Å
Control	2.98	207	161	23
	3.06	209	163	23
	3.07	210	168	21
	3.08	210	166	22
	3.37	216	169	23.5
	3.46	219	174	22.5
	3.54	220	178	21
	3.74	225	184	21.5
Mean				22.2
Test	3.98	229	186	21.5
	4.22	234	191	21.5
	4.57	240	197	21.5
	5.09	248	204	22.0
	5.18	251	209	21.0
	5.64	258	211	23.5
	5.92	262	220	21.0
	6.29	267	221	23.0
	6.97	277	233	22.0
Mean				21.9*

* Mean thickness of coat (Control + Test): 22.1±0.2.

TABLE V

Cholesterol Ester Fatty-Acid Composition of LDL Particles of Varying Molecular Weight

Diet	MW* (×10⁻⁶)	Molar percentage of cholesterol ester fatty acids						
		14:0	16:0	16:1	18:0	18:1	18:2	20:4
		%						
Control	2.98	1.0	13.3	1.7	4.5	31.2	44.6	3.7
	3.06	1.0	14.2	2.5	5.1	37.5	36.4	3.3
	3.07	2.2	12.5	2.2	4.8	32.0	44.8	1.5
	3.08	1.9	14.1	2.3	5.5	35.5	39.2	1.6
	3.37	0.7	13.3	1.7	4.5	36.8	39.4	3.6
	3.46	1.4	13.7	2.7	5.8	35.6	39.3	1.4
	3.54	1.0	12.3	2.7	5.6	43.2	33.2	2.0
	3.74	0.7	12.1	3.7	7.2	41.7	32.6	2.0
Test	4.22	0.7	15.6	3.3	8.1	47.9	20.3	4.1
	4.38	0.5	15.2	2.9	9.0	45.5	22.3	4.6
	4.57	0.7	14.8	3.4	7.7	52.0	18.2	3.2
	5.09	1.3	13.9	2.8	8.1	41.9	27.0	4.9
	5.18	1.0	15.4	3.1	9.2	46.8	21.2	3.4
	5.64	1.1	14.9	2.9	9.4	47.4	21.3	2.9
	5.92	1.1	14.0	3.7	9.6	54.2	15.3	1.8
	6.24	2.2	15.7	4.2	9.3	49.4	19.2	0.1
	6.97	0.7	15.2	3.6	10.2	53.3	15.3	1.9

* Average molecular weight of LDL particle.

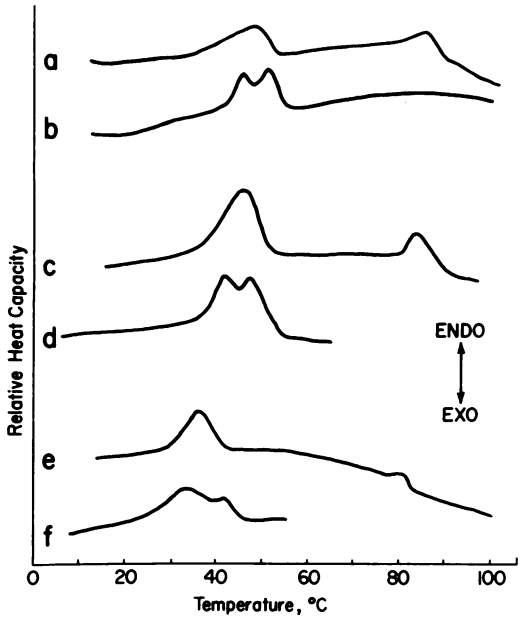

FIGURE 2 Differential scanning calorimetry heating curves of *M. fascicularis* LDL of different molecular weights. The molecular weights were *a* and *b*, 6.97×10^6; *c* and *d*, 5.18×10^6; *e* and *f*, 3.08×10^6. *a–d* are from animals on the atherogenic diet, and *e* and *f* are from an animal on the control diet. The plasma lipoproteins were isolated by centrifugation, and separated by agarose column chromatography. LDL was concentrated by vacuum dialysis to concentrations of 50–150 mg/ml and 75-μl aliquots were hermetically sealed in stainless steel differential scanning calorimetric capsules. The samples were heated at 5°C/min and cooled at 20°C/min (cooling curves not shown), with instrument sensitivities of 0.2 or 0.4 mcal/s. In *a*, *c*, and *e* the native lipoprotein is heated from 0 to 100°C. Lipoprotein disruption occurs between 80 and 90°C. The sample is then cooled and reheated from 0 to 100°C, curves *b*, *d*, and *f* (upper part of heating curves omitted for *d* and *f*). *Endo* represents the direction of endothermic transitions, while *Exo* shows the direction of exothermic transitions.

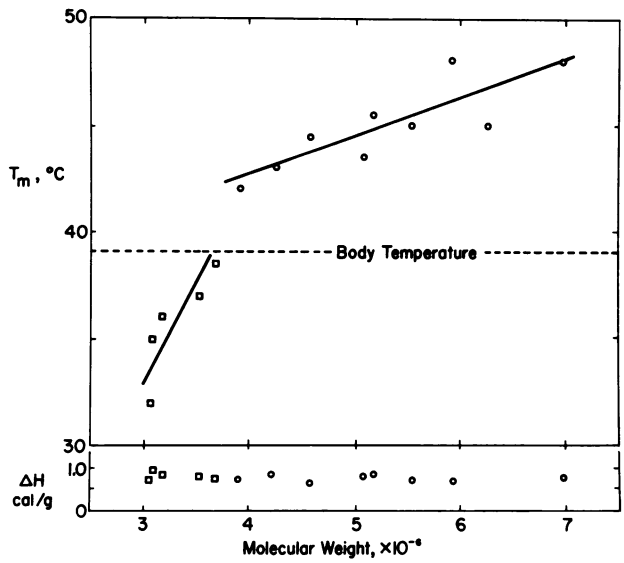

FIGURE 3 Relation between the peak temperature (T_m) above, the enthalpy (ΔH) of the cholesterol-ester transition below, and molecular weight ($\times 10^{-6}$) of *M. fascicularis* LDL. The peak temperature and enthalpy of the smectic-disordered cholesterol-ester transition of LDL were determined for animals on atherogenic diets (circles) and control diets (squares) with differential scanning calorimetry. Whereas the peak temperature of the cholesterol-ester transition correlated with molecular weight for animals on both control ($r = 0.83$) and experimental ($r = 0.77$) diets, the enthalpy of the cholesterol-ester transition was invariant. To obtain sufficient material for analysis, LDL samples of similar molecular weight were pooled in the control group. The average molecular weights of the pooled samples are shown. The samples were (2.98, 3.06 and 3.07×10^6), (3.08×10^6), (3.19×10^6), (3.46×10^6), and ($3.54, 3.74 \times 10^6$).

heat-denatured LDL showed that the peaks of this transition corresponded to smectic-cholesteric and cholesteric-liquid transitions of the liberated cholesterol esters (25). The temperatures determined by microscopy were within 1°C of the peak temperatures noted by differential scanning calorimetry.

The main determinant of LDL transition temperature was the saturation of cholesterol ester fatty acids ($r = 0.94$, controls; $r = 0.74$, experimentals, Fig. 4). Cholesterol ester fatty-acid composition was also correlated with the lipoprotein denaturation temperature ($r = 0.76$). LDL from some animals in the control group had notably lower transition temperatures than test animals with similar cholesterol ester fatty-acid saturation. The lower transition temperatures in the controls are attributed to the lower cholesterol ester:triglyceride ratio of the control group.

X-ray scattering. Irrespective of LDL molecular weight, the X-ray scattering profiles of LDL that was isolated from control and test animals, recorded at 10°C (below the thermal transition), exhibited a series of scattering maxima which included a maximum of high relative intensity at 1/36 $Å^{-1}$. This maximum at 1/36 $Å^{-1}$ was absent from the scattering profiles recorded for all LDL samples above the thermal transition.

The scattering profile (Fig. 5*a*) obtained for the control LDL (3.08×10^6 daltons) closely resembled that previously reported for human LDL (11, 15), exhibiting five, subsidiary scattering maxima with the fifth maximum at 1/36 $Å^{-1}$ having a high relative intensity. The high molecular weight LDL from animals on the test diet exhibited scattering profiles in which the positions, number, and relative intensities of the scattering maxima showed variations compared with the control LDL and human LDL. For example, the scattering profile for LDL of molecular weight 5.6×10^6 shown in Fig. 5*b* exhibits six subsidiary maxima at closer angular spacings than the maxima observed for the control LDL, consistent with a larger particle size.

In the case of human LDL and HDL isolated from

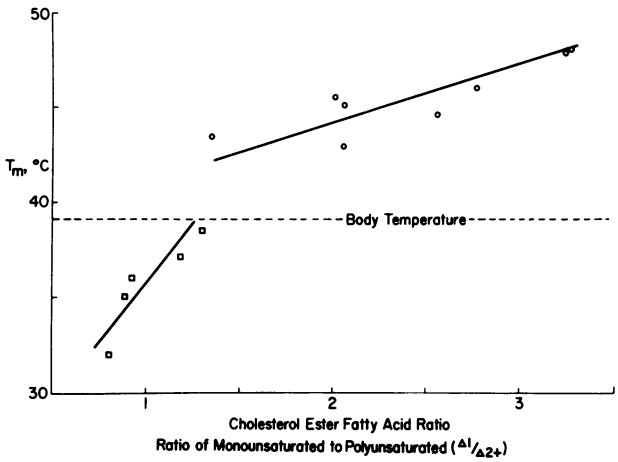

FIGURE 4 Relation between peak temperature of the cholesterol-ester transition and saturation of the cholesterol ester fatty acids of *M. fascicularis* LDL. The peak temperature (T_m) of the smectic-disordered transition of LDL cholesterol ester was determined by differential scanning calorimetry and related to an index of saturation of the cholesterol ester fatty acids (the ratio of monounsaturated to polyunsaturated cholesterol ester fatty acids). T_m correlated with saturation of cholesterol ester fatty acids on both control ($r = 0.94$) and atherogenic ($r = 0.74$) diets.

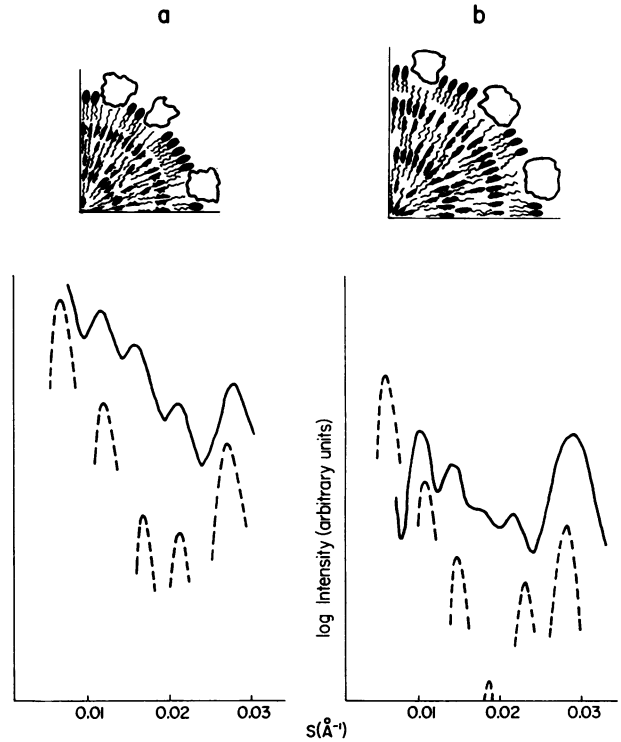

FIGURE 5 Small-angle X-ray scattering profiles for LDL isolated from control and test animals recorded at 10°C. (*a*) Control animals (LDL molecular weight 3.08×10^6), (*b*) test animal (LDL molecular weight 5.6×10^6). The solid lines are the experimentally determined profiles after subtraction of the background scattering as a result of the sample tube and buffer. The dotted lines are the calculated scattering profiles for electron density models that correspond to the cholesterol-ester organization depicted in the inserts. In the experimental profiles, the first maximum at small angles is not resolved in these densitometer traces. The schematic organization shown for the control LDL (*a*) is taken from references 16 and 28. The organization depicted for the high molecular weight LDL (*b*) is derived from that in (*a*) by the addition of one cholesterol-ester repeating unit in the radially layered organization.

cholesterol-fed swine (HDL_c) (14) the high relative intensity of the 1/36 Å^{-1} maximum in the scattering profiles observed below the thermal transition has been shown (15, 27) to arise from a radially layered arrangement of the cholesterol esters in the core of the particle. This radially layered organization results in concentric regions in the core of the particle in which the cholesterol moieties of the cholesterol esters are packed in register (13, 27). The smaller size of HDL_c (90-Å radius compared to 110-Å radius for human LDL) results in a scattering profile which exhibits four subsidiary maxima, the fourth maximum located at 1/36 Å^{-1} having a high relative intensity. This profile indicates that HDL_c has one fewer layered molecular units in the radially layered structure (27).

The observation that the sixth maximum is located at 1/36 Å^{-1} and shows a high relative intensity for the larger molecular weight LDL, demonstrates that below the thermal transition the cholesterol esters are also organized in a regular radially layered packing. The particle volume, core volume, and core diameter calculations presented in Table IV indicate that the radial dimension of LDL of molecular weight $>5 \times 10^6$ is sufficient to accommodate an additional layered molecular unit in the core. The inserts to Figs. 5*a* and *b* show schematic representations of the cholesterol ester organization below the transition in the control LDL and in an LDL of molecular weight $>\cong 5 \times 10^6$, respectively. The representation of the packing in the control LDL is identical to that previously given for human LDL. The schematic for the packing in the high molecular weight LDL was derived from that of the control LDL by the addition of one layered molecular unit in the radial cholesterol-ester organization. Also shown in Figs. 5*a* and *b* are the theoretical scattering profiles for models which correspond to the cholesterol-ester packing shown in the schematics. As in the case of human LDL (15) the calculated scattering profile for the model of the control LDL is in good agreement with the profile obtained experimentally. Furthermore, the profile derived from the model of the high molecular weight LDL which contained an additional molecular layer of cholesterol ester is in reasonable agreement with the profile obtained from LDL of molecular weight 5.6×10^6, exhibiting six subsidiary maxima with the sixth maxima at 1.36 Å^{-1}

having a high relative intensity. This observation of increased numbers of subsidiary maxima for lipoproteins of large molecular weight with the relative intensity of the maxima in the 1/36 $Å^{-1}$ region enhanced substantiates the radial nature of the layered cholesterol-ester packing in the smectic-like organization of the cholesterol esters in the particle core.

DISCUSSION

The occurrence of high molecular weight LDL has been documented previously in several species of monkeys fed atherogenic diets (4–6). With an increase of LDL molecular weight there was a large increase in the cholesterol-ester content of each particle, with smaller increases in protein, phospholipid, and free cholesterol, as would be expected for spherical particles with a core of cholesterol ester and a surface of polar lipids and protein. In the present study, we show that when the atherogenic diet of *M. fascicularis* was replaced by a control diet, the concentration of LDL cholesterol, protein, and LDL molecular weight decreases, and HDL concentration increases. These data show that the diet-induced dyslipoproteinemia found to be correlated with coronary atherosclerosis (9) is reversible. In addition, we have documented a strong correlation between LDL molecular weight and cholesterol ester fatty-acid saturation, and have shown that the atherogenic diet produced an increase in the LDL cholesterol ester:triglyceride ratio. The latter compositional factors are known to be important determinants of the structure of the LDL core lipids (9, 10, 13, 14).

LDL of *M. fascicularis* displayed a reversible thermal transition of their cholesterol esters from a smectic-like to a more disordered state, resembling a similar transition identified previously in human and swine LDL and swine HDL_c (11, 14). The evidence that the thermal transition in LDL is a result of such a structural transition of its cholesterol esters is based on analogy with the melting behavior of purified cholesterol esters and on the presence below the transition of a maximum at 1/36 $Å^{-1}$ in the X-ray scattering profile of LDL that arises from a layered arrangement of core cholesterol esters (11, 12, 14, 15). In the present study, the temperature of the cholesterol-ester transition of LDL was found to correlate with LDL molecular weight, (Fig. 2). However, this was not a result of a direct effect of particle size on the organization of cholesterol esters, as the cholesterol-ester transition temperature of thermally disrupted LDL was similar to that of the intact particle. In fact, the correlation of transition temperature and particle molecular weight resulted from a common relationship of both variables with degree of saturation of LDL cholesterol ester fatty acids.

The degree of saturation of LDL cholesterol ester fatty acids was correlated with cholesterol ester transition temperature for animals on both control and test diets (Fig. 3). The variation of cholesterol ester/triglyceride did not correlate well with transition temperature within the experimental group, because of the virtual absence of triglyceride in experimental-group LDL. In the control group LDL as well as in normal human LDL (11) both the cholesterol ester:triglyceride ratio and cholesterol ester fatty-acid composition are important in determining the LDL cholesterol-ester transition temperature. However, when particle triglyceride is very low, as occurs in several species fed atherogenic diets (2, 4, 14, 28), and perhaps in some individuals with homozygous familial hypercholesterolemia (29), cholesterol ester fatty-acid composition is the main determinant of LDL transition temperature.

An unexpected finding was the lack of correlation between the enthalpy of LDL cholesterol-ester transition and particle molecular weight, despite examination of particles with a wide range of molecular weights, from 3 to 7×10^6. In fact, even smaller particles such as HDL_c of swine (molecular weight, $1.9–2.3 \times 10^6$) show an enthalpy of the particle cholesterol-ester transition (0.83 cal/g cholesterol esters) similar to the larger LDL. An analysis of the X-ray scattering data with Fourier-Bessel transform techniques indicates that particles of increased size retain the same layered organization of cholesterol esters described previously (15, 27), but that they have added layers of cholesterol ester within the particle core. The lack of dependence of the cholesterol-ester transition enthalpy upon particle molecular weight suggests that the constraining effect of the particle on the liquid crystal melt is exerted throughout the particle core and extends for at least two or three layers of cholesterol ester. A reduction in enthalpy, dependent upon a non-melting layer of cholesterol ester contiguous with the particle surface, or upon a disordered region of lipids at the particle center, would be reflected in a progressive increase in enthalpy with increasing molecular weight. The experimental results are thus consistent with the proposed model of LDL where the reduction in melting enthalpy is a result of the persistence above the transition of a radially oriented, nematic-like arrangement of cholesterol esters (11, 15, 30). The latter is the predominant structural organization of LDL cholesterol esters in normal humans and control monkeys.

With increased molecular weight of LDL, there was an increased content of saturated and monounsaturated cholesterol ester fatty-acid particles, and a constant contribution from diunsaturated and polyunsaturated cholesterol ester fatty acids (Fig. 1). Because lecithin acyltransferase preferentially esterifies cholesterol with polyunsaturated fatty acids derived from the 2-

carbon position of phospholipid glycerol (31), these findings suggest that a relatively constant amount of LDL cholesterol esters in all samples was derived from esterification of cholesterol within the plasma. The cholesterol esters with more saturated fatty acids may have been derived from esterification of cholesterol within the liver or within the small intestinal mucosa. In the rabbit, preferential esterification and incorporation of dietary cholesterol into lymph chylomicrons and very low density lipoproteins has been shown (32). Increased dietary cholesterol may lead to lymph chylomicrons and very low density lipoproteins that have an increased particle content of cholesterol ester. Catabolism of these particles may then lead to formation of cholesterol ester-enriched remnants (33) and, ultimately, to cholesterol ester-enriched LDL particles. Although no data is available in the monkey, the high content of palmitic, stearic, and oleic acids in cholesterol esters would reflect the activity of an intestinal cholesterol-ester synthetase that preferentially utilizes more saturated and monounsaturated fatty acids, as has been described in the rat (34). If the high cholesterol test diet results in chylomicrons that contained an increased number of saturated and monounsaturated cholesterol esters then the remnant would also contain these esters.

LDL molecular weight was shown to bear a strong correlation with the development of coronary atherosclerosis in *M. fascicularis*, independent of total plasma cholesterol concentration (9). Variables of LDL composition and structure might explain this correlation. LDL molecular weight was strongly correlated with the content of saturated fatty acids of cholesterol ester ($r = 0.99$), which suggests the hypothesis that atherogenicity of the high molecular weight LDL is related to the fatty-acid composition and, thus, the physical state of its cholesterol esters. In culture of monkey aortic smooth muscle cells, abnormal Rhesus monkey LDL (35, 36) or high molecular weight *M. fascicularis* LDL (37) stimulate cholesterol ester accumulation to a greater extent than does normal monkey LDL. The cholesterol ester accumulating in response to hyperlipemic serum is thought to be derived largely from uptake of serum cholesterol ester (36). Because the affinity for binding to LDL receptors on smooth muscle cells is similar for both the high molecular weight and normal LDL,[3] accumulation of excess cholesterol ester may simply reflect the internalization of two to three times more cholesterol ester molecules per layer LDL particle. In addition, because the atherogenicity of the high molecular weight LDL may depend upon the physical state of its cholesterol esters, accumulation of cholesterol ester contained in these particles may reflect a relative inability of lysosomal acid lipase to degrade the more ordered cholesterol esters, perhaps as a result of the limited accessibility of the enzyme to the ester linkage in the smectic phase (25, 38). Thus, the structure of LDL core lipids may play an important role in determining a relative deficiency of acid lipase. The latter has been suggested by Peters and DeDuve (39), and Berberian and Fowler (40) to be a factor in the development of rabbit and human atherosclerosis.

[3] St. Clair, R. W. Personal communication.

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