Structure and Interactions of Lipids in Human Plasma Low Density Lipoproteins*

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Temperature-dependent techniques (differential scanning calorimetry, polarizing microscopy, and x-ray scattering and diffraction techniques) were used to compare the properties of human plasma low density lipoproteins (LDL) with its extracted lipid classes. Three types of thermal transitions were characterized: (a) a reversible transition in intact LDL near body temperature associated with a liquid crystalline order-disorder phase change of cholesterol esters within the particles; (b) an irreversible high temperature transition (−70–90°C) associated with LDL denaturation and release of cholesterol esters from the disrupted particles; and (c) low temperature transitions related to liquid crystalline and crystalline phase changes in these released esters. The temperature of the reversible transition in intact LDL varies among individual donors. Correlation analysis shows that the temperature of this transition negatively correlates with the amount of triglyceride relative to cholesterol ester in LDL. Studies on mixtures of cholesterol esters and triglycerides isolated from LDL show a similar effect, increasing amounts of triglyceride decreasing the temperature of the liquid → smectic liquid crystalline transition of the isolated esters. Thus, the amount of triglyceride in LDL influences the fluidity of the cholesterol esters in LDL.

The enthalpy of the reversible transition in intact LDL is 0.89 cal/g of LDL cholesterol ester. This compares with 0.89 cal/g for the liquid → liquid crystalline transition of the cholesterol esters released from denatured LDL and 1.01 cal/g for the same transition in the extracted esters. Unlike the cholesterol esters released from denatured LDL, or isolated LDL esters, cholesterol ester in the intact LDL particle does not crystallize. These findings suggest that the behavior of cholesterol esters in intact LDL is constrained relative to their behavior when freed from the restrictions of the particle. These results together with experiments on partitioning of the individual lipid classes of LDL allow us to define the distribution and interaction of lipids in the intact LDL particle.

Human serum low density lipoprotein (LDL)§ is a major carrier of serum cholesterol in man. Of the serum lipoproteins, LDL is richest in cholesterol, which contributes over one-half the particle weight. Phospholipid, small amounts of triglyceride, and apolipoprotein B make up the remainder of the particle. LDL is a quasi-spherical particle, diameter 200 to 220 Å (1), with most of the polar lipids and apoprotein molecules exposed in the aqueous environment and covering the apolar constituents located toward the center of the particle. The lipid-lipid and lipid-protein interactions in LDL are not known in detail, although we have provided evidence that the cholesterol esters of LDL are located in a core within the particle (2). Using differential scanning calorimetry (DSC) and x-ray scattering techniques, we have defined a transition in intact LDL in the range of body temperature and correlated this with a reversible phase change of cholesterol ester molecules from an ordered smectic-like liquid crystalline state to a more disordered liquid-like state (2). On the basis of the cooperativity of this transition, we suggested that a cholesterol ester rich core must exist in intact LDL (2).

Other groups have reported temperature-induced changes in solutions of intact LDL, although interpretations differed as to the physical basis for these transitions. Changes in the circular dichroism and optical rotatory dispersion spectra of LDL near physiological temperature were thought to be associated with changes in the conformation of the apoprotein in the intact particle (3, 4). Pollard and Chen (5) proposed that these changes were not related to cholesterol esters in that as much as 90% of this lipid could be removed from LDL without significant alteration of the circular dichroism spectra. However, on the basis of circular dichroism and optical rotatory dispersion studies of isolated individual LDL lipids, Chen and Kane (6) concluded that the changes observed in intact LDL could be related to thermal effects on the optical properties of these lipids. Keith et al. (7) using spin label analogues of cholesterol esters (cholestereryl 12-N-oxyl stearate) demonstrated discontinuities between 20 and 31°C on an Arrhenius plot of rotational correlation times of this probe in LDL. They suggested that this cholesterol ester-like probe was free to "tumble" in LDL at 37°C to a far greater degree than probe analogues of free cholesterol or fatty acids.

In man, elevated serum LDL is positively correlated with an increased incidence and severity of atherosclerosis (8). In abetalipoproteinemia in which LDL is virtually absent from the serum and serum cholesterol is very low, abnormalities are found in red blood cells and neural tissues suggesting that LDL and cholesterol are necessary for the normal maintenance of at least some cellular membranes (9). LDL is...
bound and taken up by a variety of tissues, such as arterial smooth muscle cells (10), human skin fibroblasts (11), and human lymphocytes (12). Inside the cell the LDL cholesterol ester is hydrolyzed and liberated free cholesterol regulates cholesterol synthesis. Therefore, LDL is important in regulation of cholesterol metabolism in extrahepatic tissues.

Dietary factors influence the composition and perhaps the metabolism of LDL. Feeding a diet high in unsaturated fat lowers serum cholesterol, LDL cholesterol, and the relative saturation of the fatty acids in LDL lipids (13). Thompson and Sigurjonsdottir (14) have recently suggested that increasing the saturation of the fatty acids in LDL (as measured by the oleic/linoleic ratio) is related to a reduction in the catalytic role of apoprotein B. Subjects on a high saturated fat diet showed a decrease in the microporous fluidity in all lipoprotein classes at a given temperature as measured by pyrene fluorescence and TEMPO paramagnetic resonance (15). Thus, the physical state of the lipid constituents may be an important determinant in the metabolism of these particles.

We have investigated the physical behavior of the isolated lipid constituents of LDL, alone and in combination, and compared their behavior to intact and thermally denatured LDL. The results suggest that (a) the amount of triglyceride present in LDL is important in determining the thermotropic properties of LDL and (b) the LDL particle imposes significant constraints on the interactions of its constituent lipids.

The results allow us to propose a model emphasizing the distribution and arrangement of the lipid molecules in LDL.

MATERIALS AND METHODS

Isolation of Lipoproteins—Blood was collected in vacuum packs containing 1 mg/ml of disodium EDTA from fasting males normolipemic subjects and 7 fasting patients with type IIA hypercholesterolemia (five males and two females). LDL from the latter group was kindly provided by Drs. S. M. Grundy (Department of Medicine, University of California, San Diego) and R. S. Leses (Arteriosclerosis Center, Massachusetts Institute of Technology). These patients were on low cholesterol, low animal fat diets at the time of sampling. Red and white cells were immediately sedimented by centrifuging in an IEC centrifuge model PR-2 at 4°C for 45 min at 2,000 rpm. 1 ml was prepared by repetitive ultracentrifugation between salt densities of 1.025 and 1.050 g/ml using density solutions of NaCl and KBr as previously described (16). All centrifuge runs were at 13°C for 18 h at 40,000 rpm in a Beckman 40.5 rotor. All solutions contained 1 mM EDTA and were adjusted to pH 8.5 using NaOH. The density solutions used for increasing densities and washing lipoproteins were verified by pyknometry. Lack of contamination with other plasma proteins and lipoproteins was assured in each preparation by immunodiffusion and immunoelectrophoresis (17). After isolation, LDL was dialyzed in the dark for 24 h against 0.19 M NaCl, 1 mM EDTA, pH = 8.5, 140, v/v at 4°C with a minimum of three changes of dialysate. When necessary, LDL was concentrated from the areas under peaks as measured by planimetry and related to the area of the crystal-liquid melting transition of an indium standard (ΔH~ = 8.60 cal/g).

Analysis—LDL protein concentration was determined by the method of Lowry et al. (18) using bovine serum albumin as a standard and correcting for the difference in tyrosine content between the two proteins. Total LDL concentration was calculated from the protein and lipid content (see below), as well as by at least duplicate dry weight determinations. Lipids were extracted quantitatively from LDL in at least 40 volumes of chloroform/methanol (2:1, v/v). Lipid composition of each LDL preparation was analyzed by quantitative thin layer chromatography using the method of Downing (19) as modified by Katz et al. (20). Cholesterol, cholesterol ester, triglyceride, and fatty acid were separated in the solvent system of hexane, diethyl ether, and acetic acid (7:3:0:1, v/v/v).

Individual phospholipids were developed in chloroform:methanol:water:acetic acid (65:25:4:1). Individual lipid classes were isolated by preparative thin layer chromatography on glass plates (20 cm) coated with 1 mm of Silica Gel G. The plates were developed to three quarters of their length in hexane:dichloroethylen ether (94:6). Following elution with chloroform:methanol (2:1, v/v), lipids were layered with nitrogen and stored at ~20°C in the dark. Prior to use, lipids were dried in a nitrogen stream and placed in a vacuum desiccator containing P2O5 for 24 h at room temperature. The purity of each isolated lipid class was verified by thin layer chromatography.

A fatty acid composition of the cholesterol ester fraction was determined using gas-liquid chromatography on a Hewlett Packard model 100 equipped with a hydrogen flame ionization detector. A 6-foot column packed with 10% SP2340 on 100/120 Supelcoport (Supelco Inc., PA.) was run at a temperature of 195°C. The cholesterol esters were hydrolyzed in 2% alcoholic KOH (21) and fatty acid methylation was accomplished by refluxing at 80°C with 14% BF3, in methanol for 30 min (22). Methyl esters were extracted into hexane. Retention times were standardized using a known mixture of methyl esters (Supelco Inc., PA.). Relative amounts of methyl esters were determined by multiplying peak height by retention time.

Mixtures of Lipoprotein Lipids—Mixtures of extracted lipid lipoproteins were made by precipitating appropriate amounts of individual lipids into DSC pans (see below), followed by the addition of 10 μl of chloroform or chloroform/methanol (2:1, v/v) to ensure mixing. Free cholesterol was added in benzene saturated with cholesterol. Samples were then dried at a vacuum desiccator for 24 h and crimped. When required, appropriate amounts of d = 1.066 solution were added before crimping.2 To ensure equilibration prior to calorimetry, microscopy, and x-ray diffraction, samples were heated to 50°C and held for 5 min. After heating, the mixture was rapidly cooled to ~20°C.

Differential Scanning Calorimetry—Calorimetric studies were performed on a Perkin Elmer DSC 2 differential scanning calorimeter at a full scale sensitivity of 0.1 to 0.2 mcal/s with heating and cooling rates of 2.5 to 10°C/min. Samples, depending on size and concentration, were hermetically sealed in aluminum (10 μl) or glass (70 μl) sample pans (Perkin-Elmer). LDL samples usually contained 1 to 3 mg of LDL and isolated lipid samples 1 to 2 mg of lipid. All aqueous samples were run with equivalent amounts of solvent in the reference pan. Aqueous samples that were cooled to ~80°C were heated at the programmed heating rate and held at 1°C until the ice liquid water transition was complete, and the heating run was then continued. Temperature calibration was performed to within ±0.2°C using indium and cyclohexane standards. Endotherms of transitions (ΔH) were calculated from the areas under peaks as measured by planimetry and related to the area of the crystal-liquid melting transition of an induction standard (ΔH~ = 8.60 cal/g).

Polarizing Light Microscopy—Samples of concentrated LDL and isolated lipid extracts were examined using a Zeiss standard NL polarizing light microscope on a controlled heating-cooling stage at rates of 1-3°C/min. The optical texture, signs of birefringence, and gross rheological changes were determined as previously described (23, 24).

X-ray Scattering/Diffraction—X-ray scattering and diffraction patterns of intact LDL, denatured LDL, and extracted lipid lipoproteins were examined for comparison with either: Elliot (25) 4-circle polaroid mirror or Franks (26) double mirror optics, utilizing nickel-filtered CuKα radiation from an Elliot GX-8 rotating anode generator. Specimens were sealed in either 1-mm diameter Lindemann glass tubes or...
Low Density Lipoprotein Structure

Table I

<table>
<thead>
<tr>
<th>LDL compositional data from normal and hypercholesterolemic donors</th>
<th>Normal donors</th>
<th>Hypercholesterolemic donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individual donors</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Protein (protein weight)</td>
<td>20.9 ± 1.7</td>
<td>20.2 ± 2.1</td>
</tr>
<tr>
<td>Cholesterol composition (weight % of total lipids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>12.5 ± 1.0</td>
<td>13.1 ± 1.6</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>56.0 ± 4.5</td>
<td>55.6 ± 3.4</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>3.2 ± 1.3</td>
<td>4.2 ± 2.0</td>
</tr>
<tr>
<td>Total phospholipids</td>
<td>20.3 ± 2.2</td>
<td>27.1 ± 2.1</td>
</tr>
<tr>
<td>Lysolecithin</td>
<td>0.3 ± 0.4</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>8.0 ± 1.3</td>
<td>9.3 ± 1.2</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>20.0 ± 2.0</td>
<td>17.0 ± 2.5</td>
</tr>
<tr>
<td>Cholesterol ester fatty acids (weight % of total methylated fatty acids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.8</td>
</tr>
<tr>
<td>16:0</td>
<td>11.4 ± 1.3</td>
<td>19.2 ± 1.5</td>
</tr>
<tr>
<td>18:1</td>
<td>3.2 ± 1.2</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>18:2</td>
<td>1.1 ± 0.8</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>18:3</td>
<td>19.5 ± 4.1</td>
<td>17.8 ± 0.3</td>
</tr>
<tr>
<td>18:4</td>
<td>57.6 ± 8.2</td>
<td>57.2 ± 7.5</td>
</tr>
<tr>
<td>Total</td>
<td>5.8 ± 2.4</td>
<td>6.5 ± 1.1</td>
</tr>
<tr>
<td>Others</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Serum lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>191 ± 45</td>
<td>360 ± 78</td>
</tr>
<tr>
<td>Triglyceride (mg/100 ml)</td>
<td>87 ± 36</td>
<td>147 ± 53</td>
</tr>
<tr>
<td>Others has to be noted</td>
<td>(38 ± 13)</td>
<td>(58 ± 20)</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± 1 S. D.

Range.

between two Mylar windows and placed in a variable temperature sample holder. Relative intensity measurements were obtained from x-ray photographs using a Joyce Loebel model III CS microdensitometer.

**Turbidity Experiments** - The absorbance of LDL solutions was studied as a function of temperature on Cary model 14 spectrophotometer (courtesy of Dr. R. Simons, Biochemistry Department, Boston University School of Medicine). In studies of the thermal denaturation of LDL (5 mg of LDL/ml), the optical absorption of the sample at 650 nm was compared with a reference solution (s = 1.006 saline).

**Statistical Analysis** - Compositional and other variables for normal and hypercholesterolemic donors were compared by nonpaired t tests. Pearson product-moment correlation and partial correlation analyses were performed to determine the associations between experimental variables. Further relationships between certain pairs of variables were determined by linear regression analysis using the method of least squares. When two variables showed a significant correlation (p < 0.05), analysis of variance for the regression was performed to show that F ratios were significant and that the slopes of the regression line were significantly different from a horizontal line (37).

**RESULTS**

**Composition of LDL**

The composition of the lipoproteins used in this study is given in Table I. There were no significant differences in either the contribution of each lipid class to the total lipid weight or the fatty acyl cholesterol ester composition between the 14 LDL samples obtained from normal donors and the 7 obtained from hypercholesterolemic donors. The protein to lipid ratio was similar in both groups. Between the two groups, only serum cholesterol (p < 0.001) and triglyceride (p < 0.05) and the ratio of LDL phosphatidylcholine to sphingomyelin (p < 0.001) were significantly different. This ratio, however, showed no correlation with any other compositional or experimental data. Following the statistical analysis of the LDL compositional and calorimetric data presented below, no further significant differences were apparent between the two donor groups. Hence, the results reported herein are derived from both donor groups.

**Studies Relating to Intact LDL**

**Transitions in Intact LDL** - The calorimetric transitions observed in intact LDL are summarized in Fig. 1. On heating intact LDL from 0-45°, an endothermic transition (Fig. 1a) is observed between 20 and 40° which is perfectly reversible on cooling (Fig. 1b). Identical transitions are obtained with repeated runs between 0 and 45° or after cooling the sample to -60° (Fig. 1c). Twenty-one LDL preparations showed qualitatively similar behavior. Although multiple samples of LDL from a single donor showed almost identical transitions, transitions of LDL taken from different individuals varied considerably (Fig. 1b). The mean onset temperature of this transition in 21 preparations was 17.3 ± 3.3° (range 11-23°), the peak temperature 30.3 ± 2.3° (range 26.0-36.5°), and the end temperature 41.0 ± 1.7° (range 36-44°). Overall, the transition width spanned 22.7 ± 2.9° (range 18-37°). These temperatures were independent of heating rate from 2.5° to 10°C/min except that the end temperature tended to decrease by 1 to 1.5° at the slowest heating rates.

Transitions were unaffected by the concentration of LDL. As shown in Fig. 2, the enthalpy of the transition showed no correlation with LDL concentration over a wide concentration range. The wider range of enthalpy values at lower concentration reflects the relatively high baseline noise to signal ratio making area measurement less accurate. Therefore, quantitative data were taken only from samples containing greater than 150 mg of LDL/ml. 3 Using electron microscopy we observed no significant difference between solutions which were concentrated to 250 mg/ml and diluted...
A similar thermal dependence has been demonstrated for a 36
obtained over an LDL concentration range of 19 to 260 mg/ml.

Furthermore, after centrifuging capillary tubes of concen-
trated LDL solutions in normal saline at 20,000 rpm for 5 h no free
lipid floated to the top of the tube. Finally, over the angular range
studied, x-ray data obtained using concentrated LDL solutions were
identical with those using samples 10 times less concentrated.

Fig. 2. Transition enthalpy ($\Delta H$) of intact LDL (heating from 0-
45°) plotted against LDL concentration in 0.19 M NaCl. (a) points
taken from separate LDL preparations; (b) points taken from differ-
et LDL preparations at different concentrations of LDL.

A diffraction spacing shown by both isolated LDL cholesterol
esters and pure cholesterol linoleate and olate when melting
from the smectic liquid crystalline phase (2).

The intensity of the scattering fringe at 36 Å in intact LDL
follows closely the transition observed by calorimetry. The
intensity of this fringe recorded at intervals from tempera-
tures below, through, and above the transition progressively
decreases until the peak temperature of the transition (Fig. 4).
At this temperature, the fringe at 36 Å has disappeared, but
reappears with the same intensity on cooling to temperatures
below the transition.

Liquid Crystalline Transitions of Isolated Lipids of LDL –
On cooling isolated LDL cholesterol ester shows different
thermal behavior compared to intact LDL. (see Fig. 5, a and d)
Unlike LDL, the isolated esters show two transitions on
cooling from 45°. As demonstrated by polarizing microscopy,
the initial smaller transition represents a liquid to cholesterol
liquid crystal phase change and this is followed by a choles-
teric to smectic liquid crystal transition. As shown in Table
IIIC, the total enthalpy of the cholesterol ester liquid-liquid
crystalline transitions (1.01 ± 0.11 Cal/g) was almost 50%
greater than that obtained with intact LDL (expressed as
calories/g of LDL cholesterol ester).

Phospholipid, triglyceride, and free cholesterol isolated
from LDL do not exhibit liquid crystalline transitions between
0 and 45° when studied by calorimetry, polarizing microscopy,
or x-ray diffraction. The single sharp 36 Å maximum is charac-
teristic of a solution of homogenous spherical particles

transition enthalpies of intact LDL are listed in Table IIA. The
enthalpy of the reversible transition was 0.38 ± 0.04 Cal/g of
LDL total lipid or 0.69 ± 0.06 Cal/g when expressed in terms of
LDL cholesterol ester. Table IIA shows that this enthalpy was
very similar for cooling transitions or for LDL which was
cooled to -60° and reheated.

X-ray scattering of LDL at temperatures below and above
the calorimetric transition shows a series of scattering fringes
characteristic of a solution of homogenous spherical particles
(Fig. 3). The relative intensity and position of each maximum
changes little from 10-45° with the notable exception of the
maximum centered at s = 2.8 × 10^{-2} Å^{-1} (equivalent to a
Bragg spacing of 36 Å), which is absent at 45°. This maximum
appears again on recollision to 10°. Identical results have been
obtained over an LDL concentration range of 19 to 260 mg/ml.
A similar thermal dependence has been demonstrated for a 36
Å diffraction spacing shown by both isolated LDL cholesterol

again to 1 mg/ml (R. Deckelbaum and E. Rogers, unpublished re-
sults). Furthermore, after centrifuging capillary tubes of concen-
trated LDL solutions in normal saline at 30,000 rpm for 5 h no free
lipid floated to the top of the tube. Finally, over the angular range
studied, x-ray data obtained using concentrated LDL solutions were
identical with those using samples 10 times less concentrated.

Fig. 3. (left). X-ray scattering curves of LDL in 0.19 M NaCl, pH
8.5, obtained after subtracting the scattering curve of the buffer
solution (Franks' double mirror optics). Arrow indicates the maxi-
num at 36 Å which is present when LDL is studied at 10° (a) but
absent at 45° (b). S = 2 sin $\theta$/\lambda (where $\theta$ is the scattering angle and
$\lambda$ is the x-ray wavelength.

### Table IIA

<table>
<thead>
<tr>
<th>Transition</th>
<th>LDL Total Lipid</th>
<th>LDL Cholesterol Ester</th>
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</thead>
<tbody>
<tr>
<td>0-45°</td>
<td>0.38 ± 0.04</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>45-0°</td>
<td>0.38 ± 0.01</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>-60-45°</td>
<td>0.39 ± 0.10</td>
<td>0.68 ± 0.14</td>
</tr>
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 transitions in intact LDL

A. Transitions in intact LDL

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Enthalpy (Cal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-45°</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>45-0°</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>-60-45°</td>
<td>0.39 ± 0.10</td>
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B. Transitions in denatured LDL

<table>
<thead>
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<th>Temperature</th>
<th>Enthalpy (Cal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-45°</td>
<td>0.50 ± 0.07</td>
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<tr>
<td>-60-45°</td>
<td>3.13 ± 0.69</td>
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C. Transitions in isolated LDL cholesterol ester

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Enthalpy (Cal/g)</th>
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</thead>
<tbody>
<tr>
<td>45-0°</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>-60-45°</td>
<td>8.33 ± 1.04</td>
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D. Transition of LDL denaturation

<table>
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<tr>
<th>Temperature</th>
<th>Enthalpy (Cal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45-100°</td>
<td>0.97 ± 0.22</td>
</tr>
</tbody>
</table>
teristic only of isolated LDL cholesterol esters in the smectic liquid crystalline phase. The other lipid components of LDL do not give rise to a similar diffraction pattern.

The effects of the other lipid classes of LDL on the liquid crystalline transitions of LDL cholesterol esters were studied by mixing these lipids with the isolated esters in similar proportions to those found in LDL. Addition of free cholesterol (Fig. 5b), in a proportion likely to be found in cholesterol esters in LDL (see below), slightly lowers the temperature of the liquid → cholesteric and cholesteric → smectic transitions of the isolated cholesterol ester. In marked contrast, triglycerides added to cholesterol ester in similar proportions to those found in LDL result in a single broad transition lower in temperature (Fig. 5c) and very similar to that obtained with intact LDL (compare Fig. 5, c and d). Thus, small amounts of LDL triglyceride (approximately 2 to 3% of the total mixture) abolish the liquid → cholesteric transition in LDL cholesterol ester and the smectic phase is formed directly on cooling the isotropic liquid. Addition of free cholesterol to the cholesterol ester-triglyceride mixture results in a slight decrease in its transition temperature without altering the shape of the transition. The transition enthalpies of these mixtures did not differ significantly from that of the pure LDL cholesterol esters.

Partitioning of Lipoprotein Lipids—Total lipid extracts of LDL were equilibrated in excess water and the resultant lipid phases separated by means of ultracentrifugation. Upon removal from the ultracentrifuge, the tube had four easily discernible layers (Fig. 6). The top layer (layer A) totally excluded water and was a clear yellow single phase oil. Just below was a white opaque layer (layer B) which contained water. The separating water layer was clear immediately below was a white opaque layer (layer B) which contained water and was a clear yellow single phase oil. The bottom layer (layer C) was a milky opaque thick liquid. The lipid composition of each layer was determined (Table III). Layer A was predominantly cholesterol ester with small amounts of triglyceride and free cholesterol but no phospholipid. Layer C was predominantly phospholipid with approximately 25% (by weight) free cholesterol, traces of cholesterol ester, and no triglyceride. The middle layer (layer B) was mainly cholesterol ester but the relative amounts of the other lipids were more variable than in layers A or C.

Polarizing light microscopy showed that the cholesterol ester-rich layer A was a single phase exhibiting liquid → liquid crystal transitions on cooling from 45°. The absence of a liquid → cholesteric transition was consistent with the presence of triglyceride. The bottom layer (layer C) was a hydrated phospholipid liquid crystalline lamellar phase in excess water which showed no transitions between 0 and 60°. The middle layer (B) was made up of two lipid phases in water, oil droplets with the melting behavior of cholesterol esters, surrounded by layers of phospholipid.

By DSC, layer A on cooling from 45° showed only a single
Taking the reciprocals of distances 2X and ZY, a mixture at cholesterol ester is unlikely to be affected by the presence of triglyceride (24). The phase equilibrium of cholesterol and found to lie on a straight line (XY) through zone III which diagram, the points for different experiments for layer A lie in which these three components partition into each phase can be determined from the reciprocal of the distances of the point to zones II and I (29). Knowing the compositions at each end of the line X and Y (Table III), the way in which these three components partition into each phase can be calculated as long as the composition of the mixture falls on the straight line (29). Triglyceride was always solubilized by the cholesterol ester oil phase. Since the solubility of cholesterol in cholesterol ester does not increase in the presence of triglyceride (24), the phase equilibrium of cholesterol and cholesterol ester is unlikely to be affected by the presence of small amounts of triglyceride.

As seen in Fig. 6, the mean value for normal lipid LDL composition (point 2) falls in zone III on the straight line. Taking the reciprocals of distances Z and ZY, a mixture at point Z would then be made up of 50.6% of the cholesterol ester-rich phase and 40.4% of the phospholipid-rich phase, Table IV lists the distribution of each lipid class in the two phases and shows that only free cholesterol partitions significantly between the two phases, although most (84%) is incorporated into LDL phospholipid.

Therefore, if the lipids in intact LDL show similar behavior to the model systems, one would predict that all LDL triglyceride would partition into the neutral cholesterol ester core, together with only relatively small amounts of free cholesterol.

**Correlations between Transitions in Intact LDL and LDL Composition**—To ascertain what effects the composition of LDL might have on the reversible transition in LDL, the transition temperatures and enthalpies were compared with a wide variety of compositional variables. As well, each variable was compared with every other variable.

As illustrated in Fig. 7A, increasing amounts of LDL triglyceride relative to LDL cholesterol ester negatively correlated with the peak transition temperature \( r = -0.61, p = 0.005 \). This relationship is mainly determined by the triglyceride content of LDL as cholesterol ester content alone did not correlate with the peak temperature. Triglyceride alone has a correlation coefficient of \( -0.60 (p = 0.006) \). In addition, both the onset and the end temperature of the transition correlated with the amount of triglyceride relative to cholesterol ester and \((f)\) arachidonate of total cholesterol esters; \((g)\) weight per cent of each of saturated, monounsaturated, and polyunsaturated cholesterol esters of total cholesterol esters; \((h)\) ratio of polyunsaturated cholesterol esters to sum of saturated plus monounsaturated cholesterol esters; \((i)\) ratio of polyunsaturated to monounsaturated cholesterol esters; \((j)\) weight per cent of total LDL lipids of free cholesterol, cholesterol ester, triglycerides, and phospholipid; \((k)\) weight per cent of free cholesterol relative to cholesterol ester; \((l)\) weight per cent of triglyceride relative to cholesterol ester; \((m)\) weight per cent of free cholesterol plus triglyceride relative to cholesterol ester; \((n)\) ratio of total phospholipid to free cholesterol; \((o)\) ratio of phosphatidylcholine to sphingomyelin; \((p)\) weight per cent protein to total particle weight; \((q)\) weight ratio of free cholesterol to protein; \((r)\) weight ratio of cholesterol ester to protein; \((s)\) weight ratio of triglyceride to protein; and \((u)\) ratio of the lecithin-sphingomyelin ratio to weight per cent protein.

Table IV

<table>
<thead>
<tr>
<th>Phase</th>
<th>Lipid amounts (mg/100 mg)</th>
<th>Per cent distribution of each lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE-rich (Zone II) (59.6%)</td>
<td>C  1.9  TG  2.3  CE  55.4  PL  0</td>
<td>15.8  100  98.6  0</td>
</tr>
<tr>
<td>PL-rich (Zone I) (40.4%)</td>
<td>C  10.1  TG  0  CE  0.8  PL  29.5</td>
<td>84.2  0  1.4  100</td>
</tr>
</tbody>
</table>

* C, cholesterol; TG, triglyceride; CE, cholesterol ester; PL, phospholipid.

Although triglyceride is not included in the condensed phase diagram (Fig. 6), the chemical analysis shows that it partitions almost entirely into the cholesterol ester-rich phase.

The relationships between the major lipid classes of LDL, free cholesterol, cholesterol ester, and phospholipid have been defined in model phase equilibrium experiments in excess water and can be expressed in a simplified condensed phase diagram (28) using triangular coordinates (Fig. 6). Plotting the relative compositions of layers A and C on this phase diagram, the points for different experiments for layer A lie in a relatively narrow range in zone II, as do the points for layer C in zone I. When the values for layer B are plotted, they are found to lie on a straight line (XY) through zone III which connects the mean values for layers A and C. The relative amount of the phases of zone II and I which constitutes points in zone III can be determined from the reciprocal of the distances of the point to zones II and I (29).

As illustrated in Fig. 7A, increasing amounts of LDL triglyceride relative to LDL cholesterol ester negatively correlated with the peak transition temperature \( r = -0.61, p = 0.005 \). This relationship is mainly determined by the triglyceride content of LDL as cholesterol ester content alone did not correlate with the peak temperature. Triglyceride alone has a correlation coefficient of \( -0.60 (p = 0.006) \). In addition, both the onset and the end temperature of the transition correlated with the amount of triglyceride relative to cholesterol ester and \((f)\) arachidonate of total cholesterol esters; \((g)\) weight per cent of each of saturated, monounsaturated, and polyunsaturated cholesterol esters of total cholesterol esters; \((h)\) ratio of polyunsaturated cholesterol esters to sum of saturated plus monounsaturated cholesterol esters; \((i)\) ratio of polyunsaturated to monounsaturated cholesterol esters; \((j)\) weight per cent of total LDL lipids of free cholesterol, cholesterol ester, triglycerides, and phospholipid; \((k)\) weight per cent of free cholesterol relative to cholesterol ester; \((l)\) weight per cent of triglyceride relative to cholesterol ester; \((m)\) weight per cent of free cholesterol plus triglyceride relative to cholesterol ester; \((n)\) ratio of total phospholipid to free cholesterol; \((o)\) ratio of phosphatidylcholine to sphingomyelin; \((p)\) weight per cent protein to total particle weight; \((q)\) weight ratio of free cholesterol to protein; \((r)\) weight ratio of cholesterol ester to protein; \((s)\) weight ratio of triglyceride to protein; and \((u)\) ratio of the lecithin-sphingomyelin ratio to weight per cent protein.
although the correlations were not as strong ($r = -0.50$, $-0.54$ and $p = 0.024$, 0.015, respectively). There were no other significant correlations ($p < 0.05$).

To prove that these relationships were not a result of an apparent effect of another variable, each variable in turn was controlled when comparing the peak transition temperature with the amount of LDL triglyceride relative to cholesterol ester or triglyceride content alone. In each case, there was no significant effect on these relationships. To determine whether a strong influence by LDL triglycerides could be masking influences of other variables, all compositional variables were compared controlling for the triglyceride content. No new correlations were evident except for a possible relationship with the amount of LDL triglyceride relative to cholesterol esters and phospholipids ($r = 0.47$, $p = 0.064$).

The transition temperature of isolated individual LDL cholesterol esters showed no relationship to the transition temperature obtained from its intact parent LDL. We found, however, that increasing amounts of isolated LDL triglyceride progressively depressed the temperature of the smectic-cholesterol transition of LDL cholesterol ester (Fig. 7B). The smectic-cholesterol transition decreased with addition of small amounts of triglyceride to cholesterol ester and disappeared with mixtures containing greater than 3% triglyceride. It seems, therefore, that the thermal behavior of intact LDL reflects the behavior of these two lipids, cholesterol esters and triglyceride, in model systems.

**Studies Relating to Denatured LDL**

**Transition in Denatured LDL**—On heating LDL to 100° an irreversible transition occurs between 65 and 90° (Fig. 8a). The mean onset of the transition at a heating rate of 0.1°/min is at 69.6 ± 3.2° (range 63–74°) with an end temperature of 88.5 ± 2.5° (range 87–93°). The peak temperature was 81.2 ± 0.8° (range 80–83°) and the mean enthalpy of this transition was 0.97 ± 0.22 cal/g of LDL protein (range 0.67–1.31 cal/g of LDL protein). Because of the gradual onset and the breadth of this transition, it was difficult to measure the onset and end transition temperatures accurately. However, a narrow range of peak temperatures was observed from both multiple samples from the same donor and samples from different donors.

Turbidometric studies were performed on LDL (5 mg/ml) at a heating rate of 0.1°/min or by increasing temperatures at 1° intervals until no further change in absorbance was observed. Beginning at 0°, no change in absorbance occurred until 62–63°. Between 63 and 67° there was a small increase in turbidity after which turbidity increased rapidly, suggesting that the transition is at least a two-step process. In all turbidometric experiments, once an increase in absorbance was recorded, it was irreversible with decreasing temperature. Since the properties of LDL are altered irreversibly following this high temperature transition, we refer to this transition as "lipoprotein denaturation."

Following LDL denaturation, different calorimetric transitions are obtained between 0 and 45°. Cooling to 0° and reheating results in broad transitions between 10 and 40° (Fig. 8b) with an enthalpy (0.89 ± 0.09 cal/g of LDL cholesterol ester) slightly larger than the low temperature transition of the intact lipoprotein (Table II). Cooling to 60° and reheating results in a transition between 5 and 45° with an enthalpy (5.63 ± 1.26 cal/g of LDL cholesterol ester) almost 10 times larger than the initial transition in intact LDL (Fig. 8c). Following either of these transitions, the high temperature transition is no longer observed with continued heating to 100° (Fig. 8, d and e).

When examined by polarizing microscopy, LDL exists as an isotropic solution between 0 and 45° (Fig. 9a). Following cooling to 60° and reheating, no changes are observed to 45°. On heating further, beginning at 65°, the solution becomes irreversibly turbid, and a second phase with a granular appearance precipitates out in close association with droplets of an oily texture (Fig. 9b). On cooling, these droplets remain isotropic until approximately 30° when progressively increasing numbers of droplets develop birefringence with a focal texture (Fig. 9c). These focal conics all have a uniaxial positive sign of birefringence, typical of smectic liquid crystals of cholesterol ester (24). If the sample is cooled further to 60° or allowed to remain at 0° for a few minutes, crystals grow from the droplets (Fig. 9d). On reheating these crystals melt to an isotropic oil over the same temperature range as the high enthalpy calorimetric transition (Fig. 8c). On cooling, the oil droplets again show the focal conic texture of cholesterol ester liquid crystals and, thus, this thermal behavior is typical of cholesterol ester crystals and liquid crystals (24).

To further associate the low temperature transitions of LDL with cholesterol esters, x-ray diffraction studies of denatured LDL and isolated cholesterol esters were compared. As shown in Fig. 10, the diffraction line at 36 Å obtained for denatured LDL at 10° (Fig. 10b) corresponds exactly to that obtained for LDL cholesterol esters in the smectic phase (Fig. 10a).

Comparing intact LDL (Fig. 10c) to denatured LDL, both at 10°, only the maximum corresponding to a Bragg spacing of 36 Å is present in both. The disappearance of all the low angle scattering fringes except for that at 36 Å following denaturation is consistent with disruption of the particle structure. In each case, the maximum at 36 Å disappears when the sample is heated above the transition observed by calorimetry and returns on cooling to temperatures below the transition.

**Crystalline Transitions of Isolated LDL Lipids and Denatured LDL**—The lipids extracted from LDL were studied over similar temperature ranges as intact and denatured LDL. Calorimetry of LDL cholesterol esters cooled to −60° and re-
Low Density Lipoprotein Structure

FIG. 9. Solution of LDL examined by polarizing light microscopy (x 100). A, 25°C (direct light) is a featureless field except for air bubbles; B, 25°C following heating to 100°C (direct light) illustrating the appearance of a droplet phase; C, 25°C following heating to 100°C (polarized light) demonstrating that droplets have characteristic smectic birefringence; D, 25°C following heating to 100°C and cooling to -60°C (polarized light) illustrating crystallization of esters.

FIG. 10. Microdensitometer tracings of x-ray photographs of (a) isolated LDL cholesterol ester in the smectic liquid crystalline state; (b) LDL solution at 10°C after heating to 100°C; and (c) intact LDL solution at 10°C. Arrows indicate maxima at spacings of 36 Å. (Because background intensity changes after LDL denaturation these three samples are compared without subtraction of background.)

heated results in a similar transition (Fig. 8d) to that shown by denatured LDL (Fig. 8c). However, the enthalpy is approximately one-third less in LDL (Table II). Addition of LDL triglyceride and free cholesterol to the isolated esters in similar proportions as in LDL results in a small decrease in the transition enthalpy, 8.33 ± 1.04 to 7.33 ± 1.03 cal/g of cholesterol ester. Microscopy studies of the LDL cholesterol esters demonstrates identical behavior to the oil droplets and crystals of denatured LDL (see Fig. 9, C and D), except that when examined in the absence of triglyceride the cholesteric liquid crystal phase can be observed with the pure esters. Furthermore, the x-ray diffraction pattern of LDL cholesterol ester in the crystalline state at 10°C following cooling to -60°C shows similarities to that of denatured LDL following a similar thermal history. Denatured LDL differs from LDL cholesterol ester in that after cooling to -60°C, in addition to the diffraction lines from cholesterol ester crystals, a 36 Å spacing is always present from the denatured sample. This suggests that some cholesterol ester remains as smectic liquid crystal in the denatured sample and may account for the lower enthalpy of the 5-45°C transition in denatured LDL compared to isolated cholesterol ester following cooling to -60°C and reheating. The phospholipid, triglyceride, and free cholesterol fractions isolated from LDL did not exhibit thermal transitions between 0 and 60°C which could account for the transitions observed in intact or denatured LDL. Although the extracted triglycerides after cooling to -60°C did show a large enthalpy transition continuing to 20-30°C, this melting process was essentially complete by -5°C. Thus, triglyceride melting does not contribute significantly to the enthalpy of the 5-45°C transition in denatured LDL.

Stability of LDL—Following the liquid → liquid crystalline transitions, the isothermal crystallization of isolated LDL cholesterol ester held at 17°C is shown in Fig. 11a. On reheating, the enthalpy of the crystal melt is equivalent to that measured from the isothermal transition. Mixtures of LDL cholesterol ester and triglyceride show similar isothermal crystallization. Intact LDL, however, shows no isothermal transition (Fig. 11b), suggesting that LDL cholesterol ester exists in a meta-

Temperature, °C

Time, min

Temperature, °C

Time, min

FIG. 11. Calorimetry of (a) isolated LDL cholesterol esters, cooling from 45-18°C and then held in the isothermal mode. Exothermic peak represents crystallization of cholesterol esters; (b) intact LDL, cooling from 45-12°C and then held in the isothermal mode. No exothermic (EXO) process occurs, even if held isothermally for more than 48 h.
stable liquid crystalline state below the transition. In fact, the melting transition obtained after keeping an intact LDL sample at 0°C for 24 h is identical with that observed after quenching the sample from 45°C to 0°C and immediately reheating. Thus, although cholesterol esters isolated from LDL and cholesterol esters extruded from denatured LDL form crystals after cooling to −60°C, or even at room temperature, crystallization of cholesterol esters is not observed in intact LDL.

**DISCUSSION**

We have defined three types of thermal transitions in human plasma low density lipoproteins: (a) a reversible transition in intact LDL encompassing body temperature which is associated with an order-disorder liquid crystalline phase change of cholesterol esters within the LDL particle; (b) an irreversible high temperature transition associated with LDL denaturation; and (c) low temperature transitions, again near physiological temperature, related to both liquid crystalline and crystalline phase changes of cholesterol esters released from the disrupted LDL particles.

The reversible transition in intact LDL has been shown by calorimetric and x-ray diffraction techniques to parallel smectic liquid crystal → liquid phase transitions of cholesterol esters released from denatured LDL, isolated LDL cholesterol esters, and pure cholesterol ester mixtures. As in model systems of cholesterol esters, the relative proportions of triglyceride appear to be important in determining transition temperatures.

Although many LDL samples used in this study were concentrated to between 150 and 250 mg of LDL/ml, our results show that the transition temperatures and enthalpies were independent of concentration. Furthermore, the transition observed in intact LDL using calorimetric and x-ray diffraction techniques is in the same temperature range as thermal transitions detected by other methods using far more dilute LDL samples (3, 4).

Because of the breadth and relatively low enthalpy of the transition in intact LDL, we were unable to study the transition at scanning rates less than 2.5°/min. In a preliminary experiment (courtesy of Drs. R. Biltonen and D. Mountcastle, Biochemistry Department, University of Virginia School of Medicine) we studied a sample of LDL (concentration 12 mg of LDL/ml) in an adiabatic calorimeter (30) at a scan rate of 0.12°/min. The same LDL preparation (concentration 190 mg/ml) was studied in the calorimeter used for this study at a rate of 10°/min. The peak melting temperatures were 28.3° and 29.0°, respectively, suggesting that even at the faster scan rate, the transition temperature was very close to the true equilibrium value.

The lipid composition of LDL influences both the temperature and the magnitude of the reversible transition in intact LDL. It is possible this broad transition represents: (a) a summation of transitions in a polydisperse population of LDL particles with a differing composition, with the transition of each individual particle restricted to a narrow temperature range; (b) all particles may have similar composition and melting behavior and the transition in each individual particle may occur over the width of the observed transition; and (c) both conditions are present. We cannot differentiate among these, although as seen in Fig. 5 addition of small amounts of triglyceride to LDL cholesterol ester significantly broadens the liquid crystal transition so that it resembles that of intact LDL.

In LDL, the peak transition temperature differed by much as 10.5° for different donors (see Fig. 7A). It might be predicted that changes in the fatty acid composition of the cholesterol ester in LDL would influence the transition temperatures. Using mixtures of cholesteryl linoleate and cholesteryl oleate (which together make up over 70% of LDL cholesterol esters), between the range of weight ratios of these two esters in LDL, the smectic liquid crystal transition varies by only 1.3°. Possibly other cholesterol esters present in small amounts have significant effects which we could not detect in our statistical analysis. Furthermore, it is unlikely that the influence of free cholesterol could be responsible for this variation. As shown in Fig. 5, cholesterol esters mixed with free cholesterol in proportions similar to those determined by the partitioning experiments have a smectic → liquid transition only 3.5° lower than that of the pure ester. As well, both the cholesteric and smectic liquid crystalline transitions are retained.

Triglycerides, however, have a significant effect on the behavior of cholesterol esters. The disappearance of the liquid → cholesteric liquid crystal transition with small amounts of triglyceride, broadening of the thermal transition, and a decrease in the liquid → smectic liquid crystal transition temperature with increasing amounts of triglyceride (Fig. 5) all indicate a disordering effect of triglyceride on the molecular organization of cholesterol esters. Triglycerides also influence the transition in the intact lipoprotein. Increasing triglyceride content lowers the transition temperature in intact LDL (Fig. 7A), presumably through the effect of triglyceride on the cholesterol esters in LDL.

Although the transition temperature of cholesterol esters in intact LDL is directly influenced by the amount of triglyceride relative to cholesterol ester, the scatter of the experimental points about the regression line in Fig. 7A suggests that other factors or combinations of factors may prove to be important. Our data does not include analyses for effects of such variables as LDL particle mean molecular weight which has been shown to vary widely among individuals (31).

The transition in intact LDL is typical of cholesterol ester liquid → liquid crystal transitions in that it demonstrates little if any supercooling and is not affected by heating rate. Following thermal denaturation the cholesterol esters extruded from LDL show some properties similar to their behavior in intact LDL in that they still undergo liquid crystalline transitions. However, the enthalpy of the transition between 0 and 45°C is now approximately 30% greater than with intact LDL. The 0–45°C transition in denatured LDL is again associated with liquid crystalline transitions of the cholesterol ester, and the greater enthalpy in denatured LDL and the isolated esters suggests some modification of the behavior of cholesterol esters in the intact particle.

In contrast to the cholesterol ester model systems and denatured LDL, evidence for crystal formation in the intact lipoprotein was not obtained (Fig. 11) even after 48 h at 0°C or after cooling to −60°C. The inability of cholesterol esters to crystallize implies that in intact LDL the particle imposes constraints on the interactions of cholesterol ester molecules within the particle and would be consistent with the increased enthalpy of the liquid crystalline transitions of isolated LDL cholesterol esters compared to the same esters inside LDL (see Table II). Furthermore, using natural abundance 13C nuclear magnetic resonance (NMR) spectroscopy, changes in the molecular motion of cholesterol esters within LDL have been studied as a func-

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* D. M. Small, unpublished results.
tion of temperature (32). Comparisons with $^1$H NMR spectra of cholesteryl linoleate and thermally denatured LDL show that at temperatures above the transition, the cholesterol esters in intact LDL are motionally restricted and appear to be in a more ordered state than that of a true isotropic liquid.

If we assume that the liquid → liquid crystalline transitions in intact LDL, denatured LDL, and the cholesterol ester–triglyceride mixtures are all reversible, then in each system at the transition temperature where the free energy change is zero, $\Delta S = \Delta H/T$ where $\Delta S$ is the change of entropy from one state to another on each side of the transition temperature $T$, and $\Delta H$ is the transition enthalpy. In intact LDL, $\Delta H$ expressed in terms of cholesterol ester is always lower than in denatured LDL or the isolated cholesterol esters (Table II) and mixtures of cholesterol ester containing up to (at least) 14% triglyceride. Therefore, the change in entropy, $\Delta S$ for the liquid-liquid crystal transition, is always less in intact LDL than in model systems, suggesting that above the transition the cholesterol esters in intact LDL must be more constrained than when removed from the lipoprotein particle.

At present the nature of these constraints cannot be precisely defined. We can speculate that placing cholesterol ester molecules in a 200 to 220 Å diameter particle results in boundary constraints which restrict their ability to move freely even above the liquid crystalline–liquid transition temperature. Alternatively, some cholesterol esters in LDL may interact directly with the surface phospholipids and apoprotein (see below).

$^1$H NMR spectra of LDL at temperatures below the calorimetric transition are very similar to that of pure cholesteryl linoleate in the smectic liquid crystalline state (32). The x-ray scattering and diffraction data provide strong evidence that the cholesterol esters in LDL are organized in a smectic-like liquid crystalline state below the transition temperature. In intact LDL, denatured LDL, isolated esters from LDL, cholesteryl linoleate, cholesterol oleate, and mixtures of the latter two esters, as well as mixtures of cholesterol ester with triglyceride all given either a strong scattering fringe or diffraction line at 36 Å, a characteristic of cholesterol esters in the smectic phase.

In x-ray scattering studies of LDL, two groups have described oscillations with a periodicity of 30 to 40 Å in the radial electron density in the core of LDL (33, 34). These studies were performed at 4° or approximately 20°, temperatures below the transition of intact LDL. A detailed analysis of the x-ray scattering profiles from LDL at 10° and 45° provides evidence that these periodic oscillations and the 36 Å fringe at 10° are explained by two layers of cholesterol ester molecules oriented radially in a smectic-like phase within the core of a single LDL particle.

In the intact particle, this cholesterol ester-rich domain must be large enough to allow the cooperative melting behavior observed by calorimetry. Shipley et al. (35) have suggested that high density lipoprotein (HDL) is a symmetrical particle with a polar shell surrounding a hydrocarbon core of approximately 70 Å diameter in which the cholesterol esters are located. We have been unable to detect either cholesterol ester–associated transitions in intact high density lipoprotein or a scattering fringe at 36 Å from this particle. Therefore, we can predict that the cholesterol ester-rich domain must be larger than 70 Å in diameter to permit cooperative melting behavior in LDL.

On the basis of our results, a schematic representation emphasizing the distribution of lipid classes in LDL is proposed (Fig. 12). Below the onset of the calorimetric transition, the cholesterol esters are radially arranged in concentric layers in a domain of approximately 144 Å in diameter. Our experiments on the partitioning of LDL lipids suggest that 15% of the free cholesterol and all of the triglyceride of LDL will be included in this cholesterol ester core. The remainder of the free cholesterol and all of the phospholipid molecules will be distributed on the outside in a shell partially surrounding the cholesterol esters. Based upon calculations of the surface coverage by phospholipids (36) and free cholesterol (37), we suggest that a fraction of the surface of LDL is covered by apoprotein B, and some apoprotein B may interact directly with core neutral lipid. Above the transition, the cholesterol ester molecules lose their smectic-like arrangement but do not assume the properties of a true liquid in that the rotational mobility of the cholesterol ester molecules is still restricted (32).

The amount of triglyceride relative to cholesterol ester is important in determining the transition temperature and may be an important factor in determining the constraint on cholesterol ester molecules at temperatures above the transition. Based on our data of this transition in intact LDL at 37°, some individuals will have a fraction of LDL cholesterol esters in a smectic-like state. This physical state of the lipids in LDL may play an important role in the metabolism of the particle. Possibly the fluidity of the lipid core may influence the surface

We realize this may be an oversimplification in that the partitioning of LDL lipids could be modified in the presence of apoprotein.

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**Fig. 12.** Schematic representation of the distribution of the lipids in LDL. A, cholesterol ester; B, triglyceride. A, below the transition in intact LDL, at 10°, the cholesterol esters are arranged in two concentric layers with a periodicity of approximately 36 Å in a smectic-like state. B, above the transition, at 45°, this layered arrangement is lost, but the organization of cholesterol ester molecules is not totally random, and some degree of motional restriction persists. In both A and B cholesterol esters are the dominant lipid in the particle core. All LDL triglycerides and approximately 15% of LDL free cholesterol are dissolved in this core. Around the neutral lipid core are the polar lipids of LDL, phospholipids, and most of the free cholesterol. Since these two lipid classes cannot cover all the surface of the neutral lipid core, we suggest that some apoprotein is adjacent to neutral lipid. Thus, the outer shell is made up of apoprotein and polar lipids mutually interacting with each other at the surface as well as with the neutral lipids in the LDL particle core. The water hydration of the LDL particle also forms part of this outer shell.
structure of LDL and alter its interaction with cell membrane receptors (11).

Dietary and disease factors also affect LDL lipid composition. Baboons fed an atherogenic diet showed a marked decrease in the contribution of triglyceride to total LDL lipids (38), and patients with homozygous familial hypercholesterolemia have significantly less triglyceride in LDL compared to normals (39). Thus, the temperature of the cholesterol ester phase transition in intact LDL may reflect its ability to interact with various tissues, such as the arterial wall, and may prove useful in predicting the extent of such interactions.

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