Thermotropic Properties and Molecular Dynamics of Cholesteryl Ester Rich Very Low Density Lipoproteins: Effect of Hydrophobic Core on Polar Surface†

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ABSTRACT: Cholesteryl ester rich very low density lipoproteins (CER-VLDL), isolated from the plasma of rabbits fed a hypercholesterolemic diet, have been studied by differential scanning calorimetry (DSC), ^{13}C nuclear magnetic resonance (NMR), and spin-label electron paramagnetic resonance (EPR) to determine the temperature-dependent dynamics of cholesteryl esters in the hydrophobic core and of phospholipids on the polar surface. Intact CER-VLDL exhibit two DSC heating endotherms; these occur at 40–42 and 45–48 °C. Cholesteryl esters isolated from CER-VLDL also exhibit two DSC endotherms; these occur at 50.0 and 55.1 °C and correspond to the smectic → cholesteric and cholesteric → isotropic liquid-crystalline phase transitions. A model mixture containing cholesteryl linoleate, oleate, and palmitate in a ratio (0.21, 0.51, and 0.28 mol fraction) similar to that in CER-VLDL exhibited comparable DSC endotherms at 45.2 and 51.5 °C. CER-VLDL at 37 °C gave ^{13}C NMR spectra that contained no resonances assignable to cholesteryl ring carbons but detectable broad resonances for some fatty acyl chain carbons, suggesting the cholesteryl esters were in a liquid-crystalline state. When the mixture was heated to 42 °C, broad ring carbon resonances became detectable; at 48 °C, they became narrow, indicating the cholesteryl esters were in an isotropic, liquid-like state. With increasing temperature over the range 38–60 °C, the resonances for cholesteryl ring carbons C3 and C6 in CER-VLDL narrowed differentially. Similar spectral changes were observed for the synthetic cholesteryl ester mixture, except they occurred at temperatures about 10 °C higher. These results indicate that the two DSC transitions in CER-VLDL do not directly correlate with the smectic → cholesteric and cholesteric → isotropic transitions exhibited by pure cholesteryl esters. (5-Doxylpalmitoyl)-phosphatidylcholine (5-DP-PC) and (12-doxylstearoyl)phosphatidylcholine (12-DS-PC) were used to probe the polar surface monolayer of CER-VLDL; the corresponding cholesteryl esters (5-DP-CE and 12-DS-CE) were used to probe the hydrophobic core. None of these probes in CER-VLDL detected an abrupt change in EPR order parameters, S, or maximum splitting, $2T_{max}$, over the temperature range 20–58 °C even though 12-DS-PC and 5-DP-PC can detect phase transitions in phospholipid bilayers and 12-DS-CE and 5-DP-CE can detect phase transitions in neat cholesteryl esters. However, 12-DS-CE and 5-DP-CE did detect a much greater acyl chain order for the neutral lipids of CER-VLDL than for those of normal triglyceride-rich VLDL. In addition, 12-DS-PC and 5-DP-PC did detect significantly greater acyl chain order for the phospholipids of CER-VLDL than for those of normal VLDL. The latter results suggest that the organization of lipids in the hydrophobic core of a lipoprotein can directly affect the dynamics of lipids in the polar surface.

Cholesterol is an essential lipid component of mammalian cells and tissues. Under abnormal conditions such as obstructive liver disease (Seidel et al., 1970) or lecithin:cholesterol acyl transferase deficiency (Forte et al., 1974), cholesterol is transported principally in its unesterified form by lipoprotein X. Under normal conditions, the major fraction of plasma cholesterol is transported as cholesteryl ester by the low-density lipoproteins (LDL)[1] (e.g., rabbit, man) or high-density lipoproteins (e.g., rat, dog). In cases where large amounts of dietary cholesterol are ingested, this cholesterol is esterified and transported initially by chylomicrons. Even though the triglycerides of these chylomicrons are readily hydrolyzed, the resulting chylomicron remnants apparently cannot be removed by the liver at a rate comparable to their formation. Hence, they accumulate at ever increasing concentrations, leading to the intra- and extracellular deposition of cholesteryl esters in the arterial wall and other tissues. Such cholesteryl ester rich very low density lipoproteins (CER-VLDL) rapidly accumulate in the plasma of rabbits fed a high cholesterol diet. Within 1 week on this diet, the $d < 1.006$ g/mL fraction is greatly elevated, and lipoproteins of higher density are decreased below the level of detection (Roth et al., 1983). The protein component of CER-VLDL consists almost exclusively of apoB and apoE, which are responsible for their binding to specific receptors and subsequent degradation (Gianturco et al., 1980).

A distinctive feature of CER-VLDL is its rather high cholesteryl ester content (60–70%) and its extremely low triglyceride content (<5%). This high cholesteryl ester/triglyceride ratio together with its large size confers on the lipoprotein certain properties that are of considerable metabolic and structural significance. For example, CER-VLDL is taken up by specific receptors on macrophages, leading to the internalization and accumulation of massive amounts of cholesteryl ester (Gianturco et al., 1982). This lipoprotein exhibits

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[1] Abbreviations: CER-VLDL, cholesteryl ester rich very low density lipoproteins isolated from hypercholesterolemic rabbit plasma by ultracentrifugation at $d = 1.006$ g/mL; HDL_2, high-density lipoprotein subfraction isolated at $d = 1.063$–1.120 g/mL; LDL, low-density lipoprotein isolated at $d = 1.019$–1.063 g/mL; 5-DP-PC, 5-doxylpalmitate esterified at the *sn*-2 position of egg phosphatidylcholine; 5-DP-CE, (5-doxylpalmitoyl)cholesteryl ester; 12-DS-CE, (12-doxylstearoyl)cholesteryl ester; EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance; CP, cholesteryl palmitate; CO, cholesteryl oleate; CL, cholesteryl linoleate.

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two DSC transitions that occur above physiological temperature and are conspicuously near the smectic → cholesteric and cholesteric → isotropic transition temperatures of cholesteryl oleate, the most abundant cholesteryl ester in CER-VLDL (Morrisett et al., 1980). Furthermore, temperature-dependent changes in the low-field ^{13}C NMR spectra suggested that these transitions were the result of phase transitions of these cholesteryl esters. Subsequent ^{13}C NMR (Kroon et al., 1982) and ^{1}H NMR (Kroon & Seidenberg, 1982) studies of rabbit CER-VLDL led to similar conclusions. In the present study, we have obtained additional ^{13}C NMR evidence about the role played by cholesteryl esters in the thermal behavior of CER-VLDL. Furthermore, cholesteryl esters and phospholipids bearing a paramagnetic reporter group on their fatty acyl chains have been used to examine separately the motions of the corresponding unlabeled acyl chains in the hydrophobic core and polar surface and to obtain new information about core–surface interactions.

Materials and Methods

Lipoproteins were obtained from male, white New Zealand rabbits fed either a normal chow diet or normal chow coated with 2% cholesterol (Sigma Chemical Co., St. Louis, MO). After 2–4 weeks on a hypercholesterolemic diet, plasma cholesterol levels typically rose to 1000–1300 mg/dL. After this time, typically 40–60 mL of blood was obtained from the animals through their ear central artery. Blood was collected in tubes containing EDTA (0.1 M) and Trasylol (Mobay Chemical Corp., 10000 Kallikrein inactivator units/mL) to retard proteolysis (Bradley et al, 1982). After centrifugation to sediment cellular components, the resulting plasma was diluted with an equal volume of 100 mM NaCl, 10 mM Tris, 1 mM NaN_3, and 1 mM EDTA, pH 7.4. Lipoproteins were floated by ultracentrifugation at $d = 1.006$ g/mL for 18 h at 10 °C. The whitish supernatant layer was collected by aspiration and was adjusted with the above buffer to the original plasma volume and recentrifuged. The supernatant was concentrated by ultrafiltration (Amicon XM-100 membrane) to 5–10 mL; the lipoprotein mixture was applied to a 2.5 × 180 cm column of Sepharose CL-2B (Pharmacia, Piscataway, NJ) and eluted at a flow rate of 30 mL/h. The desired fractions were combined, treated with Trasylol, and stored under nitrogen at 4 °C until used. Lipoproteins obtained in this manner were resistant to aggregation/precipitation and proteolysis for 2–4 weeks and were used within that time.

Lipoprotein particles were sized by quasi-elastic light scattering (Morrisett et al., 1974) and/or negative-stain electron microscopy (Hoff et al., 1973). The partial specific volume of lipoprotein fractions was determined by precision densimetry in a DMA-02 density meter (Anton Paar, Graz, Austria) (Kratky et al., 1973). The chemical composition of individual fractions was determined by a combination of wet-chemistry methods. Protein was measured by the method of Lowry et al. (1951) with bovine albumin as a standard. Free and total cholesterol were determined with the Boehringer-Mannheim assay kit based on the method of Roeschlau (1974). The difference between the total and free choleserol content was multiplied by 1.68 to obtain the cholesteryl ester content. Lipid phosphorous was determined by the procedure of Bartlett (1959) with phospholipid calculated by using a multiplication factor of 25. Triglyceride was determined with the Rapid Stat kit from Pierce Chemical Co. (Rockford, IL) based on the method of Carlson (1963). In some cases, lipids were quantitated by flame ionization of individual components separated by microscale thin-layer chromatography on silica-coated glass rods on the Iatroscan TH-10 (Ancal, Inc., Los Osos, CA) (Ackman, 1981; Mills et al., 1979). The output from this instrument was fed to an Apple II-Plus computer programmed to reduce and plot the resulting data. The efficiency of the lipid extractions was determined by adding octadecanol (Van Tornout et al., 1979) to the lipoprotein before extraction. This material migrates to a chromatographic window between cholesterol and triglyceride. The fatty acid compositions of phospholipids, triglycerides, and cholesteryl esters separated by preparative thin-layer chromatography were determined on a Hewlett-Packard 5830A gas chromatograph equipped with a Model 18850A programmer–integrater and a 6-ft circular glass column packed with 10% Silar 10C on Supelcoport, 100/120 mesh (Morrisett et al., 1977). The total cholesteryl esters of selected CER-VLDL samples were isolated by column chromatography on silica gel.

Differential scanning calorimetry was performed on a DSC-2 instrument (LKB Instruments, Rockville, MD) calibrated with an indium standard and operated at a heating rate of 2 °C/min. The temperature-dependent optical properties of neat cholesteryl ester mixtures were observed in 100-μL glass capillaries (same samples as used for EPR measurements), in NMR sample tubes slowly heated or cooled in a water bath, or on glass coverslips mounted on a temperature-controlled stage of a polarized light microscope. The type of phase present was determined by the macroscopic criteria described previously (Gray, 1962).

Natural abundance proton-decoupled Fourier-transform ^{13}C NMR spectra were obtained at 47 kG (50.3 MHz for ^{13}C) with a Bruker WP-200 spectrometer equipped with an Aspect 2000 data system. Broad-band proton decoupling centered 3.4 ppm downfield from the proton resonance of tetramethylsilane and 1.0-W decoupler power were used during acquisition of the spectra. Spectra of lipoproteins were obtained by using 1.2–1.5 mL of sample and 0.1 mL of D_2O as a lock and shim signal. Spectra of neat lipid mixtures were obtained from 400 mg of sample in a Wilmad coaxial insert placed in a 10-mm NMR tube containing D_2O. The fatty acyl CH_3 resonance was used as an internal chemical shift reference (14.1 ppm) (Hailstone, 1972; Hamilton et al., 1974). Sample temperature was controlled (±1 °C) with the Bruker B-V-T-1000 variable-temperature unit. After equilibration of the sample at the probe temperature under data collection conditions, the sample was ejected from the probe, a thermocouple inserted, and the temperature recorded at periodic intervals for several minutes. The temperatures of lipoprotein samples under experimental conditions were determined by extrapolation to zero time. For neat lipid samples, ethylene glycol was used to calibrate the temperature (Ginsburg et al., 1982). The samples were visually examined immediately after each NMR experiment to determine the phase present.

EPR spectra were obtained on a Varian E-12 spectrometer interfaced to a Data Translation Lab Datex system based on an LSI 11/2. The spectrometer was operated at 9.1 GHz and the cavity temperature governed by a variable-temperature controller. Cavity temperature was determined with a Thinc TM-401 electronic thermometer equipped with a microprobe. At a gain level of 1000 or lower, the microprobe did not contribute to the spectrum and could therefore be left in the cavity throughout an entire temperature run. When the sample signal to noise ratio required a gain much above 1000, the probe contributed significantly to the spectrum. In this case, it was necessary to remove the probe from the cavity during spectral accumulation. 5-Doxylpalmitate and 12-doxylstearate were prepared from the corresponding keto acids as described previously (Hubbell & McConnell, 1971). The

Table I: Chemical Composition (%) of VLDL Isolated Ultracentrifugally at d = 1.006 from Individual Hypercholesterolemic Rabbit Plasmas and from a Pool of Normal Rabbit Plasmas

sample	protein	PL	UC	CE	TG
CER-VLDL 1B	4.7	11.1	9.2	73.2	1.9
CER-VLDL 2A	5.9	14.1	9.8	67.8	2.5
CER-VLDL 2B	4.1	10.9	8.2	75.2	1.6
CER-VLDL 8A	3.3	13.5	11.2	68.6	3.3
CER-VLDL 8B	6.3	16.4	11.7	60.0	5.6
CER-VLDL 9A	4.6	13.5	8.8	64.8	8.2
mean ± S.D.	4.8 ± 1.0	13.2 ± 1.2	9.8 ± 1.2	68.3 ± 5.0	3.8 ± 2.3
normal VLDL	6.4	14.4	7.2	14.2	57.7

doxyl fatty acids were dehydrated with *N,N*-dicyclohexyl carbodiimide to the corresponding anhydrides, which were used to acylate either egg lysophosphatidylcholine (Patel et al., 1979a) or cholesterol (Morrisett, 1974; Patel et al., 1979b) to obtain the corresponding phosphatidylcholines or cholesteryl esters. Pure (neat) 12-DS-CE exhibited a DSC heating endotherm at 10–11 °C and cooling exotherm at 17 °C, whereas 5-DP-CE exhibited no endotherm in the range −10 to 70 °C.

Spin-labeled lipoprotein samples were prepared in the following manner. An ethanolic solution of spin-labeled phosphatidylcholine was evaporated to dryness on the wall of a 10 × 75 mm culture tube. To this was added a solution of lipoproteins. The resulting mixture was stirred gently for 1 h at 37 °C. The ratio of exogenous (spin-labeled) to endogenous (lipoprotein) phospholipid in the mixture was about 1:100. The mixture was then transferred to a 100-μL Corning capillary sealed at one end and incubated at room temperature. The equilibration of spin-labeled phospholipid into the lipoprotein could be monitored by the disappearance of the single, exchange-broadened resonance and the appearance of the typical multiline spectrum. Incubation was continued as necessary until the exchange-broadened lines disappeared or no longer interfered with measurement of $2T_{\parallel}$ and $2T_{\perp}$. The incorporation of spin-labeled cholesteryl esters into lipoproteins was achieved by using a somewhat different technique that exploited the cholesteryl ester exchange activity present in rabbit lipid-deficient plasma (Zilversmit et al., 1975). A 2-propanol solution of the ester was drawn into a 50-μL Hamilton syringe and slowly injected into a rapidly stirred solution of 100 mM NaCl and 10 mM Tris, pH 7.4, equilibrated at 50 °C. This resulted in a stable dispersion of the spin-labeled cholesteryl ester that did not precipitate over a period of several days when kept at room temperature. In a typical labeling experiment, 300 μL of dispersion (0.18 mg of spin-labeled cholesteryl ester) was added to 1000 μL of normal rabbit plasma d = 1.21 infranatant that had been dialyzed against 100 mM NaCl and 10 mM Tris, pH 7.4. This mixture was incubated at 37 °C for 5 min and then added to 200 μL of CER-VLDL (containing 6 mg of cholesteryl ester). The 1.5-mL mixture was incubated for 24 h at 37 °C and then transferred to a 2-mL nitrocellulose tube (Beckman Instruments, Palo Alto, CA) and ultracentrifuged (d = 1.006) for 18 h at 35000 rpm in a Ti-40 rotor. The spin-labeled CER-VLDL was obtained from the top 0.5 mL of supernatant and then concentrated to about 0.1 mL in a collodion bag (Schleicher & Schuell, Kene, NH). Lipoproteins labeled in this manner typically gave EPR spectra that contained no exchange-broadened components with 12-DS-PC or 12-DS-CE but occasionally some with 5-DP-PC or 5-DP-CE. The d = 1.006 g/mL infranatant fraction (containing the cholesteryl ester exchange activity) always gave a spin exchange-broadened spectrum. Approximately 30% of the initially added spin-labeled cholesteryl ester was actually incorporated into the lipoproteins. Order parameters were calculated from spectra exhibiting rapid anisotropic motion by the procedures of Hubbell & McConnell (1971) as modified by Gaffney (1976).

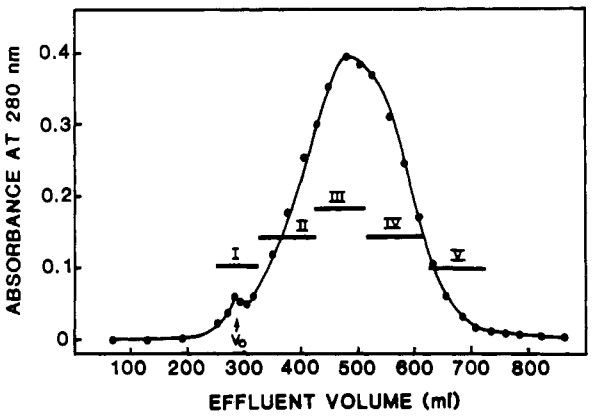

FIGURE 1: Gel-filtration chromatography of CER-VLDL on Sepharose CL-2B (2.5 × 180 cm). Conditions were 142-mg (6-mL) load, 30 mL/h, and 5 °C. Those fractions indicated by bars were pooled; chemical compositions of zones II–V are shown in Table II.

Results and Discussion

CER-VLDL Are Heterogeneous with Respect to Size and Chemical Composition. When fresh hypercholesterolemic plasma was ultracentrifuged at d = 1.006, the supernatant contained lipoproteins that were extremely rich in cholesteryl ester (68.3 ± 5.0%), and poor in triglyceride (3.8 ± 2.3%), compared to normal VLDL (Table I). There were modest differences in the compositions of CER-VLDL from different animals and in compositions of samples drawn from the same animal at different times. For example, CER-VLDL isolated from rabbit 2 after 6 weeks on the hypercholesterolemic diet (sample 2B) was 7.4% richer in cholesteryl ester than CER-VLDL isolated after only 4 weeks on the diet (2A). Rabbit 8 had been on the diet 4 weeks when sample 8A containing 68.6% cholesteryl ester was drawn; after 7 additional weeks on the diet, the fraction of cholesteryl ester had dropped to 60.0%. Hydrodynamic measurements on the d = 1.006 supernatant fractions obtained from two different animals yielded a mean hydrated density of 0.9928 g/mL, which compared favorably to a calculated hydrated density of 1.000 g/mL computed from the chemical composition.

To determine its particle-size heterogeneity, the total d = 1.006 supernatant fraction was gel filtered on a column of Sepharose CL-2B; it eluted as two partially resolved peaks (Figure 1). The first component (zone I) eluted in the void volume of the column and contained a relatively minor fraction of the total mass of phospholipid loaded. The amount of zone I material was significantly greater when the plasma was obtained from a continuously fed animal, compared to one that had been fasted overnight, suggesting this material to be chylomicrons. The second component (CER-VLDL) eluted as a broad, symmetrical peak centered at an elution volume

Table II: Chemical Composition (wt %) of Major Zones of CER-VLDL Obtained by Gel Filtration on Sepharose CL-2B (Figure 1)[a]

sample	protein	phospho-lipid	free cholesterol	cholesteryl ester	tri-glyceride
8A before chromatography	3.3	13.5	11.2	68.6	3.3
8A after chromatography					
zone II	2.4	9.8	8.9	76.5	2.3
zone III	3.1	13.7	12.2	68.7	2.3
zone IV	4.2	15.2	12.2	64.8	3.5
zone V	6.6	14.8	11.1	64.4	3.1

[a] Indicated values represent averages of triplicate determinations with micro thin-layer chromatography and flame ionization.

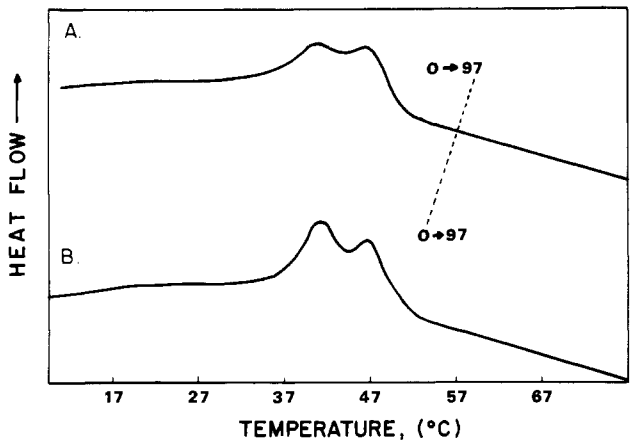

FIGURE 2: DSC thermograms of CER-VLDL: (A) native and (B) after denaturation at 97 °C. Both thermograms exhibit endotherms at 41 and 47 °C, but the enthalpy for the transition of denatured CER-VLDL is about 16% greater than that for native CER-VLDL.

of about 480 mL. Chemical analysis of the four zones II–V (Figure 1) indicated a compositional gradient across the peak (Table II). This was most apparent with the cholesteryl esters, which decreased from 76.5% in the leading zone II to 64.4% in the trailing zone V. The other components appeared to increase slightly across the peak. The relatively low triglyceride content of zone V and the absence of peak asymmetry in this region of the elution profile indicate the absence of normal VLDL, which typically elute in this region (see Figure 2B; Roth et al., 1983).

Fatty acid analysis of the cholesteryl esters in unfractionated CER-VLDL from individual preparations (Table III) revealed that $C_{18:1}$ (39–43%) was the most abundant, while $C_{16:0}$ (16–20%) and $C_{18:2}$ (20–24%) were the next most abundant fatty acid types. These results differ from those of Kroon et al. (1982), which indicated equal proportions (36% each) of $C_{18:1}$ and $C_{18:2}$ cholesteryl esters. In marked contrast to CER-VLDL, normal VLDL contained 50% $C_{18:2}$ and 27% $C_{18:1}$ but only 10% $C_{16:0}$ (Table III). While the fatty acid compositions of cholesteryl esters in CER-VLDL were quite similar for the five samples analyzed, the composition of the phospholipids varied widely but in most cases consisted primarily of $C_{18:2}$ (25–38%) with lesser amounts of $C_{18:0}$ (14–24%), $C_{18:1}$ (12–19%), and $C_{16:0}$ (14–35%). The phospholipids of normal rabbit VLDL had a fatty acid distribution very similar to that of CER-VLDL preparation 8A but quite different from that of preparations 8B, 9B, and 10A. The triglycerides of CER-VLDL also contained mainly $C_{16:0}$, $C_{18:1}$, and $C_{18:2}$, but the relative abundance of these fatty acids varied considerably among the five samples analyzed. The distribution of triglyceride fatty acids in CER-VLDL sample 9B was rather similar to that of normal VLDL.

Table III: Fatty Acid Compositions (wt %) of Cholesteryl Esters, Triglycerides, and Phospholipids in Normal VLDL Isolated from Pooled Rabbit Plasma and in CER-VLDL from Individual Hypercholesterolemic Plasmas[a]

fatty acid	normal VLDL	CER-VLDL 8A	CER-VLDL 8B	CER-VLDL 9A	CER-VLDL 9B	CER-VLDL 10A
Cholesteryl Esters						
14:0	0.43	0.35	0.53	0.32	0.64	0.78
16:0	*10.30*	*17.60*	*16.54*	*18.91*	*20.30*	*17.94*
16:1	2.44	6.23	6.29	8.23	9.10	8.27
18:0	3.55	5.92	6.12	5.64	6.21	5.69
18:1	*27.32*	*42.70*	*41.34*	*41.37*	*39.69*	*39.33*
18:2	*50.15*	*24.11*	*23.94*	*20.34*	*20.42*	*22.48*
18:3–20:4	N.D.	N.D.	0.18	1.73	0.04	0.07
20:4	0.86	0.37	0.52	N.D.	0.34	0.36
Triglycerides						
14:0	0.82	0.85	0.74	0.59	1.31	0.82
16:0	*31.48*	*20.47*	*21.95*	*10.57*	*32.70*	*27.01*
16:1	4.04	2.94	5.10	1.09	8.99	5.13
18:0	4.48	7.91	5.56	8.54	7.71	14.04
18:1	*29.10*	*31.34*	*31.11*	*27.66*	*27.32*	*30.10*
18:2	*25.46*	*31.61*	*29.10*	*42.18*	*19.53*	*19.70*
18:3	3.79	3.64	5.43	7.65	1.39	1.74
18:3–20:4	0.37	1.24	0.49	1.71	0.55	0.16
20:4	0.46	N.D.	0.50	N.D.	0.49	1.29
Phospholipids						
14:0	0.18	0.12	0.31	0.42	0.73	0.36
16:0	*16.54*	*14.04*	*25.02*	*34.82*	*25.33*	*20.34*
16:1	1.40	1.22	2.13	2.24	4.66	2.18
18:0	*23.89*	*24.18*	*14.58*	*20.33*	*14.84*	*16.28*
18:1	14.58	18.74	14.98	11.90	13.64	11.98
18:2	*33.91*	*32.89*	*34.49*	*24.96*	*33.14*	*38.22*
18:3	1.20	1.18	1.83	0.72	1.61	1.65
18:3–20:4	2.32	2.07	1.68	0.65	0.86	1.21
20:4	5.99	5.56	5.00	3.97	5.19	7.77

[a] N.D., not detectable. The most abundant species are indicated in italic type.

Thermotropic Behavior of Cholesteryl Esters in Intact CER-VLDL Is Not the Same as Their Behavior in Neat Form. The widely differing lipid compositions of triglyceride-rich normal VLDL and hypercholesterolemic cholesteryl ester-rich VLDL convey very different thermal properties on these lipoproteins. DSC detected no thermal transitions for normal VLDL in the temperature range 0–97 °C (data not shown). In contrast, the thermograms of CER-VLDL obtained with increasing temperature contained two broad endotherms, one centered at about 40–42 °C and the other about 45–48 °C (Figure 2A). When the lipoproteins were denatured by heating to 97 °C, cooled to 7 °C, then scanned again with increasing temperature, the resulting thermogram again exhibited endotherms at 40–42 and 45–48 °C. However, these endotherms were sharper, and their combined enthalpies were about 16% greater (Figure 2B). When the cholesteryl esters were isolated from CER-VLDL and examined by DSC, they exhibited two narrow endotherms, either when being heated from the smectic phase or cooled from the isotropic phase (Figure 3). The composite enthalpy associated with both of these transitions was 0.90 kcal/mol (Table IV). With the fatty acid composition of CER-VLDL cholesteryl esters (Table III) as a guide, a synthetic mixture containing the three most abundant molecular species was prepared. It contained 0.21, 0.51, and 0.28 mol fractions of CL, CO, and CP, respectively. This model mixture exhibited thermal properties remarkably similar to those of the total cholesteryl esters isolated from unfractionated CER-VLDL (Table IV, Figure 3). The results suggested that the DSC transitions seen in CER-VLDL were a result of

Table IV: Temperatures and Enthalpies of Thermal Transitions for Cholesteryl Esters Determined by Differential Scanning Calorimetry

sample	transition	temp (°C)	enthalpy (kcal mol^{-1})
cholesteryl esters isolated from unfractionated CER-VLDL (n = 2)	C1 or C2 → isotropic (heating)	44.0	6.61
		55.0	
	cholesteric ← isotropic (cooling)	52.5	0.90
	smectic ← cholesteric (cooling)	47.5	
	smectic → cholesteric (reheating)	50.0	
	cholesteric → isotropic (reheating)	55.1	
model mixture [mole fraction of cholesteryl palmitate (0.28), cholesteryl oleate (0.51), and cholesteryl linoleate (0.21)] (n = 2) (Figure 3)	C1 or C2 → isotropic (heating)	45.5	6.72
		56.5	
	cholesteric ← isotropic (cooling)	51.2	0.89
	smectic ← cholesteric (cooling)	45.0	
	smectic → cholesteric (reheating)	45.2	
	cholesteric → isotropic (reheating)	51.5	
model mixture (see above) + 1% 12-DS-CE (n = 4)	C1 or C2 → isotropic (heating)	42.2	
		59.0	
	cholesteric ← isotropic (cooling)	48.2	
	smectic ← cholesteric (cooling)	42.0	

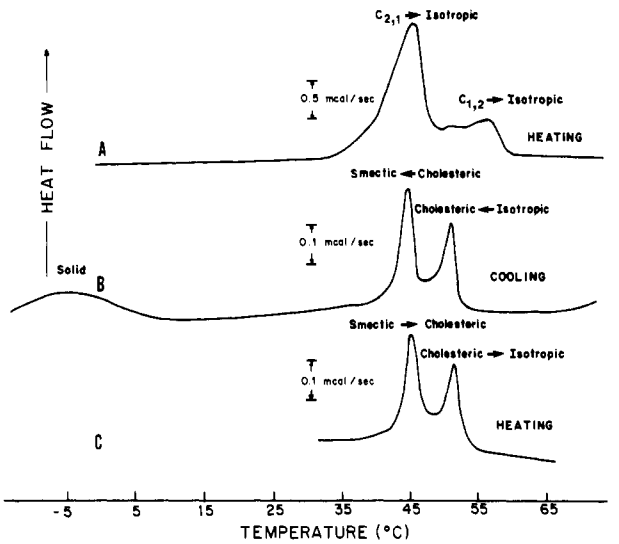

FIGURE 3: DSC thermograms of a synthetic cholesteryl ester mixture similar to that present in the cholesteryl ester fraction isolated from CER-VLDL [CL = 0.21, CO = 0.51, and CP = 0.28 (mol fraction)]. The temperatures and enthalpies of the observed phase transitions are shown in Table IV.

thermotropic transitions of the constituent cholesteryl esters.[2]

Further evidence supporting this suggestion was obtained from temperature-dependent ^{13}C NMR spectra. At temperatures above the DSC transitions, CER-VLDL gave a spectrum that consisted of narrow resonances originating from cholesteryl ring carbons and fatty acyl chains.[3] These resonances reflected primarily liquid cholesteryl esters, as shown by a close correspondence of the aliphatic regions for CER-VLDL and a liquid (isotropic) cholesteryl ester mixture (spectra not shown).

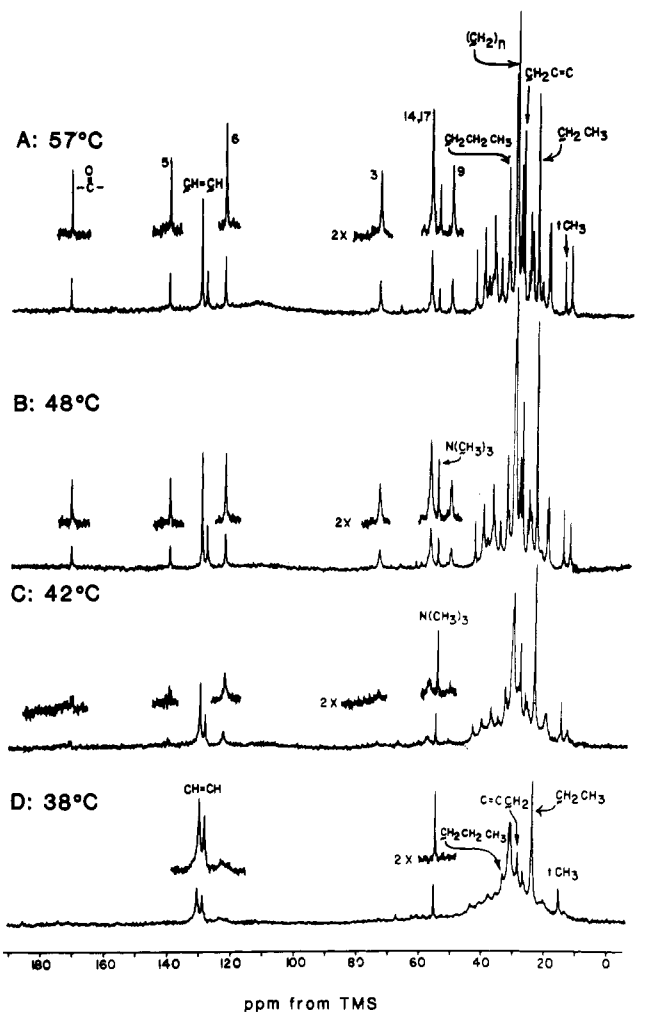

FIGURE 4: Natural abundance proton-decoupled Fourier-transform ^{13}C NMR spectra of CER-VLDL at the temperatures indicated. Spectra were recorded after 8000 scans with a 0.82-s pulse interval, a spectral width of 10 000 Hz, and 16 384 time domain points. Line broadening of 2.0 Hz was applied to improve signal to noise ratios. Insets are printed with a 2-fold vertical expansion.

^{13}C NMR spectra of two CER-VLDL samples were obtained as a function of temperature between 36 and 60 °C. The neutral lipid compositions for these were 97.0% (96.3%)

[2] Visual observation of the sample with decreasing temperature from 65 °C at a rate of 0.3 °C/min revealed the appearance at 49.7 °C of a bluish opalescence, characteristic of the cholesteric phase. At 45.0 °C, the transparent blue color was completely replaced by a colorless, cloudy appearance, typical of the smectic phase. At 42.5 °C, very small crystals began to form. The sample did not become completely crystalline until it was allowed to sit at room temperature overnight.

[3] On the basis of the average lipid composition of CER-VLDL (Table I), the cholesteryl esters should contribute about 67% of the intensity of these resonances.

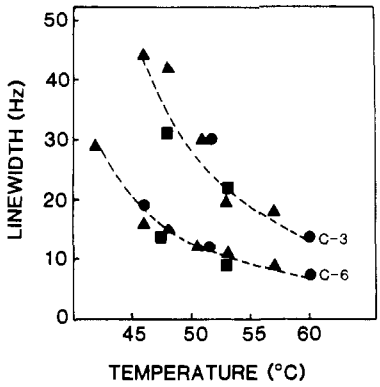

FIGURE 5: Plot of ^{13}C line width as a function of increasing temperature for the steroid ring carbons C3 and C6 in native CER-VLDL (● and ■) and heat-denatured CER-VLDL (▲).

cholesteryl ester and 3.0% (3.7%) triglyceride. Spectra for one sample were obtained upon heating from 36 to 60 °C and for the other sample upon cooling from 60 to 38 °C. No significant spectral differences were observed between the two sets of data. Complete ^{13}C NMR spectra are shown in Figure 4 at four selected temperatures corresponding to temperatures (Figure 2) well above the upper portion of the higher DSC endotherm (57 °C), near the peak of the higher (48 °C) and lower (42 °C) endotherms and near the beginning of the lower endotherm (38 °C). As a function of decreasing temperature, most resonances exhibited a continuous broadening, although the changes between 57 and 48 °C were smaller than the changes between 48 and 38 °C. In addition, broadening of steroid ring resonances was significantly greater than broadening of fatty acyl chain resonances.

Quantitative line-width results for the cholesteryl ester C6 and C3 resonances are given in Figure 5 for the two native lipoprotein samples and for a denatured sample. Of all cholesteryl ring resonances, these are the most sensitive to anisotropic motions of the ring (Hamilton et al., 1977; Quinn, 1982). The nonlinearity of the line-width vs. temperature plots and the *differential* line broadening observed for the cholesteryl ester C6 and C3 resonances from CER-VLDL are spectral features characteristic of liquid cholesteryl esters that are near a liquid → liquid-crystalline transition (Hamilton et al., 1977; Quinn, 1982; Ginsburg et al., 1982). At 42 °C, the intensities of steroid ring carbon resonances are attenuated; at 38 °C, these resonances are broadened almost beyond detection. Resonances of fatty acyl carbons near the beginning of the chain (e.g., C1 and C2) are also broadened beyond detection, whereas those resonances of carbons near the middle or end of the chain (e.g., olefinic, CH_2CH_3, and CH_2CH_3) remain relatively narrow at the lower temperatures. In addition, the phospholipid choline methyl resonance is narrow at all temperatures. Thus, the NMR results suggest that with decreasing temperature the cholesteryl esters within CER-VLDL undergo a liquid → ordered (liquid-crystalline) transition. At 42 °C, a portion of the esters are liquid (those observed in the spectrum) and a portion are liquid crystalline, while at 38 °C most of the cholesteryl esters are liquid crystalline.

The ^{13}C NMR spectra of rabbit CER-VLDL at physiological temperature is strikingly different from spectra of normal human VLDL and cholesteryl ester rich β-VLDL of type III hyperlipoproteinemic patients (Hamilton et al., 1976). In the latter cases, because of the much higher content of triglycerides relative to cholesteryl esters, spectra contain narrow resonances from the cholesteryl ring, and the C3 to C6 line-width ratio is near unity. The spectra of CER-VLDL obtained by Kroon et al. (1982) also contained observable

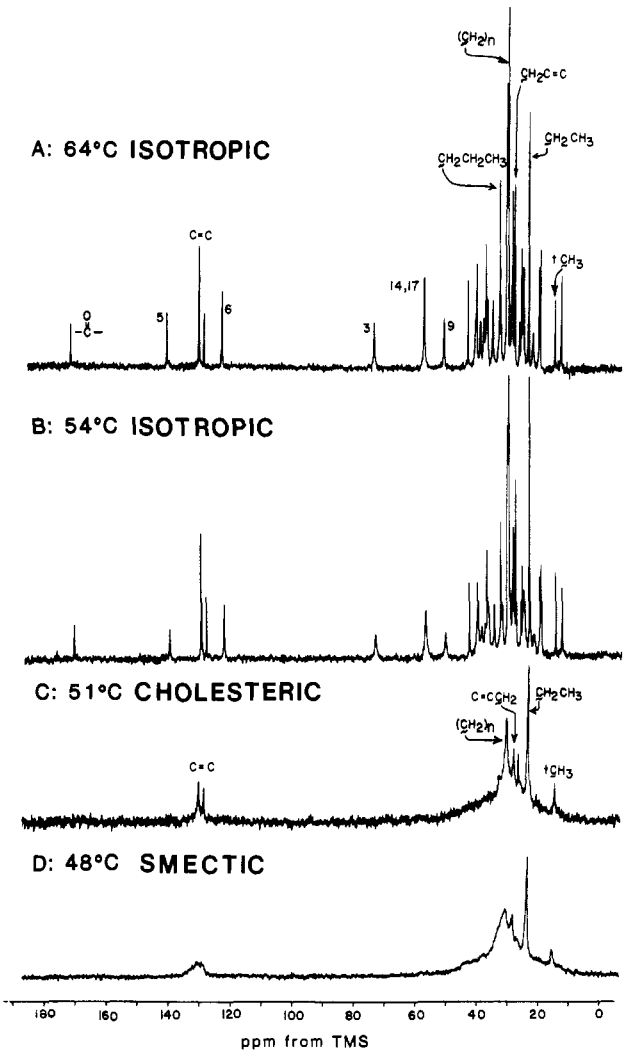

FIGURE 6: Natural abundance proton-decoupled Fourier-transform ^{13}C NMR spectra of a neat cholesterol ester mixture [CL = 0.21, CO = 0.51, and CP = 0.28 (mol fraction)] similar to that in CER-VLDL. Spectra A–C were recorded after 1000 scans with a 0.82-s pulse interval, a spectral width of 10000 Hz, and 16384 time domain points; line broadening of 2.0 Hz was applied to improve signal to noise ratios. Spectrum D was obtained as above, except after 4000 scans and with 3.0-Hz line broadening. Spectra A and B are printed with the same vertical expansion; the vertical expansions in spectra C and D are arbitrary.

steroid ring resonances at 38 °C, although they were broad. Their sample of CER-VLDL had a lower triglyceride content but a higher cholesteryl linoleate content than ours; these compositional differences would be expected to have opposing effects on the core transition temperature(s). The actual effect is not known since the DSC behavior of their sample was not reported.

In order to compare the molecular dynamics of pure cholesteryl esters with those in CER-VLDL, the temperature dependence of the model mixture CL/CO/CP (0.21:0.51:0.28) was studied. Figure 6 presents ^{13}C spectra of this mixture at selected temperatures in the isotropic liquid phase (64 and 54 °C), the cholesteric phase (51 °C), and the smectic phase (48 °C).[4] The essential features of the spectra in the different phases are the same as those reported previously for neat cholesteryl linoleate, cholesteryl oleate, and a mixture of the

[4] The small difference between the temperatures determined by NMR and DSC for the cholesteric → smectic phase transition is not considered significant and may be a result of differences in sample mass and heating/cooling rates.

two (Hamilton et al., 1977; Ginsburg et al., 1982). These features include (i) the disappearance of cholesteryl ring resonances upon cooling from the isotropic to the cholesteric phase and (ii) extreme broadening of fatty acyl olefinic resonances upon cooling from the cholesteric to the smectic phase. Although the spectra of the neat lipid mixture in the isotropic phase (Figure 6A,B) closely resemble spectra of CER-VLDL at temperatures at or above the higher DSC transition (Figure 4A,B), the spectra of this mixture in the cholesteric phase (Figure 6C) differs significantly from the spectrum of CER-VLDL near the lower (42 °C) DSC transition (Figure 4C), in that the latter still exhibits vestiges of cholesteryl ring resonances. Similarly, the spectrum of the CL/CO/CP mixture in the smectic phase differs significantly from that of CER-VLDL below the lower DSC transition in that the latter still exhibits two resolved (although attenuated) fatty acyl olefinic resonances (Figure 4D), whereas the former exhibits only a broad envelope (Figure 6D). With decreasing temperature, the CER-VLDL spectra show a *continuous* increase in line width for most resonances, and the spectrum of CER-VLDL at 38 °C (Figure 4D) more closely resembles the spectrum of the neat esters in a cholesteric phase than in a smectic phase. Thus, in contrast to the two transitions observed by DSC, only a single liquid → liquid-crystalline transition in CER-VLDL was detected by ^{13}C NMR. Furthermore, at a given temperature, the molecular motions of fatty acyl chain carbons in CER-VLDL were much greater in frequency and/or amplitude than motions of corresponding carbons in pure cholesteryl esters, implying a less rigid organization of the liquid-crystalline phase in the lipoprotein.

Spin-Labeled Cholesteryl Esters Can Detect Abrupt Changes in Acyl Chain Motions of Pure Cholesteryl Ester Mixtures near Their DSC Transition Temperatures. In CER-VLDL, about 30% of the fatty acyl carbons originate from phospholipids and 70% from cholesteryl esters. Since it was not possible to discriminate between fatty acyl carbons (other than C1 and C2) in these two molecular environments by natural abundance ^{13}C NMR, a complementary technique, spin-label EPR, was used. Spin-labeled phosphatidylcholine and cholesteryl ester bearing the paramagnetic group at either the 5 or 12 acyl chain carbon were selected as probes for comparing chain motion in the polar surface shell and hydrophobic core of CER-VLDL. The capacity of doxyl-labeled phospholipids to detect gel → liquid-crystalline transitions in bilamellar vesicles of dimyristoylphosphatidylcholine (Novosad et al., 1976) has been demonstrated. However, the capacity of doxyl-labeled cholesteryl esters to detect the smectic → cholesteric and cholesteric → isotropic transitions such as exhibited by a number of cholesteryl esters has not been established previously.

Two potential problems attend the use of these probes for studying the dynamics of cholesteryl esters in CER-VLDL: (i) the possibility that the foreign probe molecule (at the abundance used in the EPR experiments) might alter the *macroscopic* thermal behavior of the host cholesteryl esters and (ii) the possibility that the probe might perturb the local, *microscopic* envirnoment in such a manner as to misrepresent events occurring there. The first possibility was evaluated by performing DSC on the CO/CL/CP cholesteryl model mixture with and without 1% 12-DS-CE. This probe molecule decreased the C_1 (C_2) → isotropic transition from 45.5 to 42.2 °C, the isotropic → cholesteric transition from 51.2 to 48.2 °C, and the cholesteric → smectic transition from 45.0 to 42.0 °C. Hence, at the 1% level, 12-DS-CE caused a 3 °C decrease in DSC transition temperatures (Table IV). Similar decreases

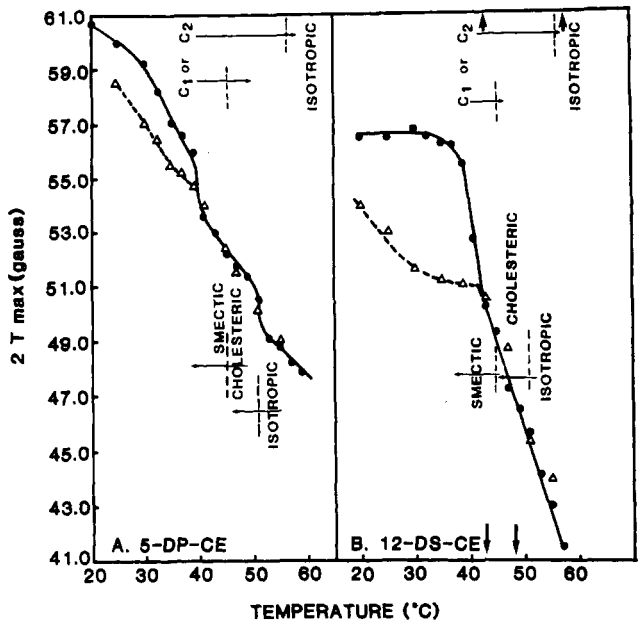

FIGURE 7: Temperature dependence of maximum splitting ($2T_{max}$) from EPR spectra of (A) (5-doxylpalmitoyl)cholesteryl ester and (B) (12-doxylstearoyl)cholesteryl ester in synthetic cholesteryl ester mixtures resembling those in CER-VLDL: CL = 0.21, CO = 0.51, and CP = 0.28 (mol fraction). Vertical dashed lines represent the DSC transition temperatures observed for the mixture containing no spin probes (Table IV). Solid arrows indicate the DSC transition temperatures observed for the mixture containing 1% spin-labeled cholesteryl ester. Data were acquired with increasing (●) and decreasing (△) temperatures.

were observed with 5-DP-CE. The second possibility was not as easily assessed. Our approach in this case was to determine the capacity of 5-DP-CE and 12-DS-CE to detect the temperature-induced transitions in the model mixture previously characterized by DSC. The plot of the total splitting between the low-field maximum and high-field minimum, $2T_{max}$, for 12-DS-CE in the model cholesteryl ester mixture exhibits a striking temperature dependence (Figure 7B). Heating the solid from 20 to 35 °C produces virtually no change in this spectral parameter. However, there is a precipitous 5-G drop at 39–41 °C, which is about 5 °C below the corresponding major DSC transition that occurs at 45.5 °C (Figure 3A). A further increase in temperature to 58 °C causes a linear, noninflected decrease in $2T_{max}$. Decreasing the temperature from 58 °C produces a linear increase in $2T_{max}$, which undergoes a sharp inflection at 41 °C, about 4 °C below the cholesteric → smectic DSC transition occurring at 45.0 °C (Figure 3B). The lower values for $2T_{max}$ obtained upon cooling the smectic phase below 40 °C compared to those values obtained upon heating the solid phase in the same temperature range demonstrate the capacity of the 12-DS-CE probe to distinguish between the metastable smectic and stable solid phases. Thermal data acquired with 5-DP-CE differed from, but complemented, those obtained with 12-DS-CE. At 20 °C, $2T_{max}$ with this probe was almost 61 G (Figure 7A). Heating the solid from this temperature resulted in continuously decreasing values for $2T_{max}$ until 40 °C was reached, where a small but reproducible inflection occurred. Again, this is about 5 °C below the solid → isotropic DSC transition occurring at 45 °C in the pure model mixture containing no spin-label (Figure 3A, Table IV) but close to the DSC transition of cholesteryl ester mixture containing 1% spin-label. A second, higher temperature inflection at 51 °C was also observed. This is 5 °C below the solid → isotropic DSC transition occurring at 56.5 °C. Upon decreasing the temperature from 60 °C,

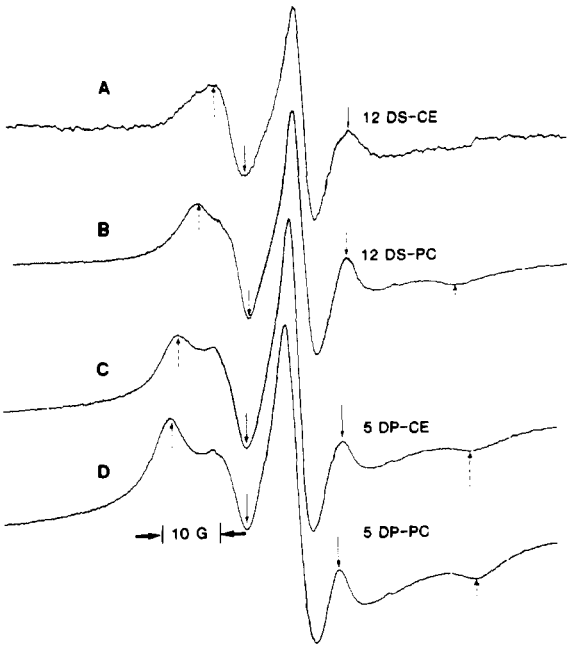

FIGURE 8: EPR spectra of CER-VLDL spin-labeled with (A) (12-doxylstearoyl)cholesteryl ester, (B) (12-doxylstearoyl)phosphatidylcholine, (C) (5-doxylpalmitoyl)cholesteryl ester, and (D) (5-doxylpalmitoyl)phosphatidylcholine: temperature 37 °C, modulation amplitude 1.0 G, power 20 mW, time constant 0.3 s. Conditions for labeling are described under Materials and Methods.

an inflection occurred at 50 °C, corresponding almost exactly with the isotropic → cholesteric transition observed by DSC. Further cooling caused a second inflection at 40 °C, about 5 °C below the cholesteric → smectic DSC transition (45.0 °C) (Figure 3B) and near the DSC transition of the mixture containing 1% spin-label. Again, it is noteworthy that the values of $2T_{max}$ obtained upon cooling below 40 °C are significantly lower than those obtained by heating in this range. In general, then, it appears that only 5-DP-CE detects the isotropic → cholesteric transition, whereas both 5-DP-CE and 12-DS-CE detect the cholesteric → smectic transitions.[5] These transitions detected by EPR occur about 5 °C below those observed by DSC for cholesteryl ester mixtures without spin-label probes but occur at about the same temperatures as those detected by DSC for the mixtures containing 1% probe molecules.

Neither Spin-Labeled Cholesteryl Esters nor Phospholipids Detect Abrupt Changes in Acyl Chain Motions of CER-VLDL Lipids. After the sensitivty of these spin-labeled lipids to temperature-dependent changes in structure and dynamics of well-defined model systems had been determined, it was possible to use them in a meaningful way to study acyl chain dynamics in their corresponding unlabeled lipid domains in intact CER-VLDL. When 12-DS-CE was incorporated into the lipoprotein, its 37 °C spectrum (Figure 8A) lacked a well-defined high-field minimum and reflected relatively rapid pseudoisotropic motion. The absence of this extremum made it impossible to calculate reliable order parameters for spectra collected over the temperature range studied. This result is in strong contrast to data obtained with the same probe in HDL_2 or HDL_3 at the same temperature (Brainard et al., 1980), which indicated a significantly more ordered environment for cholesteryl esters in HDL than in CER-VLDL. When 5-DP-CE was incorporated into CER-VLDL, the 37 °C spectrum (Figure 8C) contained well-defined extrema and reflected restricted anisotropic motion. This suggests that the cholesteryl ring more strongly restricts the motion of acyl chain carbons nearer it than those more distant and that motions within the chain are faster than mere rotation about the long molecular axis. Both phospholipid probes in CER-VLDL yielded spectra (Figure 8B,D) with larger splittings ($2T_{max}$) than those of the corresponding cholesteryl ester probes, indicating more restricted motion for phospholipid acyl chain carbons in the polar surface shell than for corresponding carbons of cholesteryl esters in the hydrophobic core.

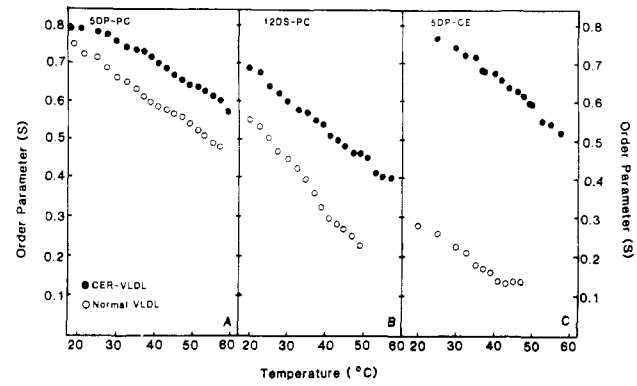

FIGURE 9: Temperature dependence of order parameters from spectra of (A) (5-doxylpalmitoyl)phosphatidylcholine, (B) (12-doxylstearoyl)phosphatidylcholine, and (C) (5-doxylpalmitoyl)cholesteryl ester in normal rabbit VLDL (open circles) and CER-VLDL sample 8A (closed circles). The chemical compositions and fatty acid analyses for these samples are given in Tables I and III, respectively. Values below 0.4 can be regarded as only rough estimates. Experimental uncertainty is about ±0.01.

The temperature dependence of order parameters obtained for 5-DP-PC, 12-DS-PC, and 5-DP-CE each incorporated into CER-VLDL and normal (triglyceride-rich) VLDL is graphically illustrated in Figure 9. Several aspects of these results are significant: (i) All probes reflected significantly higher order parameters in CER-VLDL than in normal VLDL at any given temperature. This difference was most striking for 5-DP-CE, an observation consistent with ^{13}C NMR observations that cholesteryl ester motions become more rapid and less anisotropic as the triglyceride to cholesteryl ester ratio is increased. This point is dealt with in further detail in a subsequent section. (ii) The plots reflect higher order parameters for 5-DP-PC than for 5-DP-CE at any given temperature over the range 25–55 °C, indicating somewhat more restricted motion for acyl chains in the polar surface than in the apolar core (Figure 10) for both normal VLDL and CER-VLDL. (iii) Comparison of order parameters for 5-DP- and 12-DS-PC show uniformly higher order for the doxyl moiety nearer the polar head group than for the one more distant. Hence, there appears to be an order gradient in the phospholipid monolayer of this lipoprotein similar to that demonstrated for the bilayer of vesicles (Novosad et al., 1976) and other model membranes (Hubbell & McConnell, 1971). (iv) Neither 5-DP-CE nor 12-DS-CE in CER-VLDL exhibit order parameter plots that contain temperature-dependent inflections of the magnitude seen for these probes in the neat cholesteryl ester mixtures (Figure 7A). Hence, these probes detect no thermotropic transitions in the core domain. These observations are entirely consistent with the NMR results that indicated gradual temperature-dependent changes in CER-VLDL cholesteryl ester motions and demonstrated the presence of a liquid-crystalline phase that possesed higher molecular mobilities than those of

[5] These EPR results are consistent with the ^{13}C NMR results, which indicate that resonances of carbons in the distal portion of cholesteryl ester acyl chains show only small changes in line width at the isotropic → cholesteric transition but large changes at the cholesteric → smectic transition.

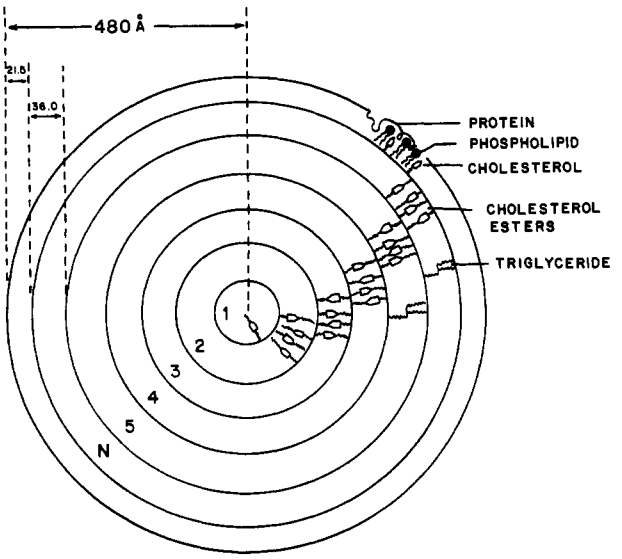

FIGURE 10: Schematic model of CER-VLDL with a 480-Å radius at or below 39 °C. An outermost shell of 21.5-Å width (Sata et al., 1972) contains 13% of the particle volume. Protein, phospholipid, and unesterified cholesterol are located primarily in this space. This leaves an inner core of 458.5-Å radius to contain the hydrophobic neutral lipids consisting primarily of cholesteryl esters, with much less amounts of triglycerides. This core is large enough to accomodate 13 concentric shells of cholesteryl ester, each 36 Å wide (Tall et al., 1978; Laggner et al., 1977).

pure cholesteryl esters at comparable temperatures. (v) Similarly, neither 5-DP-PC nor 12-DS-PC detected abrupt transitions, either inherent within the surface domain (intrinsic) or transmitted to it (extrinsic). This effect probably was not due to a lack of sensitivity of the probes since they can detect the gel → liquid-crystalline transition of pure DMPC bilayers (Novosad et al., 1976). Nor is it likely that the effect can be attributed to the difference in phospholipid layer thickness. Since the two halves of a bilayer in DMPC vesicles can undergo separate phase transitions (Sillerud & Barnett, 1982), one would anticipate that the monlayer that covers stable pseudomicelles like CER-VLDL should, in principle, be capable of undergoing the same type of transition. The absence of an inherent transition is probably due to the significant unsaturation and heterogeneity of the phospholipids (Table III) and/or to the protein and cholesterol also present in the surface shell. The absence of an extrinsic transition is probably due to the lack of coupling between the core and shell in this system. Indeed, Ginsburg et al. (1982) have reported that the egg yolk phosphatidylcholine/cholesteryl oleate system exhibits an order → disorder transition for the core cholesteryl esters but not for the surface phospholipids.

Since spin-label EPR did not detect any phase transitions of the cholesteryl esters in CER-VLDL and since ^{13}C NMR detected only a single broad transition from a liquid state to an ordered state, it is not clear what the two DSC endotherms (Figure 2) represent. The observation of two endotherms for intact CER-VLDL and for its isolated cholesteryl esters does not mean that the transitions in the intact lipoprotein correspond to isotropic → cholesteric and cholesteric → smectic transitions. In fact, the size of the average CER-VLDL particle (1000 Å; Roth et al., 1983) is too small to accommodate a true cholesteric phase, which requires a pitch of 4000 Å (Gray, 1962). Furthermore, the NMR spectra at temperatures below the lower DSC transition show that the esters in CER-VLDL do not form a smectic phase identical with that of the isolated (model mixture) esters. Thus, constraints of particle size and spherical shape influence the structural organization of the liquid-crystalline core. Such constraints may account for the lower enthalpy of the transition in CER-VLDL compared to denatured CER-VLDL and to its isolated esters. In human LDL, which has a diameter of 200 Å, the lower temperature liquid-crystalline phase has a radial smectic-like organization as determined by X-ray scattering (Atkinson et al., 1977; Laggner et al., 1977). In CER-VLDL, it is likely that a similar radial pattern is present and that the average size of the particle is such that 12 concentric shells could be accommodated (Figure 10). The two transitions detected by DSC may include transitions between different radial liquid-crystalline phases that are not detectable by ^{13}C NMR or by spin-label EPR. Alternatively, it is possible that there were two major subpopulations of CER-VLDL that, although possessing similar hydrated densities and particle sizes, have compositional differences that result in isotropic → liquid-crystalline transitions at two discrete temperatures. On the basis of data presented herein, it is not possible to distinguish between these possibilities.

Table V: Acyl Chain Order Parameters[a] (S) for Spin-Labeled Phospholipids and Cholesteryl Esters in Rabbit Lipoproteins

temp (°C)	sample[b]	5-DP-PC	12-DS-PC	5-DP-CE
30	CER-VLDL 8A	0.75	0.59	0.74
	CER-VLDL 9A	0.73	0.58	0.60
	normal VLDL	0.66	0.45	0.22
40	CER-VLDL 8A	0.70	0.52	0.67
	CER-VLDL 9A	0.67	0.48	0.58
	normal VLDL	0.58	0.31	0.14
50	CER-VLDL 8A	0.63	0.46	0.59
	CER-VLDL 9A	0.58	0.36	<0.4
	normal VLDL	0.52	0.20	<0.2

[a] Experimental uncertainty is about ±0.01. [b] See Table I for differences in composition of these samples.

Triglyceride Content of CER-VLDL Strongly Influences Acyl Chain Motions in the Core and Surface. Triglycerides are a significant source of perturbation for cholesteryl ester structural organization and thermotropic transition temperature(s) (Small, 1972; Hamilton et al., 1977). Inclusion of 4% triolein with a mixture of 72% CL and 24% CO abolishes the isotropic → cholesteric phase transition; only an isotropic → liquid-crystalline transition is observed. On the basis of these observations, we reasoned that CER-VLDL of increasing triglyceride content should have cores whose cholesteryl esters displayed decreasing order and that this decreased order might be reflected in the surface phospholipids as well. In some preparations of CER-VLDL, the triglyceride content of the total neutral lipids was above 5% (e.g., samples 8B and 9A, Table I), in which case two discrete endotherms in the DSC heating curve were not observed but rather a single, broad endotherm (data not shown). The effect of triglyceride on acyl chain order of cholesteryl esters is illustrated in Table V, which compares samples CER-VLDL 8A, CER-VLDL 9A, and normal VLDL, which contain 3.3, 8.2, and 57.7% triglyceride, respectively. When 5-DP-CE was used to probe the neutral lipid core of these lipoproteins, the 5% greater triglyceride content of sample 9A caused it to exhibit a significantly lower order parameter at 30 (0.605), 40 (0.580), and 50 °C (<0.4) compared to sample 8A (0.740, 0.675, 0.590, respectively). The much greater triglyceride content of normal VLDL caused a much greater reduction in order parameter. The possibility that this less ordered core of normal VLDL might be reflected in a less ordered surface shell was evaluated by using 5-DP-PC and 12-DS-PC. For this comparison, care was taken to use a CER-VLDL sample (8A) whose phospholipid fatty acid

composition closely matched that of normal VLDL (Table III). Figure 9A,B indicates a substantially lower order for phospholipid acyl chains in the surface of triglyceride-rich VLDL than for CER-VLDL 8A at any given temperature between 20 and 60 °C.

The present study demonstrates that the structural organization and molecular motions of the major core (cholesteryl esters) and surface (phospholipids) lipids in CER-VLDL are significantly different from corresponding features of normal VLDL. The higher degree of molecular order in the liquid-crystalline cholesteryl esters of CER-VLDL at 38 °C may have important consequences regarding the metabolism of these lipoproteins and their involvement in foam cell formation and atherosclerotic lesion development. The capacity of neutral lipids in the hydrophobic core to affect the dynamics of polar lipids at the surface has important implications. The lateral diffusion of apoproteins and lipids in the plane of the surface monolayer may be markedly affected by changes in the composition of the core during metabolism in the plasma compartment. Changes in these diffusion rates may, in turn, affect the rates of apoprotein and lipid dissociation/association at the lipid–water interface. In the case of specific proteins involved in receptor binding, the affinity with which they bind may be controlled by the dynamics of the phospholipid matrix in which they reside. Catalytic rates of lipolytic enzymes such as lipoprotein lipase, hepatic lipase, and lecithin:cholesterol acyl transferase may also be affected by these changes.

Acknowledgments

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Registry No. CP, 601-34-3; CO, 303-43-5; CL, 604-33-1; 12-DS-CE, 35203-87-3; 5-DP-CE, 69804-39-3; cholesterol, 57-88-5.

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