Low density lipoprotein particle size and core cholesteryl ester physical state affect the proton NMR magnetic environment of fatty acid methylene and methyl nuclei

John S. Parks[1] and Helmut Hauser*

Department of Comparative Medicine, Bowman Gray School of Medicine of Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157, and Department of Biochemistry,* Eidgenoessische Technische Hochschule Zurich, ETH-Zentrum, CH-8092 Zurich, Switzerland

Abstract: Recent studies using proton NMR to quantify plasma lipoprotein concentrations have shown that lipoprotein particle size affects the chemical shift of methyl and methylene resonances. The purpose of this study was to investigate the interrelationship of low density lipoprotein (LDL) size and cholesteryl ester (CE) physical state on the chemical shift of methyl and methylene protons. LDL were isolated from the plasma of nonhuman primates fed diets containing lard or fish oil to result in a wide range of LDL particle sizes and CE core transition temperatures for NMR analysis. The proportion of nuclei in the fluid state in LDL at different temperatures, measured as proton NMR peak intensity, paralleled the melting profile of LDL CE determined by differential scanning calorimetry. At 37°C the linewidths of several resolvable fatty acyl resonances, but not the choline methyl resonance, were significantly less in the fish oil LDL, indicating a less restrictive environment along the fatty acyl chain. The $(CH_2)_n$ and t-CH_3 resonances demonstrated an upfield shift (i.e., smaller chemical shift) with increasing temperature for both diet groups, but the peaks for fish oil LDL were always upfield from those of the lard LDL regardless of temperature. No change in the chemical shift of the choline CH_3 resonance was observed as a function of dietary fat or temperature. Above the transition temperature for LDL CE there was a significant positive correlation between LDL size and the chemical shift of the $(CH_2)_n$ and t-CH_3 resonances. This relationship was not statistically significant for the same LDL below the CE transition temperature. We conclude that LDL CE must be fully melted to obtain accurate results for LDL particle size distribution as well as mass quantification using proton NMR spectroscopy.—**Parks, J. S., and H. Hauser.** Low density lipoprotein particle size and core cholesteryl ester physical state affect the proton NMR magnetic environment of fatty acyl methylene and methyl nuclei. *J. Lipid Res.* 1996. **37:** 1289–1297.

Supplementary key words fish oil • lard • LDL • chemical shift • linewidth • fluidity • differential scanning calorimetry • cholesteryl ester transition temperature • nonhuman primates

Low density lipoproteins (LDL) are spherical particles composed of a surface monolayer of phospholipid, free cholesterol, and apolipoprotein(s) surrounding a hydrophobic core of cholesterol ester (CE) and triglyceride (TG) (1). The major apolipoprotein of plasma LDL is apoB-100, which is a ligand for receptor-mediated endocytosis by the LDL receptor (2). The predominant core lipid of LDL is CE. Neat CE exhibit multiple liquid-crystalline to liquid transitions depending on the fatty acyl composition of the CE (3). In intact LDL particles the CE are radially oriented in a smectic-like, liquid-crystalline array and exhibit one liquid-crystalline to liquid transition, the temperature of which is dependent on the fatty acyl composition of the CE and the amount of TG present in the particle core (4). Several studies have suggested that the LDL core composition, particularly with regard to TG content, may affect apoB conformation and LDL binding to the LDL receptor and may affect the cellular metabolism of LDL (5–8).

Recent studies have focused on the use of proton NMR to quantify lipoprotein concentrations in plasma (9–11). This procedure relies on deconvolution of the lineshape of the methyl resonance envelope to quantify the total mass of lipoproteins in plasma. Quantification of lipoprotein classes is possible because the chemical

Abbreviations: LDL, low density lipoproteins; DSC, differential scanning calorimetry; CE, cholesteryl ester(s); PL, phospholipid(s); t-CH_3, terminal methyl group; TG, triglyceride(s); TMSP, 3-(trimethyl-silyl)proprionic-2,2,3,3-d_4 acid.

[1]To whom correspondence should be addressed.

shift of the methylene $(CH_2)_n$ and terminal methyl (t-CH_3) resonances is related to lipoprotein particle size. Thus, these resonances arising from large lipoproteins such as LDL or VLDL are shifted downfield relative to those from HDL. In addition, Otvos et al. (10) showed that the quantification of lipoproteins by proton NMR was sensitive to temperature and more accurate at 40°C compared to 23°C. This was attributed to the melting of LDL core CE as the $(CH_2)_n$ and t-CH_3 signal amplitude increased with increasing temperature and LDL is the only lipoprotein species of quantitative significance in plasma that contains CE in an ordered state. However, the exact nature of the relationship of the methylene and methyl chemical shift to lipoprotein size and CE physical state is poorly understood.

The purpose of the present study was to investigate the interrelationship of LDL size and CE physical state on the chemical shift of the $(CH_2)_n$ and t-CH_3 resonances. We chose to use LDL from nonhuman primates fed diets enriched in lard or fish oil to alter the size and CE physical state based on previous studies (12). Thus, chemically defined LDL, which varied in size, were studied over a temperature range (25–57°C) in which the CE core of LDL from both diet groups ranged from liquid-crystalline to liquid. An additional advantage of using nonhuman primate LDL for these studies is the low TG content (<1%), which eliminates a confounding effect of TG on the CE phase behavior (4).

METHODS

Animals

Six adult male cynomolgus monkeys (*Macaca fascicularis*) were used for the study (n = 3 per diet group). The animals were provided through an NHLBI-sponsored nonhuman primate models program at the Bowman Gray School of Medicine and were a subset of animals used for another study (12). Experimental diets containing 40% of calories as fat with 0.26 mg cholesterol/kcal were fed to the animals for at least 6 months before blood was drawn for plasma LDL isolation. Half of the fat calories were derived from lard (lard diet group) or menhaden fish oil (fish oil group); the other half were from egg yolk or egg yolk replacement, a low cholesterol mixture that resembles egg yolk in composition (13). Both diets were balanced with regard to protein, carbohydrate, fat, cholesterol, and antioxidant content. More details regarding the experimental diets can be found in a previous publication (12).

LDL isolation and characterization

Blood samples were taken from animals after an overnight (18 h) fast. Ketamine hydrochloride (10 mg/kg) was used to sedate each animal while blood was taken from the femoral vein into chilled tubes (4°C) containing 0.1% EDTA and 0.02% NaN_3 (final concentration) at pH 7.4. Lipoproteins (d < 1.225 g/ml) were isolated from plasma by ultracentrifugation and fractionated by size using a 1.6 × 50 cm Superose 6B HPLC column equilibrated with 0.9% NaCl, 0.01% EDTA, and 0.01% NaN_3, pH 7.4, as previously described (12). This method allows the simultaneous preparative isolation of LDL and the determination of average LDL particle size, measured as LDL particle weight. The LDL peak was pooled, overlayered with argon, and stored at 4°C. Chemical compositions and fatty acid compositions were determined as described previously (12).

NMR

Aliquots of plasma LDL equivalent to 2–4 mg protein were concentrated by low speed centrifugation using Centricon 30 concentrators (Amicon, Danvers, MA). The samples were then diluted from ~50 µl to 1 ml with D_2O (99.9%; MSD Isotopes, Rahway, NJ) and reconcentrated. This procedure was repeated for a total of three D_2O washes. The samples then were brought to a volume of 0.5 ml with D_2O and 2 µmol of 3-(trimethyl-silyl) proprionic -2,2,3,3-d_4 acid (TMSP) in D_2O was added to each sample in a volume of 20 µl. The samples were transferred to 5-mm NMR tubes and gassed with argon.

Fourier transform proton NMR spectra were acquired using a Bruker 300 AM spectrometer. The D_2O in each sample served as a lock and shim signal for the analyses. Routine runs consisted of the following conditions: quadrature detection; 2500 Hz spectral width; 6.3 µs pulse width (90°); 16, 384 time domain points; 32 transients; and a 25.8 sec recycle time. A long recycle time was used to allow the TMSP protons to relax to equilibrium between pulses. The temperature of the sample was regulated between 25° and 56°C using a variable temperature unit. Sample temperature was assigned based on the chemical shift of the HDO peak according to the formula: T(°C) = (5.052 - chemical shift HDO in ppm)/0.0103 (14).

Using TMSP as an internal standard (0 ppm), proton NMR peak assignments were based on chemical shift values of resonances from previous studies (15). Peak intensities were determined from expanded plots of spectra using a HiPad digitizer interfaced to an Apple computer; peaks were digitized in triplicate and the average value was used. Errors for peak intensity were ±10%. Linewidth values were determined digitally in Hz as the width at half peak height.

After NMR analysis the LDL samples were removed from the NMR tube with a Hamilton syringe and an aliquot was taken for phosphorus assay (16). The remainder of the sample was diluted to 1.5 ml with 0.9%

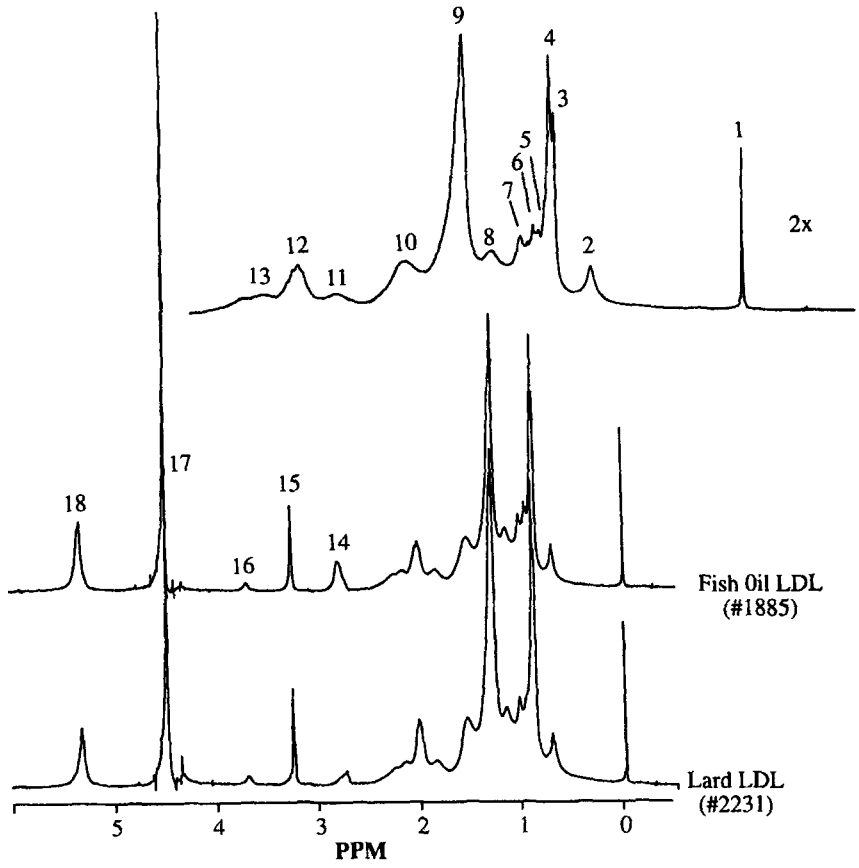

Fig. 1. Proton NMR spectra of LDL at 57°C. The spectra were obtained using a Bruker 300 AM spectrometer using the following conditions: 2500 Hz spectral width; 90° pulse width (6.3 μs); 16K time domain points; 32 transients and a 25.8 s recycle time. Peak assignments were made using previously published values (15) and TMSP as an internal standard. Plots were scaled to the internal standard (TMSP). The top scan represents a 2-fold horizontal expansion of #1885 LDL (fish oil) from -0.5 to 2.5 ppm. Each sample consisted of 4 mg LDL protein in 0.5 ml D_2O containing 2 μmol of TMSP.

NaCl, 0.01% EDTA, 0.01% NaN_3, pH 7.4, for differential scanning calorimetry analysis.

Differential scanning calorimetry

The LDL samples recovered from the NMR tube were degassed on a house vacuum line and analyzed in a Microcal MC-2 scanning calorimeter (Microcal, Inc., Northampton, MA) as described previously (17). Integrals of the scanning calorimetry curves were obtained by summing the cumulative area under the differential scanning calorimetry curve at 5°C increments from 5°C to 60°C. The data then were normalized to the total area under the curve.

RESULTS

The chemical and physical characteristics of the plasma LDL used for this study have been published (12). LDL from the fish oil group were significantly smaller than those of the lard group (4.23 ± 0.12 vs. 4.90 ± 0.13 g/μmol) and contained fewer CE (3191 ± 156 vs. 3732 ± 142), FC (790 ± 67 vs. 1136 ± 74), and phospholipid (PL) (1074 ± 42 vs. 1275 ± 30) molecules per particle. The fish oil LDL were enriched in n–3 fatty acids in the PL and CE fractions. The core CE transition temperature was also 11°C less for the fish oil group (32.3 ± 0.9 vs. 43.5 ± 0.5°C).

Figure 1 shows a high temperature (57°C) proton NMR spectrum of LDL from an animal from each diet group and the spectral assignments based on previous studies (15). A number of resonances were well resolved at high temperature, including several for cholesterol (C-18, C-21, C-19), several for esterified fatty acyl chains (t-CH_3, $(CH_2)_n$, olefinic, allylic, and double allylic) and several for the PL choline head group (choline CH_3 and choline CH_2N). The terminal CH_3 resonance was split into two peaks (peaks 3 [0.849 ppm] + 4 [0.869 ppm]) at high temperature (see expanded plot). This doublet may have resulted from the t-CH_3 of the fatty acyl groups of

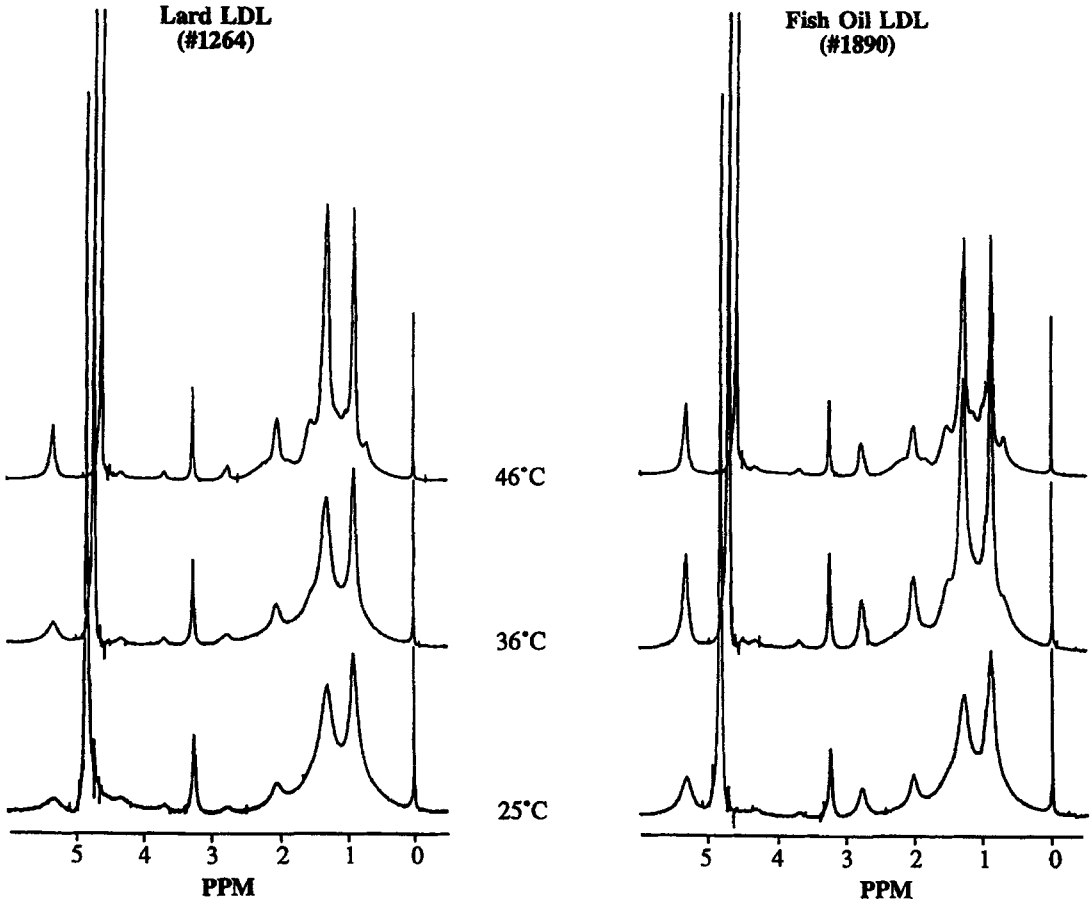

Fig. 2. Proton NMR spectra of LDL as a function of temperature. Details of experiment are given in the legend of Fig. 1 and in Methods.

PL and CE, the C_{26} and C_{27} methyl groups of free and esterified cholesterol, or spin coupling with the methylene nuclei.

Figure 2 illustrates representative spectra at selective temperatures for an animal in each diet group. At room temperature similar spectra of relatively broad lines were obtained for both diet groups. In the 0–3 ppm region only four resonances are apparent: terminal CH_3 (0.9 ppm), $(CH_2)_n$ (1.3 ppm), allylic (2.0 ppm), and double allylic (2.8 ppm). Downfield the choline CH_3 (3.3 ppm), HDO (4.5–4.8 ppm), and olefinic peaks (5.3 ppm) are clearly visible in both spectra. The choline CH_2N (3.7 ppm) and CH_2O (4.3 ppm) protons give rise to weak but detectable signals, whereas the protons of the glycerol backbone (CH_2O, 4.06 ppm) are not detectable. At 36°C there are distinct differences in the peak intensity for the two LDL samples. The resonances between 0–3 ppm and the olefinic peak (~5.3 ppm) show greater peak intensity (i.e., more area) for the fish oil LDL compared to the lard LDL. The spectra taken at 46°C are more similar in peak area between the two LDL samples than the ones taken at 36°C except for nuclei enriched in the fish oil LDL, such as the olefinic (5.3 ppm) and double allylic (2.8 ppm) nuclei. In addition, all resonances are relatively narrower, and more peak intensity is apparent in the 0–3 ppm envelope. We noted also a temperature-sensitive chemical shift of the HDO peak (4.5–4.8 ppm) relative to the TMSP internal standard (0 ppm).

To present a more quantitative analysis of the peak intensity changes with temperature, peak areas were measured by planimetry of expanded spectral plots, and the results were normalized to the peak area of the internal standard (2 μmol TMSP). **Figure 3** shows the relative intensity of several nuclei of LDL as a function of temperature between 25 and 57°C. As the 0–3 ppm envelope of the proton NMR spectrum contains several different resonances common to PL and CE, the entire envelope was integrated. The top panel of Fig. 3 shows the results. At room temperature the intensity for LDL from both diet groups was the same. Between 25°C and 40°C the 0–3 ppm envelope intensity increased for both diet groups, but the increase was greater for LDL from the fish oil group. Above 40°C there was little additional increase in intensity for the fish oil LDL, but the lard

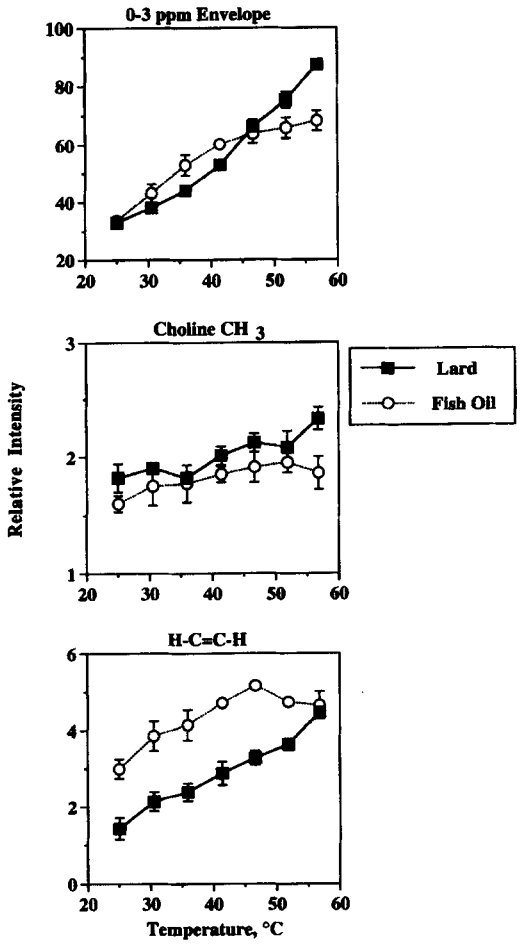

Fig. 3. Intensity of the 0–3 ppm envelope (top), the choline CH_3 (middle), and olefinic (bottom) resonances relative to that of the internal standard (2 μmol TMSP) at various temperatures. Peak intensities were determined from expanded plots using a digitizer. Details of NMR spectra are given in the Methods section. Each point represents the mean ± SEM (n = 3) for each diet group. In some cases the standard error bars are within the symbols.

LDL continued to show a nearly linear increase in intensity with increasing temperature. At 57°C the average intensity of the envelope for the lard group was 28% greater than that for the fish oil group (87 vs. 68). This likely resulted because of the 1.2-fold greater average number of lipid molecules per LDL particle in the lard group (6188 molecules) compared to the fish oil group (5121 molecules) in addition to the greater number of methylene protons on the phospholipid and cholesteryl ester fatty acyl chains of the lard LDL.

The middle panel of Fig. 3 shows the results for the well-resolved choline CH_3 resonance at 3.25 ppm. There was only a small increase in intensity with temperature for LDL from both diet groups. The values for the fish oil group were less than those for the lard group at all temperatures investigated, reflecting the lower PL/protein ratio for the fish oil group (0.96 vs. 1.04; (12)). As the amount of LDL PL in the NMR tube was quantified after each run and a known amount of TMSP was added to each tube, we calculated a choline CH_3 to TMSP theoretical intensity ratio and compared it to the measured peak intensities for these two resonances. At 57°C, the percentage of theoretical peak intensity recovered in the spectra was 101 ± 4% for the lard LDL and 92 ± 10% for the fish oil LDL, indicating that all choline CH_3 protons in the LDL samples were visible and accounted for in the spectra.

The relative intensity of the olefinic protons as a function of temperature is shown in the bottom panel of Fig. 3. At room temperature the peak intensity for the fish oil LDL was nearly twice that of the lard LDL. The olefinic peak intensity increased with temperature with both diet groups up to 40°C, after which the fish oil LDL showed no additional increase in peak intensity, whereas the lard LDL continued to show an increase up to 57°C.

LDL are known to exhibit a CE liquid-crystalline to liquid transition with a relatively broad temperature range (~20°C) that is dependent on the fatty acyl composition of the CE and the TG content of the particles (4). After the NMR runs, the CE transition temperature of the LDL samples was analyzed by differential scanning calorimetry (data not shown). The temperature at which 50% of the LDL CE molecules were melted was 33°C for the fish oil group and 40°C for the lard group. At 37°C (body temperature) 85 ± 3% of the CE in fish oil LDL had undergone a liquid-crystalline to liquid transition (i.e., melted), whereas only 36 ± 11% of the lard LDL had undergone a phase transition. At 46°C, 96 ± 4% of the CE was melted in lard LDL and 100% was melted in the fish oil LDL.

Figure 4 shows a plot of linewidth versus temperature for several resonances, including one of the interfacial surface of the LDL particle (choline CH_3), two along the fatty acyl chains of CE and PL ($(CH_2)_n$ and olefinic), and one at the terminus of the fatty acyl chain (terminal CH_3). For all resonances there was a decrease in linewidth with increasing temperature regardless of diet group. The linewidth of the $(CH_2)_n$ resonance was significantly narrower for the fish oil LDL up to 46°C after which it was similar to that of the lard LDL. The terminal CH_3 linewidth showed an interesting temperature dependence. Below 31°C and above 46°C there was little difference in the terminal CH_3 linewidth between the two types of LDL. However, at 36°C and 41°C the linewidths for the fish oil LDL were significantly less than those for the lard LDL. The choline CH_3 protons showed no diet-related difference in linewidth over the temperature range studied. Between 25°C and 41°C, the olefinic protons in the fish oil LDL were significantly narrower compared to the lard LDL. Above 36°C there

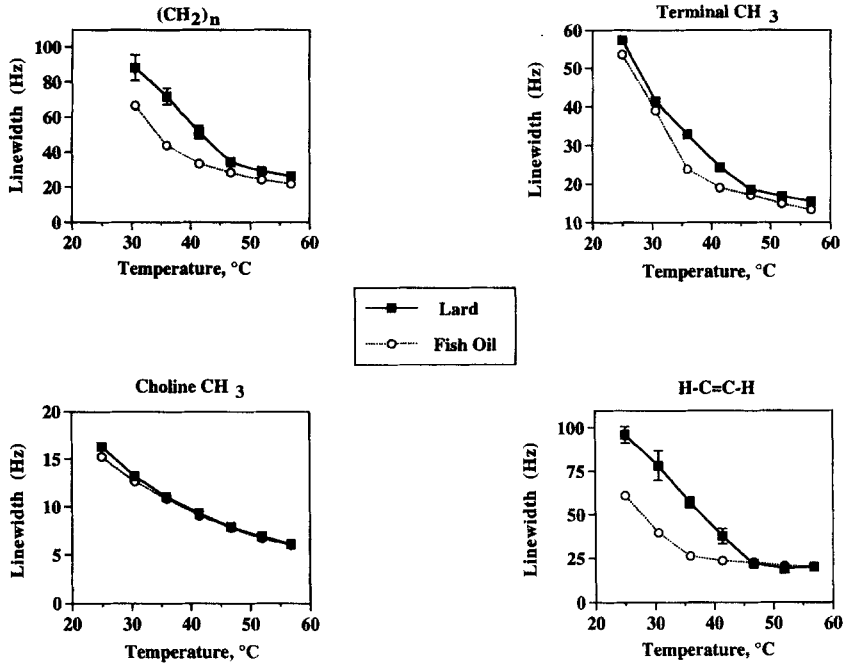

Fig. 4. Influence of diet on the linewidth of several proton NMR resonances of plasma LDL as a function of temperature. Linewidths were determined digitally. Values represent mean ± SEM (n = 3 for each diet group). In some cases the standard error bars fall within the symbol.

was no further narrowing of the olefinic resonance for the fish oil LDL, whereas the resonance for the lard LDL had a linear decrease in linewidth up to 46°C. Above 46°C there were no differences in linewidth for LDL particles from the two diet groups.

To investigate the effect of LDL particle size and CE physical state on the chemical shift of the fatty acyl $(CH_2)_n$ and t-CH_3 nuclei, we measured the chemical shift of the $(CH_2)_n$, t-CH_3, and choline CH_3 peaks relative to that of the internal standard, TMSP. The choline CH_3 nucleus served as a control as it is located on the headgroup of the PC. The results shown in **Fig. 5** illustrate two trends. First, both the $(CH_2)_n$ and terminal methyl resonances were shifted upfield (i.e., closer to TMSP) for the fish oil LDL compared to the lard LDL. Second, there was an upfield shift for these two resonances in LDL from both diet groups as temperature was increased from 25°C to 57°C. However, no such changes were observed with temperature or between diet groups in the choline CH_3 chemical shift.

Using data from individual animals we plotted LDL particle weight versus the chemical shift for the $(CH_2)_n$, t-CH_3, and choline CH_3 resonances at two different temperatures, 25°C and 46°C (**Fig. 6**). These temperatures were chosen based on our differential scanning calorimetry results which showed that LDL CE from both diet groups were liquid-crystalline at 25°C and fully melted at 46°C (data not shown). For the $(CH_2)_n$ and t-CH_3 resonances, there were positive associations between LDL size and a downfield shift in peak position. At 46°C, the association was statistically significant (P = 0.05) for both the $(CH_2)_n$ (r^2 = 0.65) and t-CH_3 (r^2 = 0.66) resonances. However, at 25°C neither of the two resonances showed a statistically significant downfield trend with increasing LDL size (r^2 = 0.10 for $(CH_2)_n$ and 0.40 for t-CH_3). The choline CH_3 resonance chemical shift was similar at both temperatures and unaffected by LDL particle size.

DISCUSSION

Our results demonstrate that LDL size and CE fatty acyl composition affect the chemical shift of the $(CH_2)_n$ and t-CH_3 resonances. These resonances were shifted upfield for the fish oil LDL at all temperatures studied between 25°–57°C compared to lard LDL. In addition, LDL from both diet groups exhibited an upfield shift with increasing temperature through the CE phase transition (Fig. 5). We also observed a significant correlation between LDL size and the chemical shift of the $(CH_2)_n$ and t-CH_3 resonances in LDL with completely melted CE cores (i.e., 46°C, Fig. 6). A relationship between lipoprotein particle size and chemical shift for the $(CH_2)_n$ and t-CH_3 resonances has been described by several investigators (9, 11). This relationship is thought to arise from differences in the ratio of surface to core constituents, so that as the amount of core is reduced

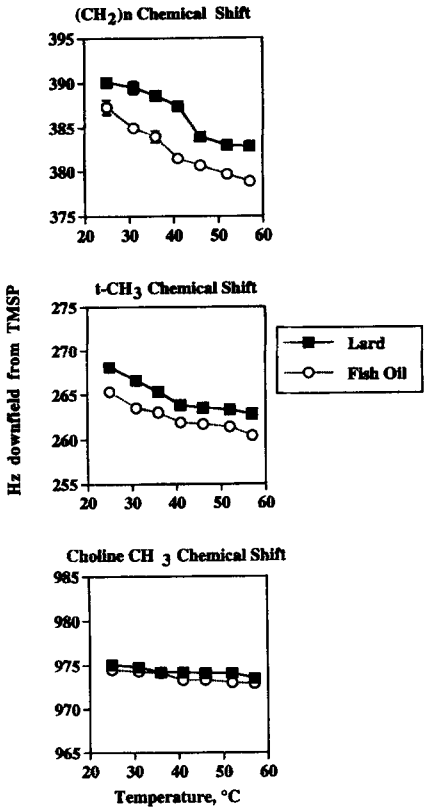

Fig. 5. Influence of diet and temperature on the chemical shift of several proton NMR resonances of plasma LDL. Chemical shift values were measured digitally relative to TMSP. Values represent mean ± SEM (n = 3 for each diet group). In most cases the standard error bars fall within the symbols.

relative to surface, the resonances are shifted upfield. Lounila et al. (18) have proposed that magnetic susceptibility anisotropy between the surface and core may explain this phenomenon. In their model, the lipid core was assumed to be isotropic and surrounded by a monolayer of PL whose magnetic susceptibility is anisotropic because of the ordered but radial orientation of the phospholipid molecules. Their derived equation for NMR frequency shift as a function of lipoprotein particle radius agreed well with the experimental results obtained for several lipoprotein subfractions of different size and predicted a curvilinear relationship between particle size and NMR frequency of several resonances, including the $(CH_2)_n$ and t-CH_3. Our data agree with their results, as the fish oil LDL, which are smaller, had resonances that are upfield from those of the lard LDL. However, the upfield shift of the $(CH_2)_n$ and t-CH_3 resonances with increasing temperature was greater than the difference in chemical shift between the two groups of LDL at any given temperature. The choline CH_3 chemical shift was constant regardless of the LDL particle size or temperature (Fig. 5). These data suggest that the proportion of fluid CE molecules within an LDL particle has as great an impact on the observed chemical shift of the $(CH_2)_n$ and t-CH_3 resonances as LDL particle size, at least over the size range of the LDL particles used in this study.

Our results have important implications regarding quantification of plasma lipoproteins by proton NMR. As noted by Otvos, Jeyarajah, and Bennett (9), quantification of lipoproteins by NMR was more accurate and precise at 40°C compared to 23°C due in large part to the increased amplitude of the methyl resonances for LDL and HDL. More recently Otvos et al. (10) have shown that this procedure can resolve and quantify subfractions of lipoprotein classes when the NMR analysis is performed at 45°C. As the $(CH_2)_n$ and t-CH_3 chemical shifts are dependent on LDL particle size as well as fraction of core CE that is melted, it is clear that the LDL core CE must be isotropic (i.e., fully melted) to get accurate results for the LDL particle size distribution as well as quantification. For example, if some LDL CE were in the liquid-crystalline state, the $(CH_2)_n$ and t-CH_3 resonances would be shifted downfield (i.e., higher ppm values) relative to the fully melted CE core and would erroneously appear to result from larger LDL subfractions. Thus, our results provide a mechanistic explanation for the spectral changes in $(CH_2)_n$ and t-CH_3 chemical shift with LDL particle size and temperature as previously noted by Otvos and coworkers (9, 10).

In the current study n–3 fatty acyl enrichment of LDL led to a substantial increase in the motional freedom of the fatty acyl chains of LDL. Although proton NMR does not allow us to separate the fatty acyl resonances of CE versus PL molecules, several observations suggest that most of the observed changes in motional freedom of the lipids were due to the CE fraction. First, the increase in signal intensity for the 0–3 ppm envelope (Fig. 2 and 3) correlated with the melting of the CE core measured by differential scanning calorimetry. At body temperature the fish oil LDL CE were close to being fully melted and the signal intensity of the 0–3 ppm envelope and the olefinic resonances had reached a maximum. However, the lard LDL CE were only 1/3 melted at 37°C and the NMR signal intensity for the 0–3 ppm and olefinic resonances continued to increase up to 57°C (higher temperatures were not investigated because of the risk of particle denaturation). Second, the linewidth of the fatty acyl chain resonances (Fig. 4) decreased as temperature increased through the CE phase transition. Above 46°C the linewidth values for both groups of LDL were nearly identical, indicating that the primary reason for the restricted motion of the resonances at lower temperature (i.e., < 46°C) was due to the liquid-crystal packing of the CE molecules. Third, in terms of lipid mass the CE fraction is the largest single lipid compo-

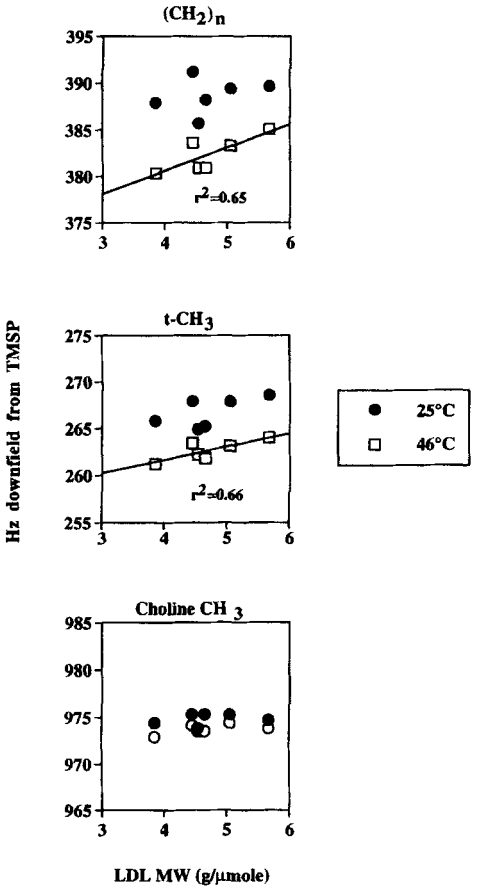

Fig. 6. Relationship of chemical shift to particle size of LDL (LDL MW) with liquid-crystalline (25°C) or liquid (melted) CE cores (46°C). Chemical shift values were measured digitally relative to TMSP. The line of best fit, determined by linear regression analysis, is shown where the regression line was statistically significant ($P = 0.05$). Each point represents an individual LDL sample from animals consuming the lard (n = 3) or fish oil diet (n = 3).

nent, accounting for just over half of the LDL particle mass (12). For these reasons, we conclude that the increase in lipid fluidity of fish oil LDL compared to lard LDL reflects primarily a change in the CE fatty acyl composition and its influence on the physical state of the CE core.

In contrast to the CE fraction, n-3 fatty acyl enrichment of the PL fraction of LDL had little observable effect on PL fluidity. Because of the overlap in CE and PL fatty acyl proton resonances, the only unique PL resonance of significant signal intensity that can be monitored by proton NMR is the choline CH_3. The intensity of the LDL choline methyl resonance was similar for both diet groups and showed only minor increases with temperature between 25-55°C (Fig. 3). Similar findings have been reported for LDL from control versus cholesterol-fed rabbits (19) and for human LDL (19). In addition, the decrease in choline methyl linewidth was the same for both diet groups (Fig. 4). All of the LDL choline methyl peak intensity could be accounted for in the spectra, within experimental error, indicating that apolipoprotein B (apoB) on the surface of the LDL particle was not restricting the motion of the PL headgroups on the NMR timescale. Yeagle et al. (20, 21) reported that about 20% of the LDL phospholipid may be immobilized by apoB using ^{31}P-NMR. Our data indicate that n-3 fatty acyl enrichment had little effect on PL headgroup motion, or alternatively, that the change in headgroup motion was faster or slower than that which can be monitored by proton NMR.

We have shown that n-3 fatty acid-enriched diets influence the biological, chemical, and physical properties of LDL and result in less coronary artery atherosclerosis in nonhuman primates compared to their lard-fed counterparts (12, 22-25). Fish oil LDL were shown to bind less avidly to cells in culture and to arterial proteoglycans (24-28). Part of the decreased binding appeared related to the decreased apolipoprotein E content of fish oil LDL (24, 25, 27). Some studies have suggested that the core composition of LDL can influence the conformation of apoB and the metabolism of the LDL particle (5-8). The results of our current study suggest that n-3 fatty acid enrichment had little effect on the motional properties of the LDL phospholipid monolayer, but had considerable effects on the CE core. However, it is unknown whether an increase in lipid fluidity of the fish oil LDL CE core can account for some of the observed differences in metabolism between fish oil versus lard LDL through direct effects on apoB conformation.

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