Proton magnetic resonance spectroscopy of plasma from patients with dyslipoproteinemia: identification of factors governing methyl and methylene proton line widths

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Abstract The line width of the proton magnetic resonance spectrum (MRS) of the composite methylene and methyl resonances of plasma has been reported as a marker for the presence of malignancy. In this study, the contribution of very low density (VLDL), low density (LDL), and high density lipoproteins (HDL) to the MRS line width was determined. This was achieved by measuring the MRS line widths for the plasma from patients with primary disorders of lipoprotein metabolism and from normal individuals. A negative correlation between plasma trigylceride levels and the average line width was observed and this was confirmed in normal plasma to which pure VLDL was added. Also, computer simulations were employed to demonstrate how the line width varies in such complex mixtures of lipoproteins. We demonstrate that the line width is governed by the relative contribution of VLDL and HDL to the composite line shape. This is particularly important when the shoulder from the HDL line lies near the half-height of the VLDL line. As changes in VLDL/HDL ratio occur in patients with malignancy, we propose that this is the basis of the narrowed MRS lines observed in the proposed test for malignancy. However, any individual with elevated VLDL will be false positive in this test.—**Herring, F. G., P. S. Phillips, and P. H. Pritchard.** Proton magnetic resonance spectroscopy of plasma from patients with dyslipoproteinemia: identification of factors governing methyl and methylene proton line widths. *J. Lipid Res.* 1989. **30:** 521–528.

Supplementary key words VLDL • HDL • triglycerides

Fossel, Carr, and McDonagh (1) have recently proposed that the proton magnetic resonance spectrum (MRS) of the composite methylene and methyl resonances of plasma may be used to detect the presence of malignancy. The average line widths of these resonances at half-height were found to be significantly narrower in the plasma from patients with cancer than those from healthy controls (1). Subsequently, the work of Bell et al. (2) showed that the lines in the proton MRS observed by Fossel et al. (1) are due to very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) in the plasma.

The presence of deranged lipid metabolism when cancer exists in humans is now well established (3). The sera of these patients often contain levels of lipoproteins that are different from those without disease (4) and the presence of cancer has been related to the presence of an RNA-containing proteolipid (5). Thus, the variation of the relative amounts of lipoproteins in human plasma is probably the underlying basis for the proton magnetic resonance test for malignancy proposed by Fossel et al. (1).

The results of the proton MRS study of Bell et al. (2) can be used to show that the shape and width of the composite spectrum observed for the methylene and methyl peaks of the lipoproteins will vary with lipoprotein composition of the plasma. The object of the present study was to investigate the proton MRS of plasma from patients with various disorders of lipid metabolism for which the lipid and lipoprotein composition is accurately known. Computer simulations are employed to show how the line width varies in these complex mixtures. Such a study provides an invaluable insight into the variation of line shape of the proton MRS of the methylene and methyl peaks with lipid and lipoprotein composition and answers, in part, the question: "Is the Fossel test simply based on variation of plasma triglycerides?"

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; MRS, magnetic resonance spectroscopy.

[1]British Columbia Cancer Registry Statistics (1986).

MATERIALS AND METHODS

Subjects

Blood samples were collected into EDTA after a 16-hr fast from 70 normolipidemic volunteers and 90 patients with primary disorders of lipid metabolism that resulted in hypertriglyceridemia and/or hypercholesterolemia. This study was approved by the Clinical Screening Committee for research involving human subjects. Plasma was removed by centrifugation at 1750 *g* for 10 min. Total cholesterol, free cholesterol, and triglycerides were determined enzymatically (6,7). HDL-cholesterol was determined as the amount of cholesterol remaining after precipitation of apoB-containing lipoproteins with heparin-$MnCl_2$ (8).

Proton magnetic resonance spectroscopy

All spectra from plasma were obtained with a Bruker AM400 spectrometer from double blind samples. The samples were not spun and the probe tuning and field shimming were optimized for the plasma samples. The water signal was suppressed by presaturation using on-resonance irradiation for 2S at approximately 0.5 W. The temperature (300K) and sample volume (0.8 ml) were rigorously controlled to avoid artefacts arising from variation of these two parameters. Line widths at half peak-height were measured as described by Fossel et al. (1) (see Fig. 1). Lactate was eliminated by careful sample preparation and did not have to be corrected for. Line broadening of 2 Hz was applied, but not corrected for. Spectra were phased using the Bruker automatic phase correction routine. This routine was surprisingly consistent and eliminated all operator bias (the Fossel line width is extremely sensitive to spectrum phase). Samples were frozen once before use. On average, this broadens the lines by 3 Hz; however, it introduces much less serious artefacts than analyzing a mixture of fresh and frozen samples. Samples were thawed and thoroughly mixed before use and allowed to equilibrate in the probe for 5 min before a spectrum was taken.

Isolation of plasma lipoproteins

High density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were isolated from normolipidemic pooled plasma by ultracentrifugation at densities 1.210–1.063, 1.063–1.006, and <1.006 g/ml, respectively (9), and recentrifuged at the upper density limit to remove remaining plasma proteins. The $d > 1.21$ g/ml fraction was defined as lipoprotein-free plasma. All fractions were dialyzed against 4 × 100 volumes of 0.15 M NaCl containing 0.1% EDTA and 0.03% sodium azide.

Addition of VLDL to plasma

VLDL was removed from a sample of plasma as described above. The infranatant containing the LDL, HDL, and the plasma proteins was then concentrated by 25% by Amicon ultrafiltration. VLDL dialyzed against 10 mM Tris-HCl containing 0.1% EDTA from pooled normal plasma was then recombined with the concentrated plasma to give final triglyceride which varied between 30 and 526 mg/dl. The remaining volume was made up with buffer alone. Proton MRS was then carried out on the resulting samples as described above.

RESULTS

The water-suppressed proton MRS of human plasma over the chemical shift range of 0–10 ppm shows a pair of prominent peaks centered at approximately 1.0 ppm which are those used in the Fossel test (**Fig. 1**). The spectra of VLDL, LDL, HDL, and delipidated protein obtained at 400 MHz are shown in **Fig. 2**. The spectra of VLDL and HDL are in agreement with those obtained at 500 MHz by Bell et al. (2). The most striking difference in these spectra is the narrowness of the VLDL methylene and methyl lines relative to HDL and LDL. In addition, the methylene peak of VLDL is quite asymmetric; this asymmetry has important consequences in the simulation of spectra (vide infra). The methyl and methylene peaks of HDL are chemically shifted upfield by about 0.05 ppm from the LDL and VLDL which have the same chemical shifts (as measured by the peak positions). The spectrum assigned as protein in **Fig. 3** is obtained from delipidated plasma; it is common to all spectra and has been tentatively assigned as being due to serum proteins. The sum of all of the spectra shown in Fig. 2 will lead to a typical spectrum shown in Fig. 3 where the assignments are indicated. It is apparent from the above results, and those of Bell et al. (2), that the measured line widths will depend markedly on the relative amounts of lipoproteins.

Frequency distribution analysis of the MRS line widths obtained for all individuals studied (**Fig. 4**) demonstrated that plasma from patients with hyperlipidemia (triglycerides > 200 mg/dl or total cholesterol greater than 220 mg/dl) had on average narrower line widths than are seen for normal controls (P, 0.001; Student's t-test). The mean line widths given in **Table 1** show that significant narrowing occurs in patients with increased triglyceride. However, increase in LDL-cholesterol levels in patients with hypercholesterolemia did not result in significant changes in line width. This suggests that the narrow line widths are related to generalized changes in plasma lipoprotein levels rather than disease-specific changes. Cor-

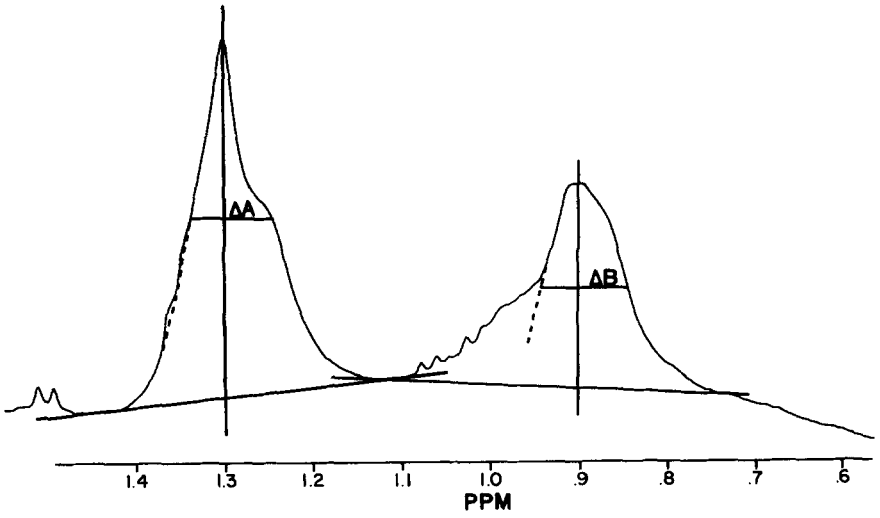

Fig. 1. Measuring the Fossel line width. The dashed lines show the construction used to correct the line widths for lactate and protein (where necessary). The solid lines show the construction used to correct for the baseline and to get the line widths. The Fossel line width is the average of the two line widths A and B.

relation analysis of the Fossel Average with all of the lipid values measured indicated a strong negative correlation (r = 0.6) with plasma triglyceride up to 200 mg/dl. This was observed in both the normal individuals and in the patients that were studied. The relationship between triglycerides and the Fossel Average of all individuals studied is shown in **Fig. 5**. This figure also indicates that above approximately 200 mg/dl the relationship between the line width and triglyceride level is no longer observed.

These results suggest that the variation in line width is due to varying amounts of VLDL which, along with chylomicrons, is the major triglyceride-carrying lipoprotein. This was confirmed by a reconstitution experiment in which varying amounts of VLDL were added to plasma containing a fixed amount of HDL, LDL, and protein. The spectra obtained are shown in **Fig. 6** and the Fossel Averages measured from these spectra are shown in **Fig. 7** (panel A). Clearly, as the amount of triglyceride increases, the Fossel Average decreases. The results of this reconstitution experiment clearly demonstrate how increases in VLDL will cause the Fossel Average to decrease. The slope of this relationship was similar to that observed for native plasma from both the hyperlipidemic and normal patients (Fig. 5). However, the minimum line width observed appears to be lower. This indicates that some factors, other than the mass of VLDL, also contribute to the line widths. It is well established that reciprocal changes in HDL and VLDL levels occur in patients with hypertriglyceridemia (10). Therefore, it seems likely that relative changes in this ratio, along with changes in LDL levels, govern the line widths measured.

The effect of the interrelationships between the amount of VLDL, LDL, and HDL on the proton MRS line

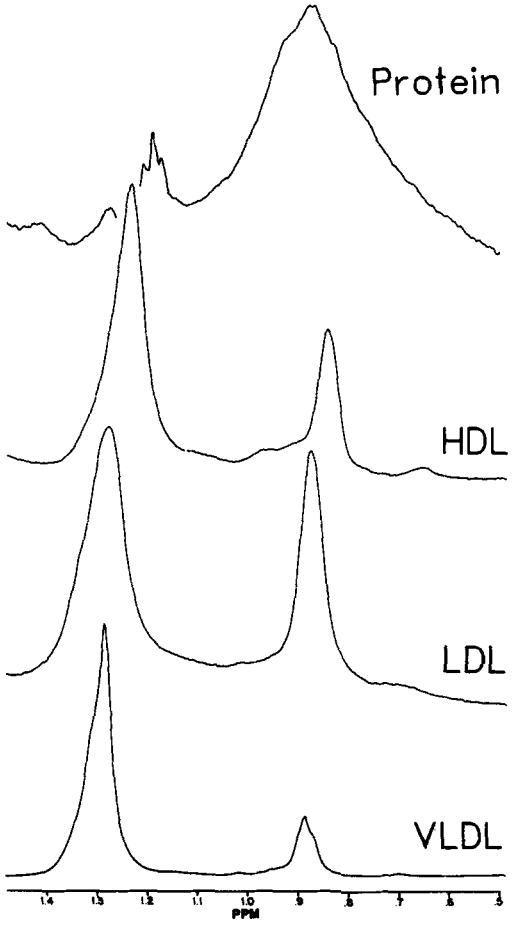

Fig. 2. Individual spectra of the various plasma components in the 0.5–1.5 ppm region of the proton magnetic resonance spectrum. In a normal plasma sample, the LDL, VLDL, and HDL components are approximately the same amplitude; the protein component is usually about 25% of the total amplitude.

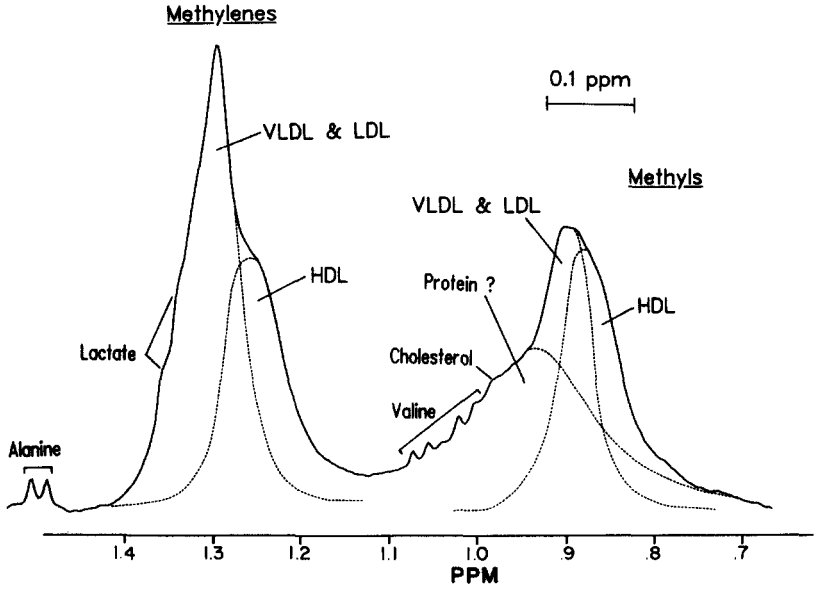

Fig. 3. Spectral assignments were made from the isolated lipoprotein spectra and delipidated plasma (Fig. 2) and from the assignments described by Bell et al. (2).

widths were studied using computer simulations. Simulated spectra were generated by using the line position and line widths taken from the MRS spectra of the individual plasma lipoproteins (Fig. 2); the values used in the simulations are given in **Table 2.** The simulated spectra are simply an addition of the spectra of HDL, VLDL, and LDL generated using these parameters and using a Lorentzian line shape. In order to determine the relative contribution of each component spectra to the total spectrum, it was necessary to adjust the amplitudes of each individual spectrum (**Fig. 8**). This was accomplished by fitting simulated spectra to the spectra obtained experimentally from samples of known lipid concentrations. This procedure, while not giving quantitative compostional analysis, provided a basis upon which the variation of line width with differing contributions from each component could be studied.

In order to study the effect of different amounts of specific lipoprotein classes on the line shape, a variety of simulations were performed for various combinations of simulated VLDL, LDL, HDL, and protein spectra. The gross features of all experimental spectra can be accounted for by a combination of the VLDL and HDL spectra. However, the line widths for both the methyl and methylene resonances for such spectra are too low, but this can also be easily accounted for as follows. The width of the methyl peak may be increased by incorporating the protein component into the spectrum using an approximate amplitude determined from the experimental spectra (Fig. 2; Table 2). The width of the methylene peak can be increased by incorporating LDL into the simulation. This also raises the amplitude of the methyl peak which is also too low in the simpler simulations containing no LDL. In addition, we find it is essential to incorporate the asymmetry of the methylene peak of the VLDL in order to be able to simulate the methylene region of the lipoprotein spectra correctly. The influence of the LDL level is more complicated. Its strongest effect is to broaden the methylene line from approximately 20 Hz to 30 Hz. This effect is almost constant for fractional peak heights for LDL of above 0.2 (Fig. 8). However, the shape of the line is also strongly affected and decreases the influence of

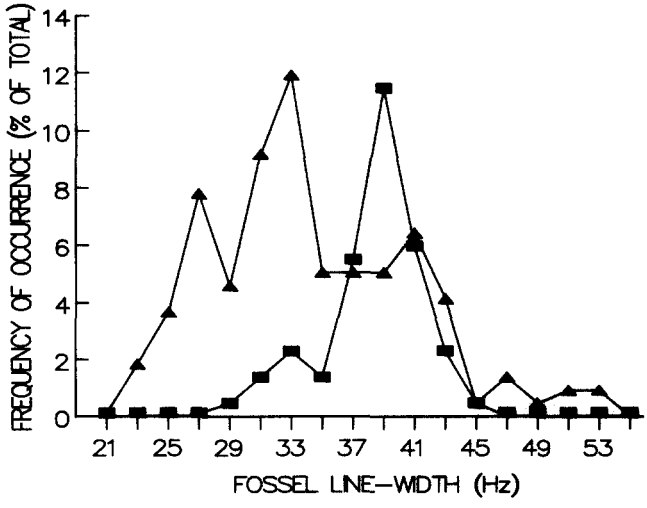

Fig. 4. Distribution of line widths for samples taken from hyperlipidemic patients (solid triangles) and normal individuals (solid squares).

TABLE 1. Plasma lipid levels and line width measurements in patients with primary hyperlipidemia

Group	n	Fossel Average	Total Cholesterol	Triglyceride	LDL Cholesterol	HDL Cholesterol
				mg/dl		
Normal	13	37.6 ± 3.8[a]	162 ± 28	88 ± 42	89 ± 21	56 ± 8.5
Familial hypercholesterolemia	35	33.6 ± 5.0*	326 ± 60***	165 ± 86*	227 ± 81***	47 ± 13*
Familial combined hyperlipidemia	6	28.5 ± 2.4***	295 ± 32***	309 ± 149*	172 ± 25***	41 ± 10**
Dysbetahyperlipidemia	4	28.9 ± 1.9***	469 ± 112***	663 ± 151***	73 ± 25	33 ± 0.5***

Significance of difference from controls (determined by Student's *t*-test): *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.
[a]Data are expressed as means ± 1 standard deviation.

VLDL on the line width such that the more LDL present, the greater the amount of VLDL required to narrow the line. Also, samples with high LDL levels will not show line widths less than 30 Hz, whereas samples with low LDL levels (with respect to VLDL levels) could show line widths of 20 Hz or less.

Using the simulation procedures described above and by correlating spectra of samples with known triglyceride levels, it is possible to simulate the reconstruction experiment described earlier. In order to compare the simulated spectra to the experimental data, the ratio of the MRS peak heights originating from VLDL to those of HDL was related to the triglyceride level. The variation of the simulated spectra for given changes in triglyceride content is shown in Fig. 7 (panel B). The fit for the variations of line width for the methylene line is good, but that for the methyl line is less satisfactory. In general, it seems that the width of the HDL methyl peak is greater in plasma than in its isolated state on which the simulations are based. Line widths determined from these simulated data are shown in Fig. 7 (panel B) and may be compared to the experimental data. Comparison of panels A and B of Fig. 7 clearly demonstrates the efficacy of the simulation procedures adopted here and supports the fact that the relative lipoprotein composition dramatically affects the line widths.

DISCUSSION

The present study has shown that for a variety of patients with dyslipoproteinemias the average line width of the methylene and methyl peaks from the proton MRS of plasma decreases with increasing triglyceride content. This observation was confirmed by a reconstruction experiment in which the level of VLDL-triglyceride was varied whilst keeping the HDL, LDL, and protein concentrations constant. Subsequent simulations demonstrated that the observed line widths are due to a complicated interplay between the various levels of VLDL, LDL, HDL, and protein.

Any given ^{1}H-MRS spectrum will be a composite of the VLDL, HDL, LDL, and protein spectra. This will bear a multivariate relationship to the actual relative lipoprotein levels in the sample. In practice, the situation is much simplified if we restrict ourselves to the average behavior of the samples. Normal plasma appears to have fairly constant amounts of LDL and protein. However, many hyperlipidemic individuals have elevated levels of VLDL (and hence triglyceride) which gives rise to a narrow line. Patients with cancer have slightly elevated triglyceride levels and also depressed HDL levels with respect to healthy individuals (3), which also gives rise to narrow

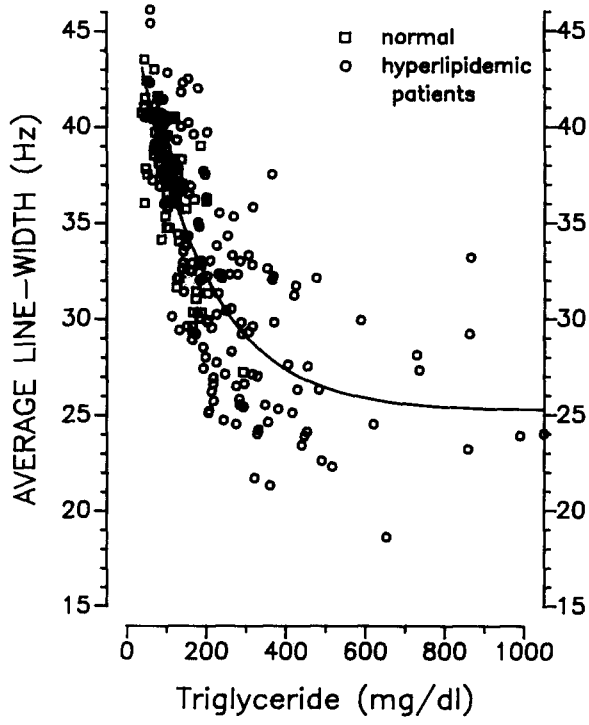

Fig. 5. Relationship between line width and triglyceride levels in all patients studied. The line is to guide the eye.

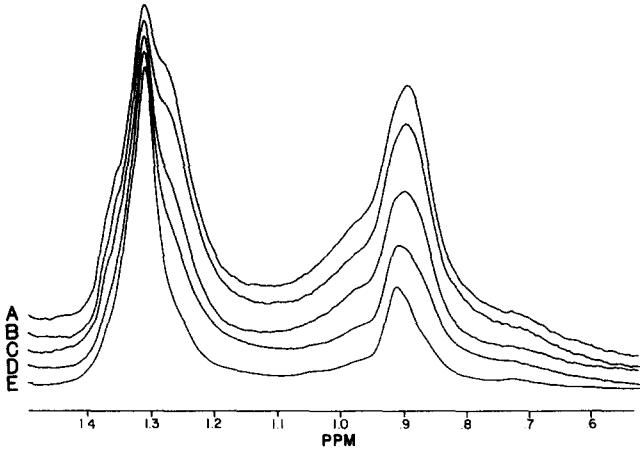

Fig. 6. The spectrum for the reconstitution experiment. The triglyceride concentrations (mg/dl) are: A = 5.0, B = 64, C = 93, D = 281, E = 526. The average line widths are shown in Fig. 7.

lines (11). However, in the latter case, the HDL and VLDL levels are similar to the LDL levels, so the line is broadened with respect to that from hyperlipidemic patients. The line width is mainly controlled by the HDL level and if it is high will give a broad line of 40 Hz or more. Conversely, Wilding et al. (11) have recently reported that in cancer patients with triglycerides above 200 mg/dl, the line width is dominated by the VLDL; hence, the line width is narrow. In addition, the data of Fossel et al. (1) indicate an abrupt decrease in line width between normal and cancer patients. Thus, our data suggest that this is controlled by the amount of HDL relative to VLDL. This is demonstrated by Fig. 8 in which the width of the methylene line depends almost entirely on where the shoulder from HDL lies with respect to the half-height of the VLDL line (similar arguments apply for the methyl leak, but the effect is less clear).

From the above discussion it is clear that the line shape is composite in nature and is controlled by the relative concentration of VLDL, LDL and HDL. Thus, narrow line widths are observed in samples with elevated VLDL and decreased HDL such as is found in plasma of patients with abnormal lipoprotein metabolism (**Table 1**). As a similar lipoprotein profile often occurs in malignancy (3), it is probable that this is the cause of the narrowed line widths observed in cancer patients by Fossel et al. (1). This suggestion is supported by the simulations which provide an adequate rationalization of what we have observed experimentally, by titration of VLDL into plasma and in hyperlipidemic patients. For example, elevated triglyceride levels resulted in narrower line widths. Since HDL levels are decreased in patients with cancer in some instances (3), this probably accounts for the observed line narrowing in these individuals. However, any individual with elevated VLDL will test false positive which would invalidate the Fossel test as a tool for screening for cancer.

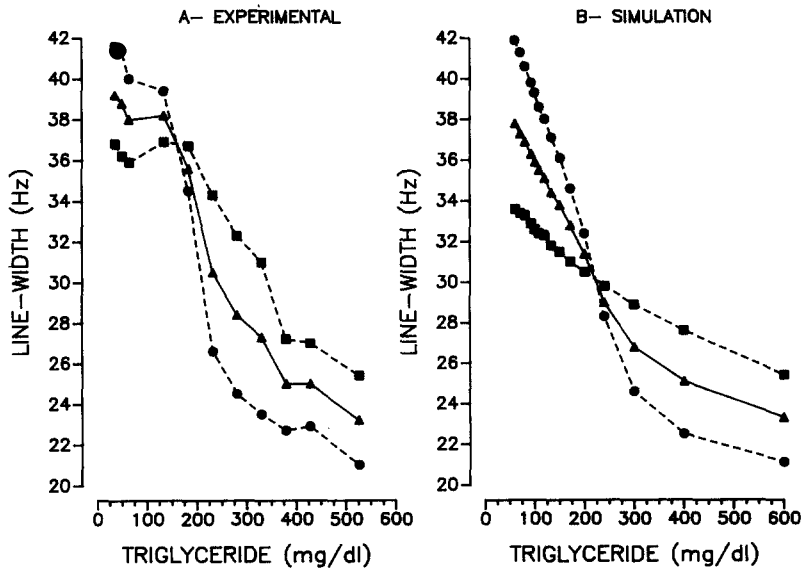

Fig. 7. Comparison of the experimental and simulated data for the reconstruction experiment. The average line width is shown as the solid line with triangles. The dashed line with squares is for the methyl peak and the other dashed line (with circles) is for the methylene peak. Note the difference in sensitivity of the two peaks to the triglyceride concentration. The data in panel B was generated after simulation of the effect of VLDL and LDL on the line shape as described in Fig. 8.

TABLE 2. Observed spectral parameters for plasma lipoprotein fractions

Peak	Position (Hz)	Width (Hz)	Relative Amplitude
VLDL methyl	160	17	0.24
VLDL methylene	0	12	1.00
VLDL methylene (shoulder)	−9	20	0.6
LDL methyl	163	21	0.92
LDL methylene	2	36	1.00
HDL methyl	175	18	0.44
HDL methylene	19	28	1.00
Protein	135	70	

The peak parameters are taken from spectra and used for the simulations. Amplitudes are the relative values for the methylene and the corresponding methyl peak in each sample. All positions are upfield of the VLDL methylene set at 0 Hz.

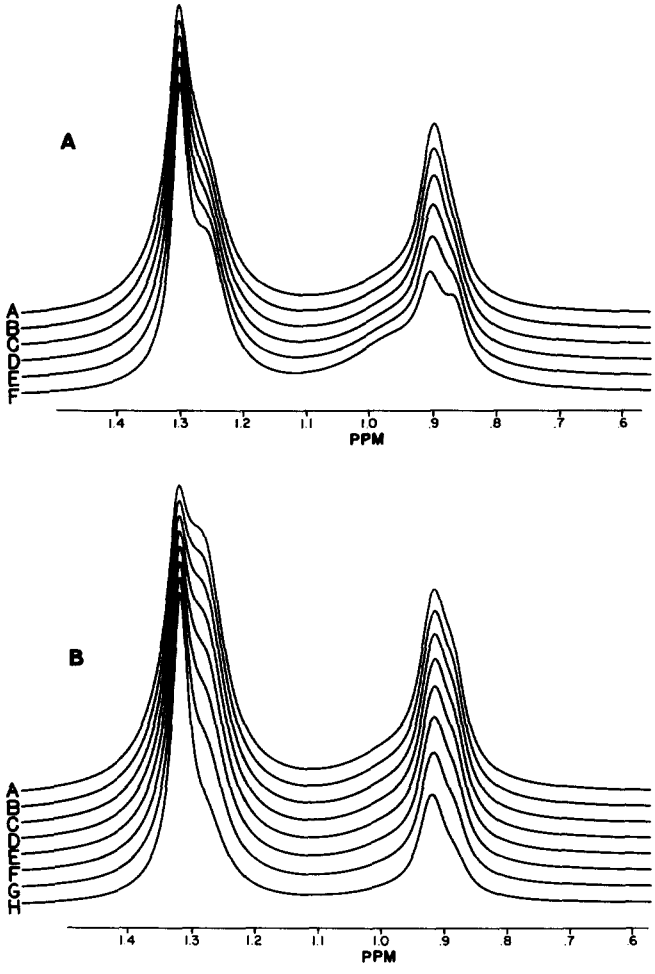

Fig. 8. A simulation of the effect of LDL and VLDL on the composite lipid spectrum. A: The amplitude ratios for VLDL:HDL:protein are 1.0:0.5:0.2. The amplitude ratios of the LDL component are A = 1.1, B = 0.9, C = 0.7, D = 0.5, E = 0.3, and F = 0.1. B: The ratio of the signal amplitudes for the LDL, HDL, and protein was 1.0:1.0:0.4, respectively. The VLDL component was varied as follows: A = 0.6, B = 0.7, C = 0.8, D = 1.0, E = 1.3, F = 1.7, G = 2.5, and H = 5.0.

This is particularly relevant when one considers that the age- and sex-adjusted incidence of cancer[1] is approximately 300 cases/100,000 whereas there will be approximately 15,000 persons per 100,000 with triglycerides greater than 200 mg/dl (12). Also, in certain circumstances, the relative levels of VLDL, HDL, LDL, and protein are such that the apparent width, at half height, of the methyl peak is increased to 70 Hz. Thus, the occurrence of false negative results is also possible. These observations suggest that any study of the relationship between Fossel line widths and malignancy should include an analysis of the lipoprotein content of the test plasma. Such a study is currently underway in our own laboratory.

Despite the cautions described in this paper, the use of MRS to analyze variations in plasma lipoproteins in malignancy and other disease states has considerable potential. However, careful analysis of line shapes as described here is required if valuable information is to be obtained.

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