

# Comparison of Localized Proton NMR Signals of Skeletal Muscle and Fat Tissue *in Vivo*: Two Lipid Compartments in Muscle Tissue

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***In vivo* <sup>1</sup>H NMR spectra of small volumes-of-interest (VOI) were localized in human soleus muscle (8 ml) and compared with volume selective spectra of subcutaneous fat tissue and femoral yellow bone marrow (2 ml). All examinations were performed by the double spin echo (PRESS) localization technique. To provide comparability, spectra of different tissues were recorded using identical sequence timing. Clearly improved resolution of the lipid signals of muscle tissue was obtained using long echo times TE > 200 ms. The spectra of muscle tissue exhibit lipid signals that stem from two compartments with a difference of their resonance frequencies of about 0.2 ppm (Larmor frequency difference 12–13 Hz at 1.5 T). The existence of two fatty acid compartments is supported by measurements of the relaxation times and line shape analysis. Both compartments contain fatty acids or triglycerides with similar composition. Probably one compartment corresponds to fat cells within muscle tissue, the other compartment with lower Larmor frequency is located within muscle cells.**

**Key words:** NMR; proton spectroscopy; localized spectroscopy; muscle tissue.

## INTRODUCTION

NMR spectroscopy of skeletal and heart muscle have been previously performed by many investigators in order to achieve noninvasive insight in metabolism of intact muscle tissue of healthy volunteers and patients with neuromuscular or vascular diseases. NMR signals of different nuclei have been recorded using various techniques for spatial localization:

Volume selective proton spectra from skeletal muscle tissue have been reported by several authors since 1986 (1–9). Strong lipid signals appear in a range between 0.8 ppm and 2.8 ppm (1–9), the distribution and assignments of the lipid signals are not consistent in all spectra of these investigators (e.g., (6) in comparison with (7, 8)). Additionally signals from unsaturated fatty acids occur at 5.3 to 5.9 ppm (1–9). Further <sup>1</sup>H signals of muscle tissue stem from creatin/phosphocreatin at 3.0 to 3.1 ppm (1, 3, 4, 7–9) and 3.9 ppm (3, 4, 8), from cholin at 3.2 ppm (1, 3–5, 7–9), and very small signals from aromatic amino

acids (His) appear at 7.0 ppm (1, 5, 8) and 8.0 ppm (1, 5, 7, 8). The histidine proton signals are pH sensitive (1, 8).

Slice-selective methods like DRESS (1, 3, 5, 6) and fully localized techniques, e.g., SPARS (2, 4) and STEAM (4, 6–9), have been used for the localization of large volumes-of-interest (VOI) of at least 27 cm<sup>3</sup>. The echo times ranged from 20 ms (8) to 400 ms (3). Proton NMR is not adequate to study the energy metabolism, which is concerned in neuromuscular and vascular diseases. Creatin and phosphocreatin signals are not distinguishable in proton spectra. Many investigations of high-energy phosphate metabolism of muscle tissue have been performed using <sup>31</sup>P spectroscopy (e.g., 10–14).

However, techniques in proton NMR spectroscopy have been improved in the last years and the use of <sup>1</sup>H for muscle spectroscopy offers some advantages:

1. Protons provide high signal per volume. Small VOI (minimum approximately 1 cm<sup>3</sup>) are sufficient to obtain highly resolved proton spectra in short measurement times at fields of 1.5 T. Examinations of such small volume elements that are chosen in homogeneous tissue regions without fasciae and large vessels are expected to exhibit new spectral information, especially using the PRESS localization sequence (15–21) providing high signal-to-noise ratio.
2. Radiofrequency pulses can be irradiated by the body coil of the imager resulting in good B<sub>1</sub> field homogeneity. Deviations from the intended flip angles are reduced and double spin echo sequences with high signal-to-noise ratio can be properly adjusted.
3. Various coils for signal reception are available for most of the imagers.
4. Improvement of the B<sub>0</sub> homogeneity (shimming) and the adjustment of the volume selective spectroscopy sequence following proton NMR imaging for determination of the VOI are carried out for protons more easily than for other nuclei.

These points show that a new approach to proton spectroscopy of muscle tissue and an evaluation of the signals that can be recorded and resolved using optimized measurement parameters is promising.

The signals in the range between 0.8 and 2.8 ppm are classified as signals from different protons of fatty acids. To elucidate the assignments of different signals, spectra from fat tissue of subcutis and of yellow bone marrow are compared with the results from human calf muscle using the same measurement parameters for <sup>1</sup>H spectroscopy. Especially the signals of triglycerids and of nonesterified

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fatty acids are important for examinations of the lipid metabolism within the muscle cells. The mitochondrial oxidation of fatty acids provides acetyl CoA to supply substrate for the Krebs cycle.

## METHODS

Volume selective proton spectroscopy was performed on a Siemens (Erlangen, FRG) Magnetom 63 whole body imager working at 1.5 T. The body coil was used for rf-excitation and surface coils ( $^1\text{H}$  Helmholtz coils of 170 mm diameter and an oval spine coil of 120 mm \* 230 mm from Siemens) for signal reception.

Volume selective spectra of a cubic VOI of  $(13\text{ mm})^3$  or  $(20\text{ mm})^3$  were recorded by a  $90^\circ - 180^\circ - 180^\circ$  double spin echo sequence, PRESS (15-21). The timing of the pulse train was according to  $90^\circ - t_1 - 180^\circ - t_1 - t_2 - 180^\circ - t_2$  - acquisition with fixed  $t_1 = 7\text{ ms}$  and variable  $t_2$  for echo times  $\text{TE} \geq 50\text{ ms}$ . The volume selection gradient strength was  $0.002\text{ T/m}$  and the length of the Hamming filtered sinc pulses was 2.56 ms resulting in a bandwidth of 1100 Hz for a cubic VOI of  $(13\text{ mm})^3$  and 1700 Hz for a VOI of  $(20\text{ mm})^3$ . We found in test measurements that the contamination from signals coming from outside of the VOI is negligible ( $<2\%$ ). The Larmor frequency difference between water and lipid signals is 215 Hz at 1.5 T. This chemical shift difference results in a localization difference between the VOI of water and lipids of about 2.5 mm. The size of a VOI of  $(20\text{ mm})^3$  in the soleus muscle of human calf is shown in Fig. 1a. Figure 3 exhibits a VOI of  $(13\text{ mm})^3$  within subcutis and yellow bone marrow. The coordinates of the VOI were determined by standard imaging in two perpendicular slices.

The echo time TE of the localization sequence was set to 50, 150, 250, and 350 ms in all cases presented. For evaluation of the transverse relaxation times  $T_2$ , 128 scans for muscle tissue and 32 scans for fat tissue were accumulated with a repetition time TR of 2 s for each spectrum to achieve good signal-to-noise ratio within a reasonable measuring time of 4.5 min for muscle and 1.2 min for fat tissue, respectively.

The longitudinal relaxation times  $T_1$  of the signals were evaluated using an inversion recovery method. A  $180^\circ$  rectangular inversion pulse with a length of 500  $\mu\text{s}$  is followed by the double spin echo sequence after inversion times  $\text{TI} = 50, 100, 200, 400, 800, 2000\text{ ms}$ . TR was chosen  $3\text{ s} + \text{TE}$  in all measurements to obtain unchanged intervals between acquisition and inversion pulse of the next scan for different TI. TE for each series of spectra is fixed to  $\text{TE} = 50\text{ ms}$  or  $\text{TE} = 350\text{ ms}$ . One hundred twenty-eight scans were added for each muscle spectrum, 32 scans were used for the spectra from fat tissue. Recording a large number of scans for highly resolved spectra is possible since time stability of the imager is very good: typically a shift of only 1 Hz/h was found experimentally (22).

For spectroscopy of muscle tissue, water suppression was performed by a Hamming filtered sinc pulse of 35.84 ms with a bandwidth of 70 Hz, followed by a spoiling

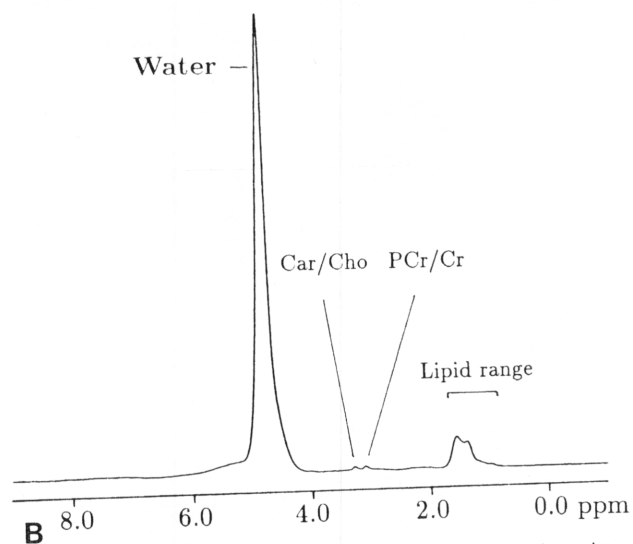
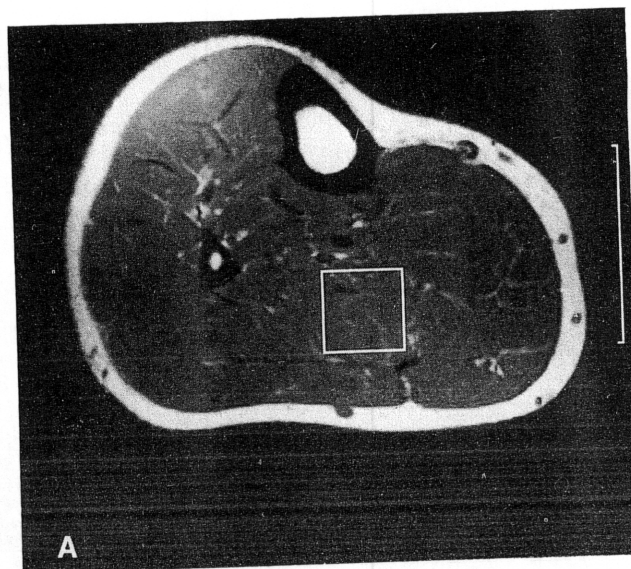


FIG. 1. (a) A volume element of  $(2\text{ cm})^3$  is marked within the soleus muscle in the calf of a 31-year-old healthy male volunteer. (b) The  $^1\text{H}$  spectrum from the VOI given in (a) without water suppression. Measuring parameters: 16 scans,  $\text{TR} = 2\text{ s}$ ,  $\text{TE} = 50\text{ ms}$ .

gradient. Transmitter frequency was adjusted to the water resonance before optimizing the pulse angle for minimal water signal from the volume element. The spectra for  $T_1$  measurements are not water suppressed.

For all spectra, 1 k complex data points were recorded using a dwell time of 1 ms resulting in a spectral width of  $\pm 500\text{ Hz}$ . Spectral processing involved multiplication of the original data by a rectangular function of 300 ms length, and additionally Gaussian multiplication with half height at 150 ms to improve signal-to-noise ratio without considerable line broadening. The resulting data were Fourier transformed before zero and first order phasing. No zero filling or baseline correction was applied. Localized shimming was necessary to obtain well resolved spectra.

## RESULTS

 $^1\text{H}$  Signals of Human Calf Muscle Tissue

Human calf muscles of 11 healthy volunteers and of three patients with fatty degeneration of skeletal muscle were examined to obtain spectra of homogeneous VOI of  $(20\text{ mm})^3$ . A volume element within the soleus muscle is shown in Fig. 1a. Such a small VOI can be selected in regions with very small content of connective tissue, fatty septums, and large vessels. The spectrum in Fig. 1b from this location is not water suppressed. The water signal amplitude is 14 times higher than the maximum signals of other metabolites. The water resonance was set to 4.77 ppm for this and all following spectra. Water-suppressed spectra from 128 scans are presented in Fig. 2. Small volume elements of  $(20\text{ mm})^3$  provide good signal-to-noise ratio using the double spin echo method. In the spectra with echo times from TE = 50 ms to TE = 350 ms, better resolution of the signals within "Lip 3" is obtained for long echo times. Four different signals become clearly resolved for TE = 350 ms. This phenomenon is visible in the localized spectra from calf muscle of all our healthy volunteers, if a VOI without fatty septums and large vessels was selected. Pathological muscle tissue with considerable fatty degeneration did not always allow to re-

solve four signals in spectra of long echo times. The signals at 1.6 and 1.4 ppm at the left-hand side of "Lip 3" do not show monoexponential transverse relaxation. The percentual signal loss of these spectral lines from TE = 50 ms to TE = 150 ms is stronger than from TE = 250 ms to TE = 350 ms. Probably both lines are composed of protons with different  $T_2$  and different linewidths resulting in improved signal resolution in spectra of longer echo times. The right-hand signals within "Lip 3" and the signals from creatine/phosphocreatine and *N*-trimethyl groups do not show such a multiexponential relaxation behavior. Olefinic protons exhibit signals at 5.3 to 5.7 ppm (Lip 1). The signals between 2.0 and 2.8 ppm (Lip 2) correspond to  $\alpha$ - or  $\beta$ -methylene protons of triglycerids or fatty acids. Histidine resonances were recorded at 8.1 and 7.1 ppm. The intensities of these signals in comparison with the other lines vary for different volunteers.

$B_0$  homogeneity within the *muscle cells* in the volume of interest can be evaluated from the linewidth of creatine methyl signals at 3.0 to 3.1 ppm and betaines (cholin, carnitin) signals at 3.2 to 3.3 ppm; 4.5 Hz full width at half-height (FWHH) is evaluated from the spectra.  $T_2$  values of approximately 160 ms cause linewidths of  $1/\pi \cdot T_2 = 2\text{ Hz}$ . Field inhomogeneity amounts to 2.5 Hz

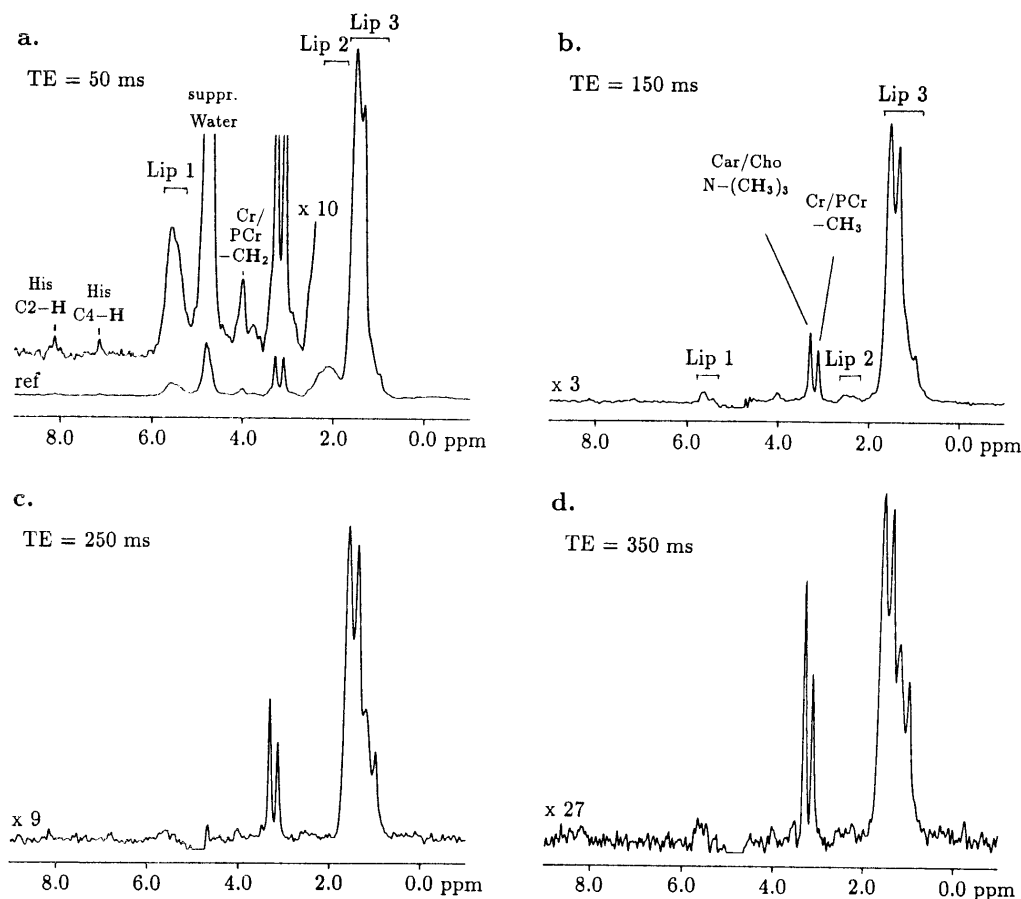


FIG. 2.  $^1\text{H}$  spectra from a VOI of  $(2\text{ cm})^3$  within soleus muscle as shown in Fig. 1a. All water-suppressed spectra stem from the same volume element of the 31-year-old male volunteer with unchanged pulse amplitudes, 128 scans, TR = 2 s. (a) TE = 50 ms, (b) TE = 150 ms, (c) TE = 250 ms, (d) TE = 350 ms.

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within the muscle cells of the VOI. In the following, spectra from fat tissue are reported to elucidate the curious lipid signal distribution in the muscle spectra. Signals which stem from fat cells between the muscle cells are expected to show similar signals as fat cells in subcutis or in yellow bone marrow.

### $^1\text{H}$ Signals of Human Fat Tissue

*In vivo* signals of lipids in gluteal subcutaneous fat and of femoral yellow bone marrow were recorded in three volunteers using the double spin echo method. Typical volume elements of  $(13 \text{ mm})^3$  are marked in Fig. 3. Echo

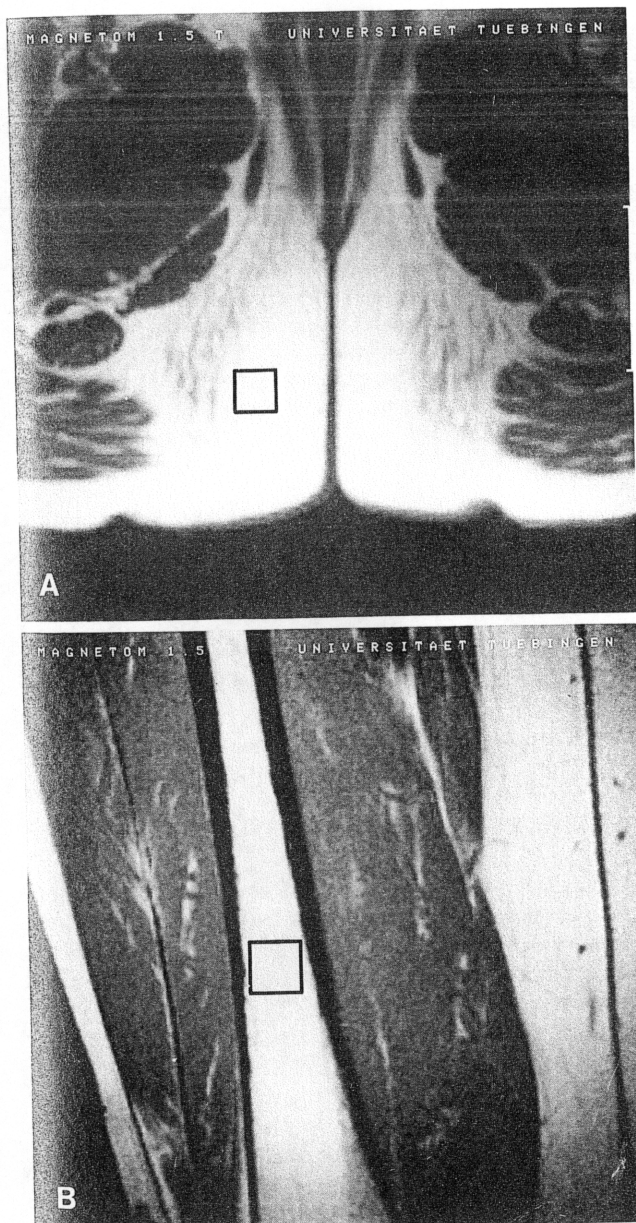


FIG. 3. (a) A VOI of  $(1.3 \text{ cm})^3$  for volume selective  $^1\text{H}$  spectroscopy is marked within gluteal subcutaneous fat tissue of a 25-year-old female volunteer. (b) Yellow bone marrow in the femur of a 25-year-old female volunteer was examined by volume selective spectroscopy of a VOI of  $(1.3 \text{ cm})^3$ .

times TE and repetition times TR are chosen according to the muscle examinations. Water suppression was not performed because the water content is very small in these tissues.

Figure 4 exhibits spectra with echo times between 50 and 350 ms from subcutaneous fat in correspondence to the spectra from muscle tissue in Fig. 2. The lipid signals from fat tissue are different from muscle tissue. For long echo times, only two signals are exhibited in the range between 0.8 and 2.8 ppm, the well-known signals of methylene  $(\text{CH}_2)_n$  and methyl  $\text{CH}_3$  protons of fatty acids (23, 24). Transverse relaxation of the methylene protons is not monoexponential and parallels findings in the left-hand signals within "Lip 3" from skeletal muscle. Transverse relaxation of the methyl protons is similar to the behaviour of the right-hand signals within "Lip 3" in Fig. 2. Signals of different  $\alpha$ - and  $\beta$ -methylene groups (23, 24) are well resolved. Additionally a smaller line remains for longer echo times from olefinic protons at 5.6 ppm. The linewidth of the latter signal is clearly smaller than in spectra from muscle tissue. The olefinic triplet shows phase anomalies due to J-coupling (21). The linewidths in the spectra and  $T_2$  relaxation data of the signals allow to calculate the  $B_0$  inhomogeneity within the VOI of  $(13 \text{ mm})^3$  to 3 to 4 Hz (FWHH).

Spectra of TE = 150 ms and TE = 350 ms from yellow bone marrow are shown in Fig. 5. The size of the volume element was chosen to 2 ml as for the examinations of the subcutis. Yellow bone marrow in the femur is less homogeneous and its osseous limitation is located in the edges of the VOI. Susceptibility effects of the micro-trabeculae may also cause broader lines in the spectra.  $B_0$  inhomogeneity of 5 to 6 Hz was obtained in bone marrow. Signal ratios between methylene and methyl signals and the relaxation behavior are very similar to subcutaneous fat, the olefinic signals are slightly decreased in femoral yellow bone marrow. Interindividual differences are small. In conclusion the length of the fatty acid chains are similar in subcutaneous and bone marrow fat, the olefinic part is slightly decreased in bone marrow.

### Classification of the Lipid Signals

Lipid signals from muscle tissue show components which are not found in fat tissue. The Larmor frequency difference between methylene and methyl signals in fat tissue is 25 Hz at 1.5 T (0.4 ppm). The muscle spectra show four well-resolved signals within "Lip 3" (see Fig. 2) which are nearly equidistant. There is a difference between the resonances of about 12.5 Hz. Therefore, it is reasonable that two of the four signals from muscle tissue are comparable with the methylene and methyl signals from fat tissue. Ensured classification of the signals using the water signal as reference is impossible since the water content of fat tissue is too small. Therefore, the correct assignments were determined making use of a spectrum of skeletal muscle with evident fatty degeneration (histologically confirmed) which was recorded by the same technique on our imager. This tissue had a clearly increased amount of lipid cells between the muscle cells.

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FIG. 4.  $^1\text{H}$  spectra from a VOI of  $(1.3\text{ cm})^3$  within subcutaneous fat tissue as marked in Fig. 3a. The spectra are not water-suppressed and stem from the same volume element of the 25-year-old female volunteer with unchanged pulse amplitudes, 32 scans, TR = 2 s. (a) TE = 50 ms, (b) TE = 150 ms, (c) TE = 250 ms, (d) TE = 350 ms.

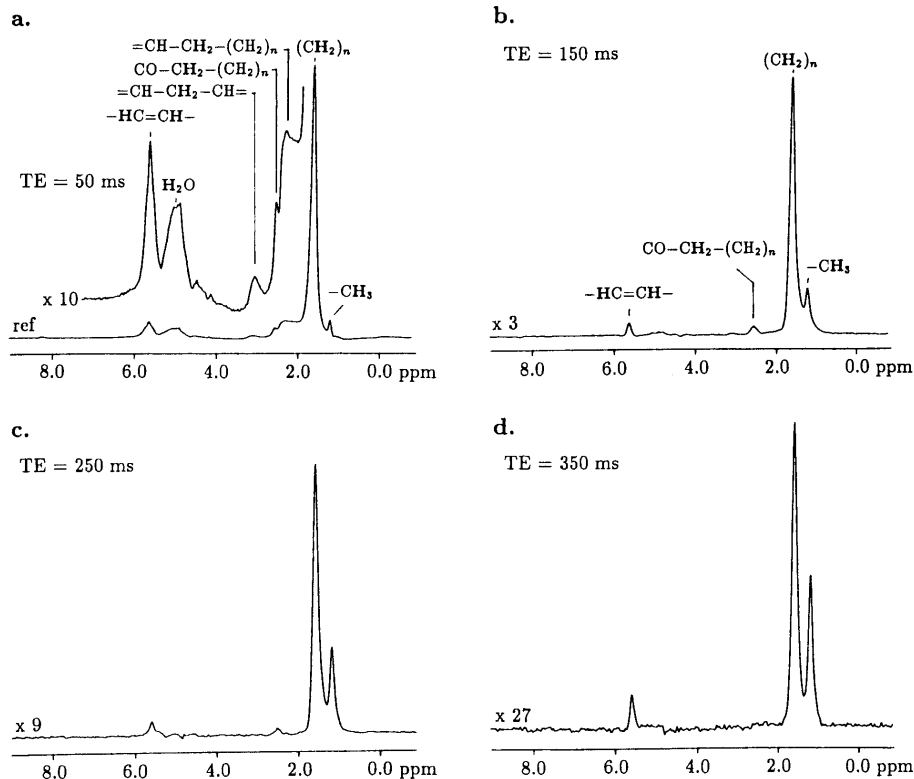
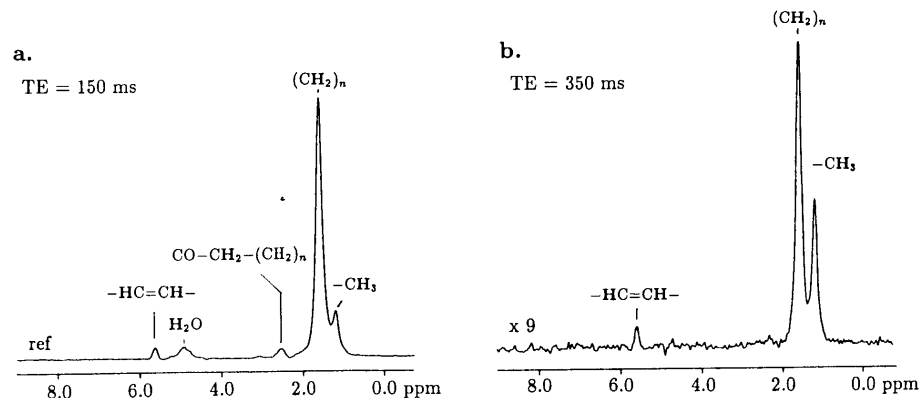


FIG. 5.  $^1\text{H}$  spectra from VOI of  $(1.3\text{ cm})^3$  within femoral yellow bone marrow as shown in Fig. 3b. Both spectra were recorded without water suppression from the same volume element of the 25-year-old female volunteer using unchanged pulse amplitudes, 32 scans, TR = 2 s. (a) TE = 150 ms, (b) TE = 350 ms.



Strong lipid signals from fat cells and also smaller signals from creatines and betaines of remaining muscle cells result in spectra as shown in Fig. 6. Comparison with a spectrum from normal muscle tissue with equal measurement parameters shows that the methylene signal from the fat cells correspond with signal 1 of normal muscle tissue. Methyl signals from fat cells are in conformity with signal 3. The signals 2 and 4 are specific for muscle cells. To obtain a proper assignment of the resonances, spectra from fat tissue and normal calf muscle are compared again. Spectra of equal echo time TE = 150 ms and pulse timing of the localization sequence are shown in Fig. 7. Each proton resonance from the triglycerids is accompanied by another resonance which is offset in Larmor frequency by 12 to 13 Hz. The signals marked with an *A* are in accordance with the signals from fat cells. The signals of similar intensity which are offset in

Larmor frequency are marked with *B*. This phenomenon was visible in well resolved spectra of all healthy volunteers examined. Since all signals from fatty acids are accompanied by resonances which are offset in Larmor frequency, there must be two compartments *A* and *B* containing similar composition of saturated and unsaturated fatty acids or triglycerids. Compartment *A* corresponds to fat cells within muscle tissue. Signals from compartment *B* seem to be related to muscle cells. Lipid vacuols located within cytoplasm between myofibrils of skeletal muscle may be responsible for the compartment *B* signals with their reduced Larmor frequency of 12 to 13 Hz at 1.5 T.

#### Relaxation Times

The relaxation behavior of the signals in spectra from muscle tissue and fat tissue was evaluated to assess or

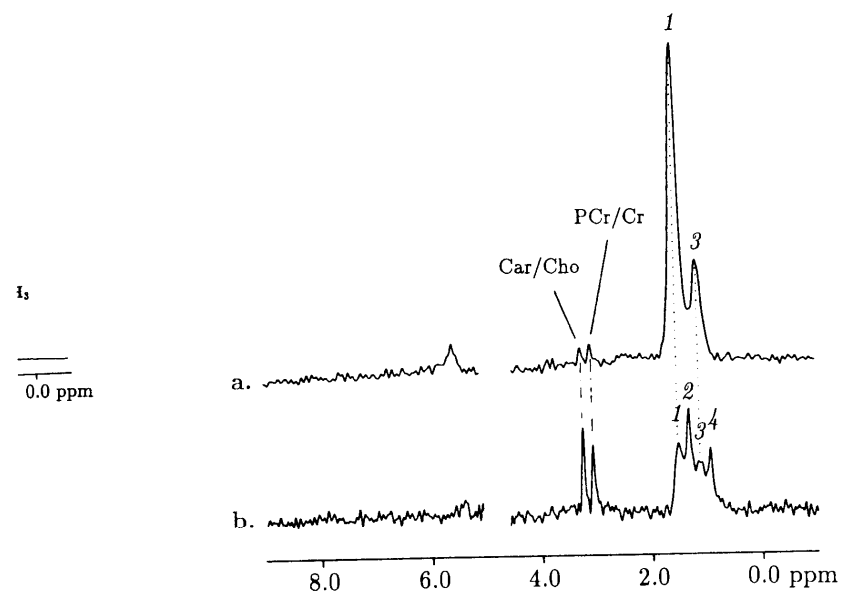


FIG. 6. Comparison of spectra: (a) Musculus rectus femoris of a 60-year-old male patient with fatty degeneration (histologically confirmed). (b) musculus soleus of a 36-year-old healthy male volunteer. Both spectra were obtained using water suppression, 128 scans, TR = 2 s, TE = 400 ms, and VOI of (2 cm)<sup>3</sup>. The correspondence of the lipid signal from fat cells with the signals 1 and 3 from normal calf muscle tissue in the range between 0.8 and 1.8 ppm is demonstrated.

contradict the statements about spectral components of two different compartments in skeletal calf muscle. Transverse relaxation data were obtained from the spectra with echo times TE = 50, 150, 250, 350 ms as shown in Figs. 2, 4, 5. Longitudinal relaxation times  $T_1$  were determined using the inversion recovery technique as described in Methods section. Figure 8a shows spectra from muscle tissue with fixed TE = 50 ms, Fig. 8b exhibits spectra with identical measurement parameters from yellow bone marrow of the femur. Longitudinal relaxation of the muscle tissue signals in the lipid ranges "Lip 1," "Lip 2," and "Lip 3" (see Fig. 2) are not clearly different from relaxation of the signals which stem from femoral yellow bone marrow. The signals in the range between 0.8 and 1.8 ppm are better resolved using long echo times (see Fig. 2) and show less superposition of each other. Therefore muscle and fat tissue signals have been recorded using the inversion recovery method with fixed TE = 350 ms. Figure 8c shows  $T_1$  relaxation times of all four muscle signals within "Lip 3" in comparison with subcutaneous fat signals in Fig. 8d. Identical measurement parameters were used. The spectra in Fig. 8c show that  $T_1$  of the left-hand components at 1.4 and 1.6 ppm are similar to the methylene groups from fat tissue (Fig. 8d). The muscle signals at 1.0 and 1.2 ppm exhibit  $T_1$  according to methyl protons within fat tissue. The statement of the existence of two fatty acid compartments A and B is supported by these measurements.

The evaluated relaxation times  $T_1$  using echo times TE = 50 ms and TE = 350 ms as well as  $T_2$  data are given in Table 1 for the lipid signals and in Table 2 for the other

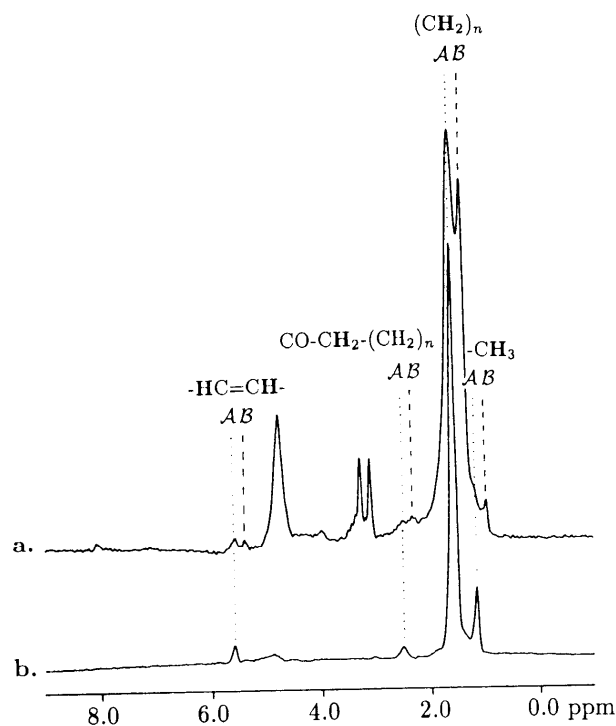


FIG. 7. Comparison of spectra: (a) Calf muscle spectrum from a healthy 36-year-old male volunteer (water suppression, 128 scans, TR = 2 s, TE = 100 ms, VOI of (2 cm)<sup>3</sup>). (b) Subcutaneous fat of a 28-year-old male healthy volunteer (32 scans, TR = 2 s, TE = 100 ms, VOI of (1.3 cm)<sup>3</sup>). Characteristic signals from fatty acids occur double in the spectrum from calf muscle with a Larmor frequency difference of about 12 to 13 Hz.

muscle signals. The water resonance shows longer  $T_1$  in the inversion recovery measurement, when a fixed echo time TE = 350 ms was used instead of TE = 50 ms. The VOI and the pulse amplitudes were unchanged for the measurements with different TE in Fig. 8a and Fig. 8c. Probably water has several components within muscle tissue. Water components with short  $T_2$ , which disappeared for TE = 350 ms, may have shorter  $T_1$ . Methylene signals show multiexponential transverse relaxation and also negligible dependence of  $T_1$  on the echo time chosen. Figures 8b and 8d must not be compared directly, since the VOI was different.  $T_1$  data of lipids in Table 1 were obtained using the same VOI for TE = 50 ms and TE = 350 ms in all tissues. The standard deviation of the evaluated relaxation times is about 10% considering the strong spectral signals. The values are similar to data from Bruhn *et al.* (8). Relaxation times from J-coupled protons or from signals which are clearly influenced by neighbouring lines are marked in the tables and result in standard deviations of more than 10%.

### Signal Shape

The signal shape between 0.8 and 1.8 ppm ("Lip 3" in Fig. 2) were analysed in spectra from muscle tissue (Fig. 9) and yellow bone marrow (Fig. 10). The measured spectra from human calf muscle for TE = 150 ms in Fig. 9a can

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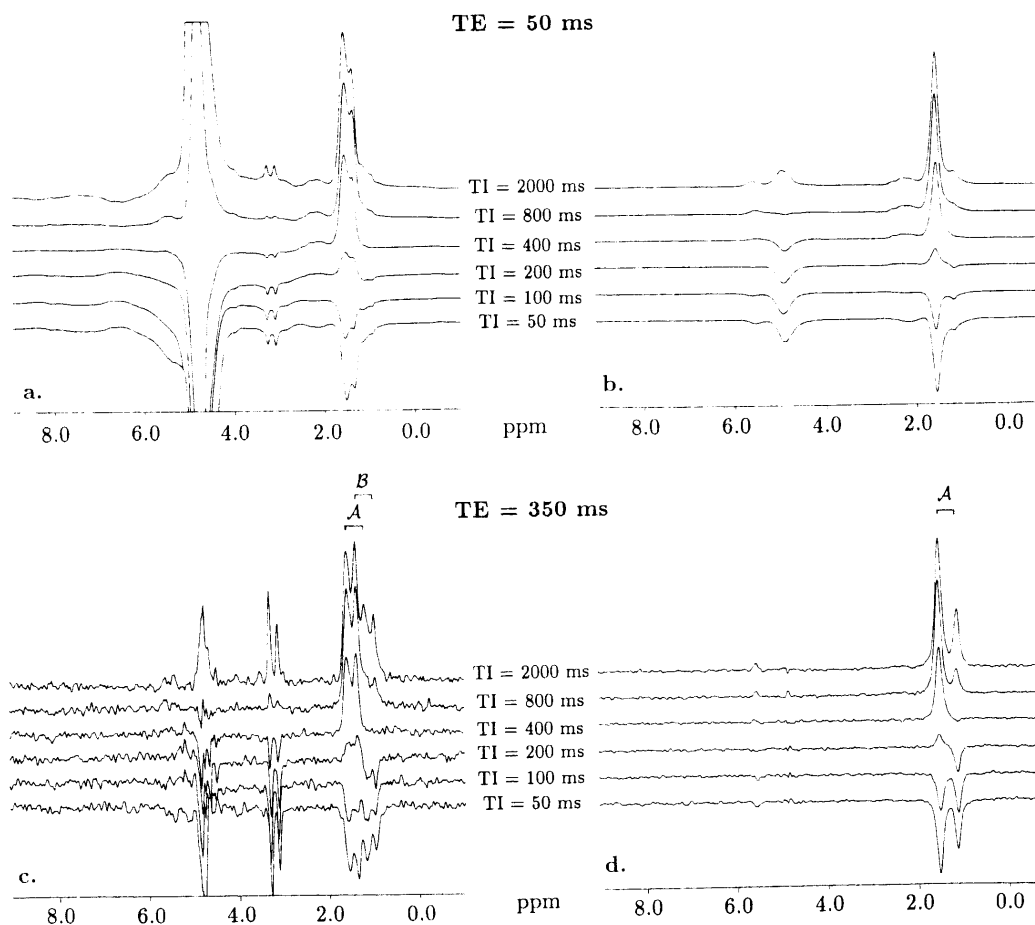


FIG. 8.  $T_1$  measurements using the inversion recovery method with inversion times  $TI = 50, 100, 200, 400, 800, 2000$  ms: (a) Calf muscle tissue of a 31-year-old healthy male volunteer,  $TE = 50$  ms, 128 scans,  $TR = 3000$  ms +  $TI$ ,  $VOI$  of  $(2 \text{ cm})^3$ . (b) Yellow bone marrow of a 25-year-old healthy female volunteer,  $TE = 50$  ms, 128 scans,  $TR = 3000$  ms +  $TI$ ,  $VOI$  of  $(1.3 \text{ cm})^3$ . (c) Calf muscle tissue of a 31-year-old healthy male volunteer,  $TE = 350$  ms, 128 scans,  $TR = 3000$  ms +  $TI$ ,  $VOI$  of  $(2 \text{ cm})^3$ . (d) Subcutaneous gluteal fat tissue of a 25-year-old healthy female volunteer,  $TE = 350$  ms, 32 scans,  $TR = 3000$  ms +  $TI$ ,  $VOI$  of  $(1.3 \text{ cm})^3$ .

be fitted by superposition of four Lorentzian lines with 12.5 Hz frequency intervals between the lines and linewidths of 11.0 Hz (line 1), 10.5 Hz (2), 9.5 Hz (3), and 9.0 Hz (4) as shown in Fig. 9b. The relative amplitudes are given in the caption. The signals of spectra taken with longer echo time  $TE = 350$  ms are also reproduced by superposition of Lorentzian lines. In this case, the amplitudes of the components are more similar and the linewidths are reduced. Both findings improve the separation of four lines in the spectra using long echo times. Recent measurements of rabbit skeletal muscle myosin *in vitro* (25, 26) show  $^1\text{H}$  spectra with narrow lines at approximately 0.8 to 1.0 ppm. These signals may occur in localized spectra *in vivo*, but signals from amino acids do not predominate the line at 1.0 ppm in the muscle spectra. Additional lines beside the main methyl signal are multiplet components caused by J-coupling effects with neighboring methylene groups.

The signals of yellow bone marrow (see Fig. 10a for  $TE = 150$  ms; Fig. 9c for  $TE = 350$  ms) can be fitted by two Lorentzian lines with a frequency difference of 25 Hz as shown in Fig. 10.

The signal maximum in the spectra is not exactly the center of the Lorentzian lines due to superposition from neighbouring signals. Altogether the statements about fatty acid signals from one compartment A in fat tissue, but two compartments A and B in normal skeletal muscle are supported by these signal shape analysis.

## DISCUSSION

The results from double spin echo (PRESS) localized  $^1\text{H}$  spectroscopy show well resolved spectra from small volume elements of  $(2 \text{ cm})^3$  in human calf muscle and  $(1.3 \text{ cm})^3$  in subcutaneous and bone marrow fat. The use of relatively long echo times  $TE$  is advantageous to get highly resolved spectral signals between 0.8 and 1.8 ppm. High signal-to-noise ratio was provided by a Helmholtz coil of 170-mm diameter and volume selection by a double spin echo sequence. This technique profits from increased signal of spin echoes in comparison with STEAM techniques.

TABLE 1  
Transverse ( $T_2$ ) and Longitudinal ( $T_1$ ) Relaxation Times of Lipid Protons in Calf Muscle, Subcutaneous Fat, and Yellow Bone Marrow at 1.5 T *in Vivo*

Signal	$\delta$ (ppm)		Skeletal muscle (ms)	Subcutaneous fat (ms)	Yellow bone marrow (ms)
"Lip 1"		$T_2$	70 <sup>a</sup>	70 <sup>a</sup>	80 <sup>a</sup>
Unsaturated F.A.	5.3-5.7	$T_1^{50ms}$		300	390
-HC=CH-		$T_1^{350ms}$			
Polyunsaturated fatty acids		$T_2$		≈60	
	2.9-3.1	$T_1^{50ms}$		230	≈240
		$T_1^{350ms}$			
=CH-CH <sub>2</sub> -CH=		$T_2$	≈110 <sup>b</sup>	80 <sup>b</sup>	≈110 <sup>b</sup>
"Lip 2"	2.3-2.6	$T_1^{50ms}$		150 <sup>b</sup>	
Ester		$T_1^{350ms}$			
-CO-CH <sub>2</sub> -CH=		$T_2$	≈30 <sup>b</sup>	≈30 <sup>b</sup>	≈30 <sup>b</sup>
"Lip 2"	2.0-2.3	$T_1^{50ms}$		230	≈240
Unsaturated F.A.		$T_1^{350ms}$			
=CH-CH <sub>2</sub> -(CH <sub>2</sub> ) <sub>n</sub> -		$T_2$	75	90	85
Saturated chains	1.5-1.6	$T_1^{50ms}$	270	260	270
(CH <sub>2</sub> ) <sub>n</sub>		$T_1^{350ms}$	≈270	280	270
→ Signal 1 in "Lip 3"		$T_2$	85 <sup>b</sup>		
Saturated chains	1.3-1.4	$T_1^{50ms}$	280 <sup>b</sup>		
(CH <sub>2</sub> ) <sub>n</sub>		$T_1^{350ms}$	≈270		
→ Signal 2 in "Lip 3"		$T_2$	≈150 <sup>ab</sup>	≈200 <sup>ab</sup>	≈170 <sup>ab</sup>
Methyl groups	1.1-1.2	$T_1^{50ms}$	≈400 <sup>b</sup>	≈800 <sup>b</sup>	≈500 <sup>b</sup>
-(CH <sub>2</sub> ) <sub>n</sub> -CH <sub>3</sub>		$T_1^{350ms}$	≈700	780	720
→ Signal 3 in "Lip 3"		$T_2$	≈200 <sup>ab</sup>		
Methyl groups	0.9-1.0	$T_1^{50ms}$	≈600 <sup>b</sup>		
-(CH <sub>2</sub> ) <sub>n</sub> -CH <sub>3</sub>		$T_1^{350ms}$	≈800		
→ Signal 4 in "Lip 3"					

$T_1^{50ms}$ : Evaluation of  $T_1$  by Inversion Recovery using fixed TE = 50 ms.

$T_1^{350ms}$ : Evaluation of  $T_1$  by Inversion Recovery using fixed TE = 350 ms.

<sup>a</sup> Evaluation of these relaxation times is clearly influenced by J-coupling effects.

<sup>b</sup> Evaluation of these relaxation times is clearly influenced by neighboring signals.

If muscle marrow of a -year-old -year-old

TABLE 2  
Transverse ( $T_2$ ) and Longitudinal ( $T_1$ ) Relaxation Times of Protons in Non-Lipid Metabolites in Human Calf Muscle at 1.5 T *in Vivo*

Signal	$\delta$	$T_2$ (ms)	$T_1^{50ms}$ (ms)	$T_1^{350ms}$ (ms)
His C2-H	8.0-8.1	≈100 <sup>a</sup>		
His C4-H	7.0-7.1	≈50 <sup>a</sup>		
Water H <sub>2</sub> O	4.77	50	1100	≈1500
Cr/PCr CH <sub>2</sub>	3.9-4.0	110	≈800	
Cholin N-(CH <sub>3</sub> ) <sub>3</sub>	3.2-3.3	190	1100	≈1100
Cr/PCr CH <sub>3</sub>	3.0-3.1	140	1100	≈1100

$T_1^{50ms}$ : Evaluation of  $T_1$  by Inversion Recovery using fixed TE = 50 ms.

$T_1^{350ms}$ : Evaluation of  $T_1$  by Inversion Recovery using fixed TE = 350 ms.

<sup>a</sup> Evaluation of these relaxation times is clearly influenced by J-coupling effects.

The VOI of only (2 cm)<sup>3</sup> can be located within homogeneous regions of calf muscle tissue without fatty septums and large vessels. Such VOI result in spectra which are not dominated from lipid signals of fatty regions. However, differences of the lipid signal strength from different VOI within the same healthy human soleus muscle were found.

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Comparison of signals from skeletal muscle tissue and fat tissue shows that muscle tissue contains two compartments of triglycerids or fatty acids with a resonance frequency shift of 0.2 ppm (12-13 Hz at 1.5 T). One of these compartments is according to the lipids within fat cells. The other compartment with lower Larmor frequency contains lipids with similar composition (similar length of the fatty acid chains). Probably the second compartment of lipids is located within the cytoplasm of muscle cells. The reason for the Larmor frequency shift of 12-13 Hz for *in vivo* measurements is not yet clearly determined. Susceptibility effects within muscle cells may be responsible.

Altogether the measurements show that localized proton spectroscopy with optimized measuring parameters can offer new interesting insights in physiological and pathological lipid metabolism of muscle tissue *in vivo*.

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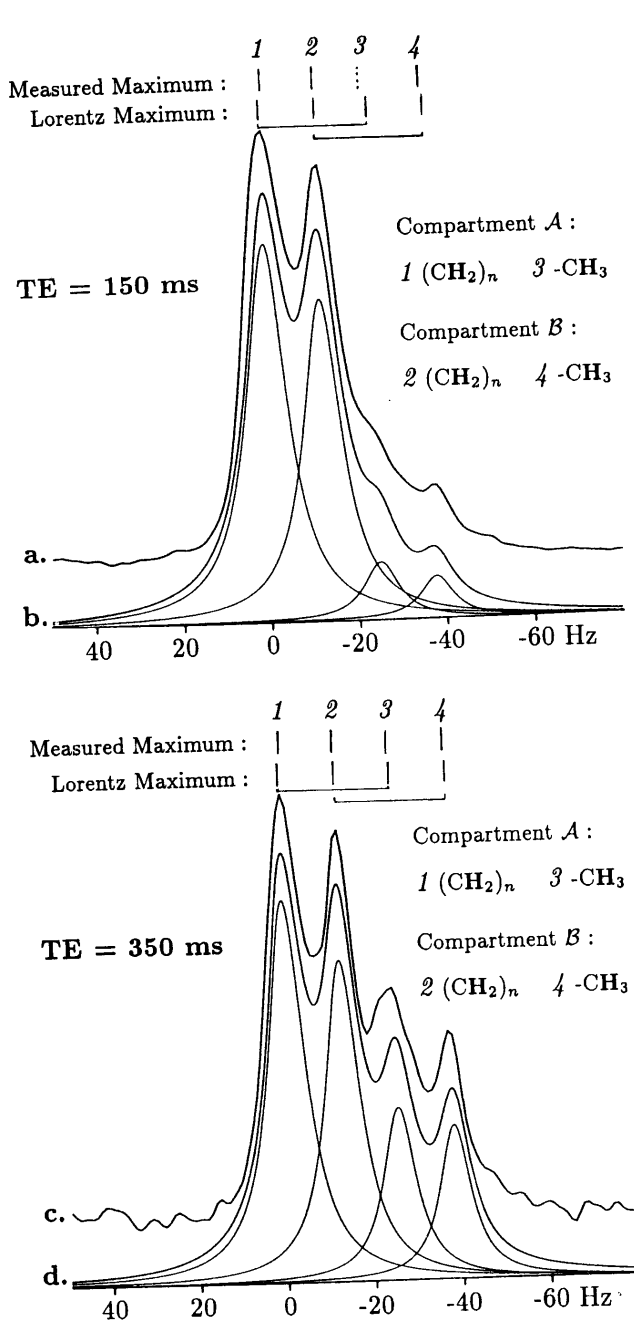


FIG. 9. Spectral signals from muscle tissue of a 31-year-old healthy male volunteer between 0.3 and 2.4 ppm (see Fig. 2b and 2d). The left-hand line at 1.6 ppm is set to 0 Hz. The signals in the measured spectra (a and c) are fitted with four Lorentzian curves with intervals of 12.5 Hz (b and d). Following amplitude ratios and linewidths were optimum fits: (a,b) TE = 150 ms. Lines 1 : 2 : 3 : 4, amplitudes 1.00:0.85:0.15:0.11, full linewidths at half height FWHH 11.0 Hz:10.5 Hz:9.5 Hz:9.0 Hz. (c,d) TE = 350 ms. Lines 1 : 2 : 3 : 4, amplitudes 1.00:0.84:0.45:0.40, FWHH 10.0 Hz:9.5 Hz:9.0 Hz:8.5 Hz.

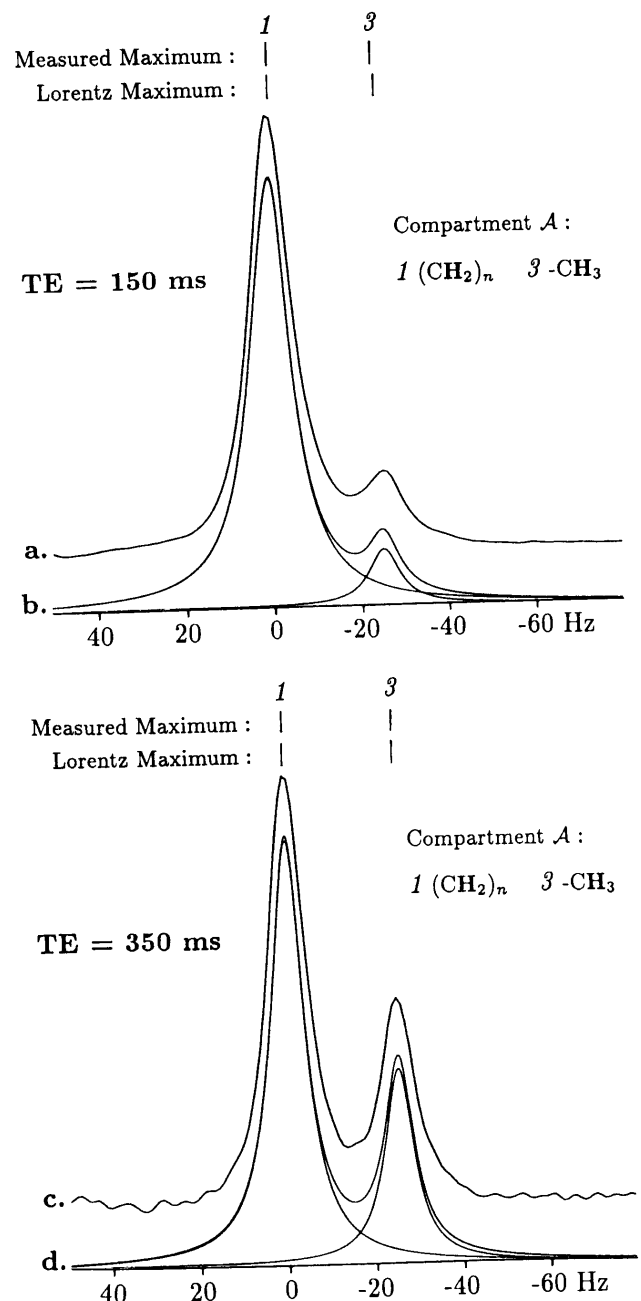


FIG. 10. Spectral signals from yellow femoral bone marrow of a 25-year-old healthy female volunteer between 0.3 and 2.4 ppm (see Fig. 5). The left-hand line at 1.6 ppm is set to 0 Hz. The signals in the measured spectra (a and c) are fitted with two Lorentzian curves with 25 Hz difference (b and d). Following amplitude ratios and linewidths were optimum: (a,b) TE = 150 ms. Lines 1 : 3, amplitudes 1.00:0.13, FWHH 11.0 Hz:8.5 Hz. (c,d) TE = 350 ms. Lines 1 : 3, amplitudes 1.00:0.46, FWHH 9.0 Hz:8.0 Hz.

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