Journal of Magnetic Resonance **154,** 228–235 (2002)
doi:10.1006/jmre.2001.2481, available online at http://www.idealibrary.com on IDEAL®

Separation of Intra- and Extramyocellular Lipid Signals in Proton MR Spectra by Determination of Their Magnetic Field Distribution[1]

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Received May 29, 2001; revised October 30, 2001; published online January 16, 2002

In skeletal musculature intramyocellular (IMCL) and extramyocellular lipids (EMCL) are stored in compartments of different geometry and experience different magnetic field strengths due to geometrical susceptibility effects. The effect is strong enough to—at least partly—separate IMCL and EMCL contributions in ^{1}H MR spectroscopy, despite IMCL and EMCL consisting of the same substances. The assessment of intramyocellular lipid stores in skeletal musculature by ^{1}H MR spectroscopy plays an important role for studying physiological and pathological aspects of lipid metabolism. Therefore, a method using mathematical tools of Fourier analysis is developed to obtain the magnetic field distribution (MFD) from the measured spectra by deconvolution. A reference lipid spectrum is required which was recorded in tibial yellow bone marrow. It is shown that the separation of IMCL contributions can be performed more precisely—compared to other methods—based on the MFD. Examples of deconvolution in model systems elucidate the principle. Applications of the proposed approach on *in vivo* examinations in m. soleus and m. tibialis anterior are presented. Fitting the IMCL part of the MFD by a Gaussian lineshape with a linewidth kept fixed with respect to the linewidth of creatine and with the assumption of a smooth but not necessarily symmetrical shape for the EMCL part, the only free fit parameter, the amplitude of the IMCL part, is definite and subtraction leads to the EMCL part in the MFD. This procedure is especially justified for the soleus muscle showing a severely asymmetrical distribution which might lead to a marked overestimation of IMCL using common line fitting procedures.

Key Words: **^{1}H MR spectroscopy; skeletal muscle; intramyocellular lipids; deconvolution; magnetic field distribution.**

INTRODUCTION

Volume-selective ^{1}H MR spectroscopy has been shown to be a noninvasive tool which allows one to assess the lipid content of human skeletal muscles (*1–3*). The signal consists of components from intramyocellular (IMCL) and extramyocellular lipids (EMCL), leading to a relatively complex signal pattern in the recorded spectra (*2–4*). Since IMCL are actively involved in lipid metabolism with turnover rates of several hours for substrate utilization, an accurate assessment of the IMCL signal by ^{1}H MR spectroscopy is desirable for studying physiological and pathological aspects of lipid metabolism. In clinical studies, a negative correlation between intramyocellular lipid content and insulin resistance was found recently (*5–7*). Since decreased insulin sensitivity is an important predictor for type 2 diabetes, ^{1}H MR measurements of IMCL will be a helpful tool for primary recognition. Additionally, IMCL serve as an energy depot for workload depending on duration and intensity of exercise (*3, 8, 9*), and, for this reason, the regulation of IMCL is also of interest in sports medicine.

IMCL show relatively homogenous spatial distribution in musculature, whereas EMCL are more inhomogenously located in the septa between the muscle bundles (*10*). The latter are relatively inert in lipid metabolism and can only serve as a long-term fat depot. IMCL are settled as little droplets with a diameter of about 0.5 μm in close apposition to mitochondria in the cytoplasm of myocytes (*3*). Since the droplets have a rather spherical shape with a homogenous distribution throughout muscle fibers, signals of IMCL are independent of muscle orientation relative to the magnetic field and show a regular symmetrical distribution of the experienced magnetic field. In contrast, different geometrical arrangements of EMCL relative to the magnetic field lead to orientation-dependent anisotropic susceptibility effects (*3, 11, 12*). The EMCL compartments are determined mainly by the more tubular structures of muscle fibers. For this reason the compartments of EMCL can show a rather inhomogenous and even asymmetrical distribution of the magnetic field due to a tube-like geometry combined with anisotropic susceptibility effects as was similarly found for geometric models of trabecular bone (*13*) and lung tissue (*14*).

The lipid signal positions in spectra from musculature depend on both the chemical shifts and the local magnetic field distribution caused by bulk magnetic susceptibility (BMS) effects. The difference of the mean Larmor frequency in IMCL and EMCL compartments is relatively small and amounts to approximately 0.15 ppm for the soleus muscle (S) and 0.25 ppm for the tibialis anterior muscle (TA) at 1.5 T. It depends on the muscle fiber orientation relative to the static magnetic field and is maximal when the orientation of the fibers is parallel to the magnetic field (*3, 4*). The superposition of the IMCL and EMCL signal components in the spectra hinders an accurate quantitative evaluation

[1] This work was supported by a grant from the Deutsche Forschungsgemeinschaft JA 1005-1-1.

1090-7807/02 $35.00

of both portions. Usually, strategies for assessing IMCL signals comprise integration of the spectral signal intensity within fixed frequency ranges or signal fitting with Gaussian and/or Lorentzian lineshapes. However, both methods will be critical for a pronounced asymmetrical field distribution.

The aim of the present study was to develop a new approach using the fact that the muscular lipid signals can be described as the convolution between the magnetic field distribution (MFD) of the lipid compartments and a characteristic line pattern of lipids describing chemical shift modulations. This approach is reasonable, because from a biochemical point of view it is clear that the mentioned characteristic line pattern of lipids must exist: IMCL, EMCL, and adipose tissue as subcutaneous fat tissue or tibial bone marrow lipids (BM) are known to consist mainly of the same composition of fatty acid triglycerides (*15–17*). For this reason, spectra of yellow bone marrow recorded with identical measurement parameters can be used to assess the required characteristic line pattern of lipids. Mathematical tools of Fourier analysis were used to calculate the underlying MFD of lipids in skeletal musculature. The aim of this work is to show that an easier interpretation and determination of IMCL and EMCL components in the pattern of the MFD is possible rather than in the more complicated lipid signals in the recorded spectra.

THEORY

In contrast to aqueous solutions with one single compartment, tissues show several intra- and extracellular compartments with variable geometry, volume, concentrations of substances, and magnetic susceptibilities. The frequency shifts in a spectrum recorded *in vivo* depend, first, on the characteristic chemical shifts and, second, on the distribution of the magnetic field experienced by the substance of interest. If it were possible to record a spectrum of the substance in a homogenous magnetic field, the frequency shifts in the spectrum would only depend on the chemical shifts and the linewidth would be minimal. These minimal linewidths $\Delta w_{\min}$ are determined by $\Delta w_{\min} = 1/\pi \cdot T2$. This theoretical spectrum of the substance expressing only chemical shift modulations will be called Θ. In terms of Fourier analysis, the recorded spectral signals of a substance in tissue (E) can be written as the convolution ($*$) between the magnetic field distribution (MFD) experienced by the substance of interest and Θ:

$$E = \mathrm{MFD} * \Theta. \quad [1]$$

In contrast to Θ, expressing the chemical shift modulations, the MFD describes frequency shifts caused by BMS effects. If it is possible to determine Θ indirectly by recording a reference spectrum (R) out of a compartment with the same chemical composition as the substance of interest but with a rather homogenous magnetic field distribution, calculation of the MFD from a recorded spectrum of a substance will be feasible. Since *in vivo* conditions do not provide an ideal homogenous field, the linewidths in R are broadened and a normal distribution (G) for variation of the magnetic field is assumed. This leads to

$$R = G * \Theta. \quad [2]$$

The inverse Fourier transform of G again yields a Gaussian function $g(t) = e^{-(t/T_g)^2}$ (up to a scaling factor) with time constant T_g depending on the line broadening in R and the quality of shimming. T_g can numerically be determined with aid of $T2$ and the measured linewidth Δw of a dominating peak in a reference spectrum in homogenous compartments (e.g., the methylene group ($(-CH_2)_n$) of lipids in BM). Using the well-known convolution theorem of Fourier analysis, i.e., convolution in the frequency domain corresponds to multiplication in the time domain, inverse Fourier transformation of Eqs. [1] and [2] leads to

$$e = \frac{1}{N} \cdot \mathrm{mfd} \cdot \theta \quad [3]$$

and

$$r = \frac{1}{N} \cdot g \cdot \theta \quad [4]$$

in the time domain, where the small letters denote the inverse Fourier transforms of the corresponding capital letters and N is a scaling factor depending on the definition of Fourier transformation. Combination of Eqs. [3] and [4] leads to

$$\mathrm{mfd} = N \cdot e/\theta = g \cdot e/r. \quad [5]$$

No problem will occur if the magnitude of r approaches zero. Since both e and r can be written as a sum of functions which decays exponentially with a linear argument in time, the Gaussian function g with an exponential decay quadratically in time dominates the behavior of mfd for large times for which the function mfd converges to zero. Fourier transformation of mfd yields the desired magnetic field distribution MFD. Figure 1 shows the whole procedure schematically.

METHODS

Spectroscopic *in vitro* and *in vivo* experiments were carried out in order to test the proposed method for the assessment of the MFD. All measurements were performed on a 1.5-T whole-body system (Magnetom Vision, Siemens, Erlangen, Germany). For volume selection, a single-voxel STEAM sequence (TR = 2000 ms, TE = 10 ms, TM = 15 ms) was applied. Data processing was executed on a personal computer using the Matlab Signal Processing Toolbox (MathWorks Inc., USA). The recorded time domain signals from the investigated spectrum E and the reference spectrum R of both *in vitro* and *in vivo* examinations were filtered by a Gauss function with a half-width of 150 ms and underwent a fast Fourier transformation followed by baseline correction and constant and linear phase correction.

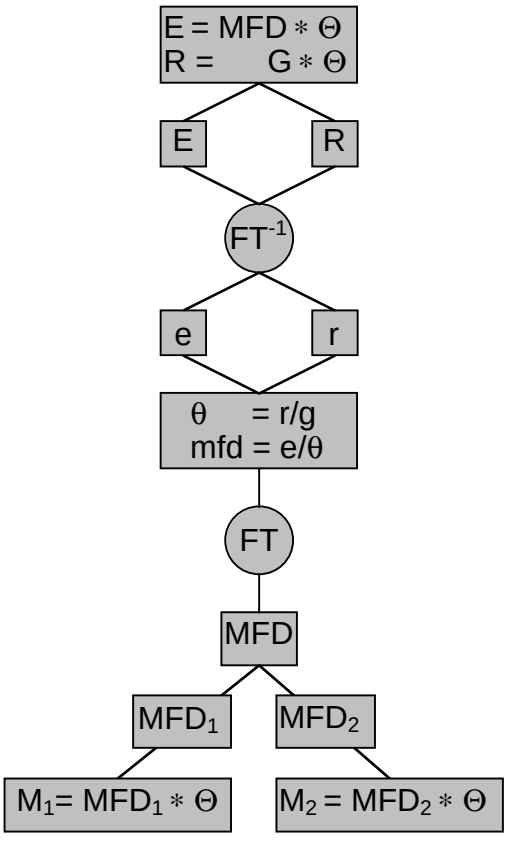

FIG. 1. Scheme of the procedure for calculation of the magnetic field distribution. MFD magnetic field distribution; E, lipid signals for evaluation; R, reference lipid signals; G, Gaussian distribution; Θ, theoretical lipid signals; FT, Fourier transform; FT^{-1}, inverse Fourier transform; MFD_1, MFD_2, ..., portions of MFD; M_1, M_2, ..., portions of M.

In Vitro Examinations

A glass sphere with a diameter of 100 mm was filled with vegetable oil providing a rather homogenous magnetic field for recording a reference spectrum. For simulation of several different lipid compartments with a more inhomogenous field distribution, two glass tubes with a diameter of 8 mm were filled with vegetable oil and closed at both ends with silicone. The tubes were hanged with nylon threads in a Plexiglas frame and surrounded by air. One of the tubes with a length of 20 cm was always positioned parallel to the static magnetic field B_0 and the other one with a length of 15 cm was positioned under different angles ($\alpha = 45^\circ$, 60°, 75°, and 90°) to B_0 and different distances ($d = 0, 5$, and 10 mm) to the first tube at the crossing. The spectra were measured using the standard head coil of the manufacturer for radiofrequency (RF) irradiation and signal acquisition. Voxel size was chosen to $20 \times 20 \times 40$ mm. Voxel position was at the center of the glass sphere or at the crossing of the two tubes, respectively. For the signals from the oil-filled sphere serving as reference for lipids, $T2$ of the dominating methylene groups ($(-CH_2)_n$) at 1.5 T was determined by the aid of a multi-spin-echo sequence and amounted to $T2 \approx 68$ ms. The corresponding linewidth $\Delta w \approx 7.5$ Hz of the methylene groups in the spectrum of R leads to a time constant $T_g \approx 166$ ms for g. Calculation of the MFD yields the field distributions MFD_1 and MFD_2 of both tubes, which could be easily separated. Convolution of MFD_1 or MFD_2 and the reference spectrum R gave the contribution of each tube to the measured spectrum.

In Vivo Examinations

A frequency-selective prepulse was used for suppression of the water signal. Additionally, a reference spectrum was recorded from tibial bone marrow (BM). Voxel size was always chosen to $11 \times 11 \times 20$ mm, with the 20-mm extent along the leg axis. All derivations under Theory were based on the assumption that only signals from one interesting substance occur in the spectra. This precondition is not fulfilled for the entire spectra recorded *in vivo*. Muscular spectra also show signals from water, creatine, and choline; marrow spectra show additional water signals. For this reason, it was necessary to restrict the calculations on lipids to the chemical shift range between 0.5 and 2.5 ppm containing the main signals of fatty acid triglycerides and no further signal components. Irrelevant parts of the spectra were cut off after baseline and phase correction. The cutoff was done smoothly by fitting the flanks at 0.5 and 2.5 ppm with an exponential function as shown in Fig. 2 by examples from bone marrow and musculature. Since the molecular composition of the lipids (fatty acids and triglycerides in the chemical shift range of 0.5–2.5 ppm) in IMCL, EMCL, and BM are nearly identical and BM is located in a rather homogenous field, the procedure described under Theory can be used to calculate the MFD of lipids in TA and S. For the signals from BM serving as reference for lipids, $T2$ of the dominating methylene groups ($(-CH_2)_n$) at 1.5 T is known and amounts to $T2 \approx 80$ ms (*18*).

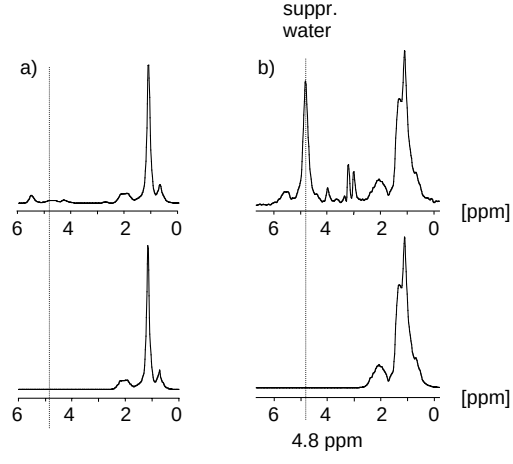

FIG. 2. Spectra acquired from tibial bone marrow (a) and m. soleus (b). Upper row, original spectra; lower row, cut-off spectra.

TABLE 1

The Full Widths at Half-Maximum Δw_1 and Δw_2 (ppm) in Both Tubes and the Mean Frequency Difference $\Delta\nu$ (ppm) Assessed for Different Orientations (α) and Distances (d) in the Arrangement

	$d = 0$ mm			$d = 5$ mm			$d = 10$ mm		
α	Δw_1	Δw_2	$\Delta\nu$	Δw_1	Δw_2	$\Delta\nu$	Δw_1	Δw_2	$\Delta\nu$
45°	0.06	0.47	1.80	0.07	0.26	1.99	0.07	0.09	2.12
60°	0.07	0.76	2.88	0.07	0.37	3.03	0.07	0.12	2.99
75°	0.07	0.84	3.29	0.06	0.27	3.59	0.06	0.17	3.65
90°	0.06	0.84	3.79	0.07	0.32	3.82	0.07	0.13	3.88

The corresponding linewidth $\Delta w \approx 8$ Hz of the methylene groups in the spectrum of BM leads to $T_g \approx 70$ ms.

This allows one to calculate the total MFD of lipids in musculature by the proposed procedure. To separate IMCL and EMCL in the MFD prior knowledge is necessary because of the overlapping of the two peaks. Two methods were used to split up both parts:

Method 1. The IMCL and EMCL part were separated by fitting the MFD with two Gaussian lineshapes without further restrictions about amplitude and linewidth.

Method 2. In spectra out of S and TA with a dominating IMCL signal, a linear correlation was found between the linewidth of the methylene peak of IMCL (Δw_{IMCL}) and creatine (Δw_{Cr}) which amounts $\Delta w_{\mathrm{Cr}}/\Delta w_{\mathrm{IMCL}} = 0.91 \pm 0.03$. Therefore, demanding a smooth but not necessarily symmetrical decay at the right side of the EMCL part and the linewidths of the IMCL part kept fixed with respect to creatine, the IMCL part is fitted by a Gaussian lineshape, varying one free fit parameter for the amplitude until the difference between the MFD and the Gaussian lineshape gets an as smooth as possible decay. Although a disadvantage of this method may be the subjective and visual view of the smoothness, this fit procedure is rather stable due to the fact that the amplitude is the only free fit parameter resulting in clearly smaller deviations as demonstrated in Table 2.

TABLE 2

Mean Values and Standard Deviations of the Reproducibility Measurements of the IMCL Content of Three Subjects (A, B, C) for the Tibialis Anterior Muscle (TA) and Soleus Muscle (S) Evaluated with Method 1 and Method 2, Respectively, as Described under Methods

	TA		S	
	Method 1	Method 2	Method 1	Method 2
A	1.57 ± 0.25	1.60 ± 0.26	6.25 ± 1.19	3.32 ± 0.18
B	1.94 ± 0.52	1.77 ± 0.53	4.95 ± 1.48	2.13 ± 0.04
C	2.13 ± 0.04	1.95 ± 0.14	6.80 ± 1.48	4.32 ± 0.38

Note. The IMCL content was normalized to the creatine content determined by a fit with a Lorentzian lineshape.

Both methods worked well for TA. For S, however, the first fitting procedure led to clearly larger linewidths in the IMCL part compared to the IMCL part in TA. In some cases, the residual of fitting even contained a distinct additional signal toward higher frequencies on the right side of the IMCL peak in the MFD. Assuming a narrow Gaussian distribution for the IMCL, this additional signal must belong to the EMCL part, since there are only lipid signals present in this frequency range (1.5–0.5 ppm). In this case the IMCL part is located within the right slope of the EMCL part.

For the determination of intra- and interindividual reproducibility, spectra out of the tibialis anterior muscle and the soleus muscle of three subjects were measured three consecutive times with a break of about 15 min between the first and second measurement and a break of 1 week between the second and third measurement. The voxel was set at identical positions in the muscles for all three measurements. All subjects gave their informed consent before the investigation.

RESULTS

In Vitro Examinations

The spectra from the spherical oil sample were well resolved (Fig. 3a). The experiments with crossing tubes showed a marked variability of the spectral pattern depending on the orientation of the tubes and on the distance between the tubes at the crossing. Decreasing distance of the tubes at the crossing points led to a more inhomogenous field distribution, especially in the tube oblique to B_0. The mean Larmor frequency difference of both tubes was increasing with the orientation angle of the second tube with a maximum at 90°. Figure 3b shows an example of

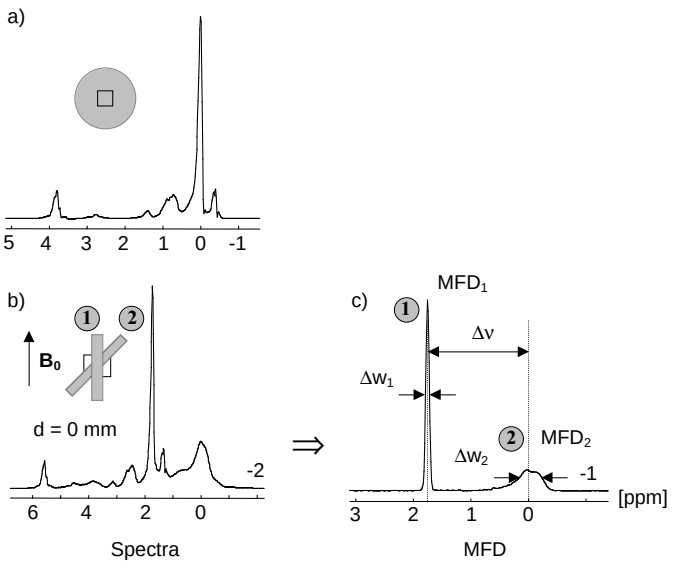

FIG. 3. Spectra acquired from the sphere (a), the tubes for the case $\alpha = 45°$ and $d = 0$ mm (b), and the corresponding MFD (c).

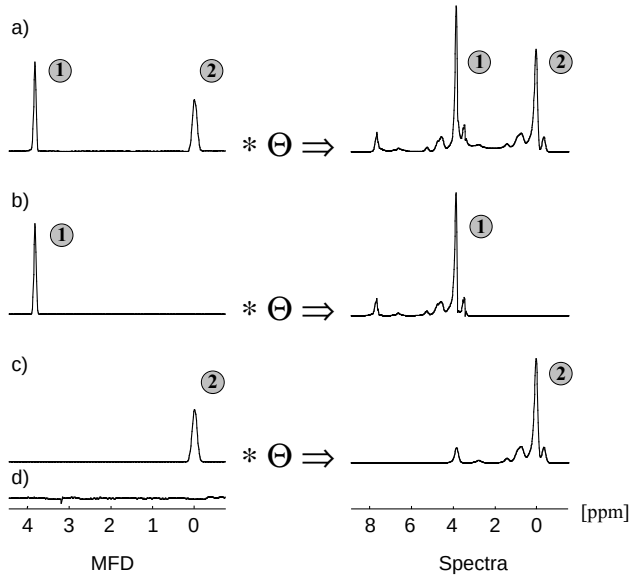

FIG. 4. Convolution of the total MFD (a), MFD_1 (b), and MFD_2 (c) with reference spectrum R for the case $\alpha = 90^\circ$ and $d = 10$ mm. The residual of fitting the MFD is shown in (d).

spectra from a voxel covering both tubes at the crossing point. The proposed deconvolution procedure for a spectrum from the crossing tubes (E) was applied using the spectrum from the sphere as reference. Figure 3c shows the resulting total MFD of E. The field distributions MFD_1 and MFD_2 of both tubes are well separated. The full widths at half-maximum Δw_1 and Δw_2 in both tubes and the mean frequency difference $\Delta \nu$ assessed for different orientations (α) and distances (d) in the tube arrangement are listed in Table 1. Calculating the convolution of only one part of the total MFD (MFD_1 or MFD_2) and the reference spectrum R, the contribution of each tube to the measured spectrum can be simulated as shown in Fig. 4.

In Vivo Examinations

The MFD for lipids in skeletal musculature was assessed by spectra from the tibialis anterior muscle and the soleus muscle, using the lipid spectrum from tibial bone marrow as reference R. Figure 5 shows a recorded spectrum R serving for referencing, muscular spectra E, and calculated MFD. The assignment of the partial MFDs of IMCL (MFD_{IMCL}) and EMCL (MFD_{EMCL}) was based on prior knowledge about a narrow and symmetrical shape of MFD_{IMCL} due to its spherical geometry. The rest of the MFD is expected to represent MFD_{EMCL}. Especially MFD_{EMCL} in S shows an asymmetrical distribution in Fig. 5d which was similar in other examples. Furthermore, most spectra from S confirmed MFD_{IMCL} to be symmetrical and narrow and located inside the range of MFD_{EMCL}. Convolution of only MFD_{IMCL} or MFD_{EMCL} and the function Θ leads to a selective presentation of the IMCL or EMCL contributions in the measured spectrum. This simulation of "partial spectra" is demonstrated in Fig. 6 for a measured spectrum from the soleus muscle for both Method 1 and Method 2. In the soleus muscle the superposition of EMCL signals on IMCL signals was found to be clearly more pronounced than expected. The reason is the asymmetrical lineshape of MFD_{EMCL} with a broad slope toward lower field (Fig. 5c). This phenomenon is hidden in the recorded spectra. For this reason, most IMCL values from S derived by fixed integration borders or by fitting with symmetrical lineshapes are overestimated. This is demonstrated in Table 2, which shows the results of the intra- and interindividual studies. The IMCL in the soleus muscle could be measured with a better reproducibility as shown by the distinctly smaller standard deviations and a clearly

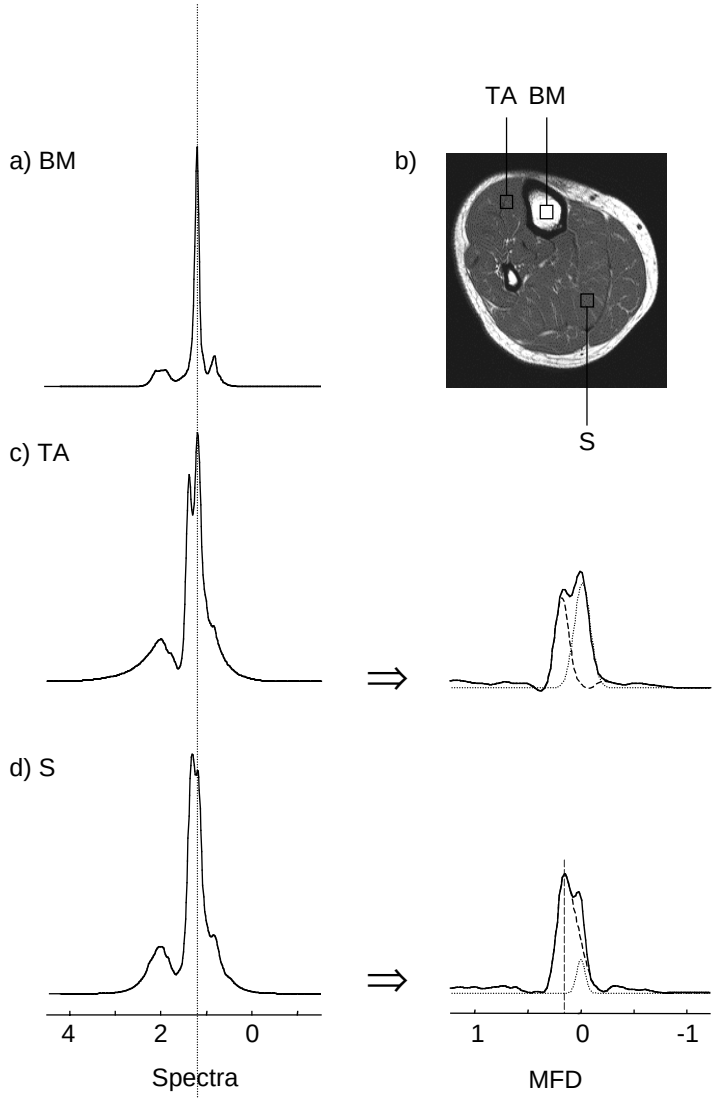

FIG. 5. Cut-off spectra acquired from tibial bone marrow (a), m. tibialis anterior (c), and m. soleus (d) of the voxels are shown in (b). In the right column of (c) and (d) the corresponding total MFD (solid line), the MFD_{EMCL} (dashed line), and the MFD_{IMCL} (dotted line) are depicted. The vertical dashed line elucidates the asymmetrical lineshape of MFD_{EMCL}.

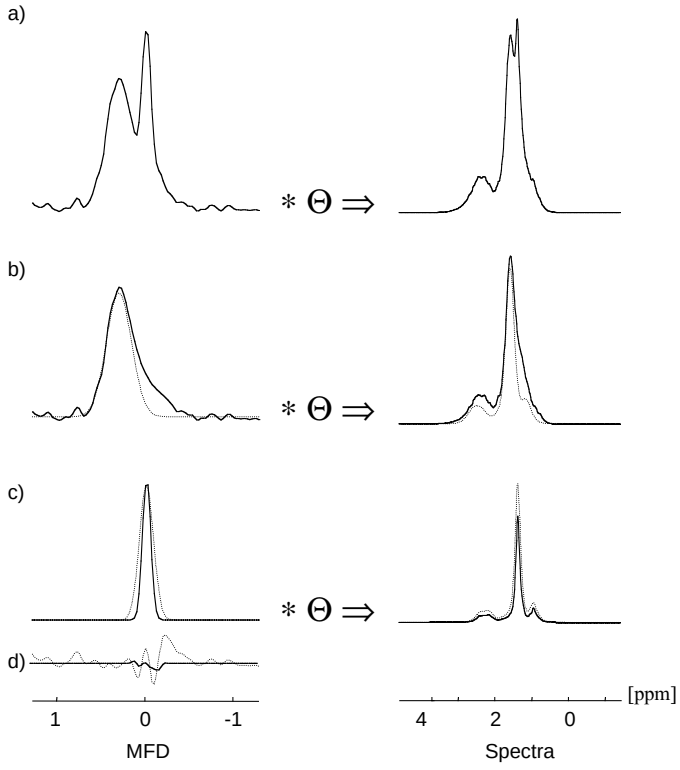

FIG. 6. In the left column the total MFD (a), the MFD_{EMCL} (b), the MFD_{IMCL} (c), and the residual of fitting the MFD (d) are depicted. In the right column, the corresponding convolutions with theoretical lipid spectrum Θ are shown. The dashed lines in (b), (c), and (d) show the fit with Method 1 (two Gaussians) and the solid lines the fit with Method 2.

lower content in comparison to the method with Gaussian fitting. In contrast, the IMCL content in the tibialis anterior shows no significant difference for both methods.

DISCUSSION

In a former work the principle of deconvolution has been used for measuring the contribution of trabecular bone structure and density of the upper femur to the effective transverse relaxation time in trabecular yellow bone marrow (*17*). Using a signal out of subcutaneous tissue as a reference and assuming a line broadening function with an exponential decay, line broadening could be estimated by division and fitting in time domain. The work presented here applies a similar procedure assuming a corresponding spectral composition of EMCL, IMCL, and tibial bone marrow lipids. As in (*17*) the assumption of a similar chemical composition of the muscle lipids and the lipids in BM is crucial for the procedure. However, it is known that the chemical composition of muscle lipids and adipose tissue as subcutaneous fat or tibial bone marrow lipids is nearly the same (*15–17*). No trabecular structures exist in the mid-tibia in contrast to the upper femur and only fatty acid triglycerides are measured in the rather homogenous surroundings. This indicates that all the requirements presented under Theory are fulfilled. The presented work focused on the explicit lineshape of the MFD experienced by a substance in living tissue rather than $T2^*$ measurements which would only provide very unspecific and rough information about line broadening caused by both lipid compartments together. To the knowledge of the authors this is the first direct demonstration of the MFD of a substance in living tissue, allowing a pure visualization of susceptibility effects.

The aim for the development of the deconvolution procedure was to simplify the evaluation and interpretation of spectra recorded from human musculature. It is known from earlier studies that musculature shows at least two different lipid containing compartments with a clearly visible frequency shift in the spectra of about 0.2 ppm (*2, 3*). The reason for the different magnetic field strength was shown to be the geometric arrangement of the IMCL and EMCL compartments and the susceptibility of the surrounding tissue components (*3*). Original spectra recorded from musculature showed a line pattern depending on the MFD and on the chemical shift as well, and distinguishing IMCL and EMCL components is not trivial. Even sophisticated, especially edited one-dimensional or two-dimensional, techniques of localized ^{1}H MR spectroscopy, which provide more details about the composition of musculature *in vivo* (*19*), do not allow one to distinguish between portions of EMCL and IMCL, since the molecular composition in these tissue compartments is nearly identical.

For both the proposed method and standard fitting methods, acquisition and postprocessing of the data have to be performed carefully. Since water is the dominating signal in muscle tissue, water suppression is essential. The voxel has to be chosen carefully such that contaminating components of subcutaneous fat tissue or macroscopic fatty septa are excluded or minimized and mainly muscle tissue is included. A local shim is necessary to get optimized homogeneity within the voxel and high signal intensity to avoid problems with signal-to-noise ratio. Furthermore, thorough phase correction for both the muscle spectrum and the bone marrow spectrum has to be done to avoid a mixture of the real and imaginary parts. The signals from residual water, choline, creatine, and other metabolites are excluded by a cut-off procedure as described under Methods.

Both portions, EMCL and IMCL, can be distinguished in the "simple" MFD. Two main peaks of both contributions are visible. It was confirmed that the field distribution of IMCL is nearly symmetrical and narrow for all muscle groups and voxel positions examined. In contrast, for EMCL a frequently asymmetrical distribution of the magnetic field is revealed by the MFD; i.e., most of the EMCL experience a higher magnetic field as neighboring IMCL, but there are some EMCL portions with the same or even a lower magnetic field strength, compared to the IMCL in the examined voxel. The asymmetry of the field distribution of EMCL was pronounced in the S, but not so evident in the TA. However, asymmetrical lineshapes seem

to be rather the rule than the exception as several models for heterogenous tissue show (*13, 14*). Highly resolved muscular spectra recorded *in vivo* at 4 T confirmed the mentioned asymmetry of EMCL signals in S (*20, 21*). In contrast to EMCL layers and muscle fibers in the TA orientated nearly parallel to the axis of the leg and the static magnetic field B_0, the oblique arrangements of the muscle fiber bundles and the EMCL compartments in S are expected to be the reason for the marked asymmetry in the feathered soleus muscle with many fiber intersections.

The results show that usual fitting procedures with defined lineshapes are not suitable for an adequate and quantitative assessment of IMCL and EMCL portions with their partly irregular and asymmetrical field distributions, and the proposed technique with deconvolution of the spectral line pattern by a reference spectrum worked well in musculature. However, it should be mentioned that several conditions have to be fulfilled for a correct application: all signals in the analyzed frequency range in the spectrum of interest and in the reference spectrum must stem from material with the same chemical components. This precondition seems to be fulfilled with very good approximation for the fatty acid triglycerids in IMCL, EMCL, and fatty bone marrow. A potential problem could be additional signal contributions from proteins or lactate in musculature. However, there is no evidence in the literature that considerable signal components from metabolites other than lipids occur in human muscle spectra in the chemical shift range between 0.5 and 2.5 ppm.

One may ask whether such effort is worthwhile in order to assess the content of lipids in myocytes more precisely. But as described in the Introduction a number of metabolic studies have shown IMCL being involved in the pathogenesis of type 2 diabetes (*5–7*). The turnover of IMCL during one bout of long lasting and submaximal exercise (*8, 9*) or lipid infusion under high insulin levels (*22*) was shown to be often more than 50%. These dramatic signal changes could be assessed undoubtedly, indicating the relevance of muscular lipids in endocrinology and sports medicine. The next step for future studies is the assessment of lipid metabolism under more physiological conditions. For testing smaller effects of nutrition, training, or short-term workload, clearly smaller changes in the IMCL content must be measurable with minimal errors. Usual techniques are quite critical and a precision of better than 10% in the IMCL content is often hard to obtain, if the voxel position (and the EMCL contribution in the spectrum) has slightly changed. For this reason, an increased precision in the quantitative analysis of IMCL leads to a better applicability of MR muscle spectroscopy in a clinical environment.

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