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Temperature Dependence of Relaxation Times in Proton Components of Fatty Acids

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We examined the temperature dependence of relaxation times in proton components of fatty acids in various samples *in vitro* at 11 tesla as a standard calibration data for quantitative temperature imaging of fat. The spin-lattice relaxation time, T_1 , of both the methylene (CH_2) chain and terminal methyl (CH_3) was linearly related to temperature ($r > 0.98$, $P < 0.001$) in samples of animal fat. The temperature coefficients for the 2 primary proton components differed significantly; in 5 bovine fat samples, the coefficient at 30°C was 1.79 ± 0.07 (%/°C) for methylene and 2.98 ± 0.38 (%/°C) for methyl. Numerical simulations based on such a difference demonstrated the possibility of considerable error from inconsistent ratios in fatty acid components when calibrating and estimating temperature. The error reached 3.3°C per 15°C in temperature elevation when we used a pure CH_2 signal for calibration and observed the signal with 18% of CH_3 to estimate temperature. These findings suggested that separating the fatty acid components would significantly improve accuracy in quantitative thermometry for fat. Use of the T_1 of CH_2 seems promising in terms of reliability and reproducibility in measuring temperature of fat.

Keywords: *fat, magnetic resonance, proton, relaxation time, temperature*

Introduction

Noninvasive magnetic resonance (MR) temperature imaging of the breast is desirable for thermal therapies, such as high intensity focused ultrasound (HIFU) treatment, to ensure heat deposition to the target tumor and protect surrounding normal tissue. Key for such imaging is a reliable technique for measuring the temperature of adipose tissues. Although water proton chemical shift or proton resonance frequency (PRF) shift methods are widely used to monitor the temperature of tissues with high water content, such as uterine fibroids, they are not sufficient for the breast, which comprises mammary gland and fat. Adequate water content in a voxel permits measurement of the phase shift

of the water signal by suppression of the fat signal, but voxels with very low or no water content should be measured using fat signal. A study using T_1 ¹ to measure fat temperature clearly demonstrated the temperature dependence of the T_1 of the total fat signal, but the temperature dependence was not constant among different specimens.¹ Another study that used proton density to measure fat temperature detected temperature dependence of proton density that seemed to follow the Boltzmann distribution model, but the dependence was not stable and differed in the heating and cooling processes.² Deviation in results in a third study that evaluated the stability of water-fat chemical shifts using line scan echo-planar spectroscopic imaging (LSEPSI)³ demonstrated that difference in these shifts is not a useful indicator of breast temperature. Moreover, this type of spectroscopic technique requires adequate water and fat content.

The previous studies were conducted at relatively

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low magnetic fields (0.5 to 1.5T) to observe total fat signal without separating the fatty acid components. Food science and bariatric research indicate that fat or oil comprises a number of different fatty acids.⁴ In the MR observation, the different molecular groups of each fatty acid yielded different chemical shift components.⁵ The dominant components were methylene (CH₂) and methyl radicals (CH₃). As well, several chemical shift components originate from various unsaturated and saturated components that would cause the temperature dependence of the MR parameters of “fat” to be a mixture of the dependence of the parameters of the different molecular forms. Chemical research has investigated the temperature dependence of the MR parameters of ganglioside⁶ or cholesteryl ester.⁷ However, we believe only an investigation of protein-lipid interactions by Deese and Dratz⁸ has evaluated the temperature dependence of the MR parameters of each chemical shift component of animal fat.

Based on this background, we examined the temperature dependence of the MR parameters of proton components in fatty acids in various fat and oil samples *in vitro* at 11T to obtain a reliable calibration data for quantitative temperature imaging. From experimental results, we performed numerical simulation to examine the effect of the coexistence of the different components.

Materials and Methods

Experiments⁹

We measured the relaxation time of olive oil resolved in deuterated chloroform (CDCl₃) with tetramethylsilane (TMS) as a chemical shift reference reagent, pure olive oil with TMS, mayonnaise, and edible bovine and porcine fats *in vitro*. We obtained bovine and porcine samples from various farms across Japan. We placed each sample in a glass tube of 5-mm outer diameter. For mayonnaise or animal fat, we filled tubes as tightly as possible with small portions (~2 to 3 mm in size) to avoid air bubbles in the sample volume. When visual inspection revealed bubbles, we manually removed them using a syringe with a long hollow needle. The samples were set in an 11T spectrometer (Bruker AV500, XWIN-NMR™ ver 3.0, Rheinstetten, Germany). Only olive oil samples were subjected to spinning. Samples were first maintained at room temperature, then raised to 30, 40, 50 and 60°C, and finally lowered at the same temperature steps using an air blower device equipped with the spectrometer. At each temperature setting, we measured the T_1 of the different chemical shift compo-

nents of the fatty acids using a traditional inversion recovery (IR) sequence with 2 signal acquisitions at 8 different inversion times (TI), 200, 400, 600, 800, 1000, 1200, 1400, and 16000 ms, and repetition time (TR) of 20 s. We then processed data with Marquardt-Levenberg nonlinear least-mean-square software equipped with the spectrometer system. We used the following equation¹⁰ to model the experimental IR signal:

$$S = S_0 + q \cdot \exp(-t/T_1), \quad (1)$$

where S is a signal, S_0 is the signal from thermal equilibrium magnetization, and q is a term that accounts for imperfect magnetization inversion by the 180° pulse.¹⁰ We similarly measured T_2 values with the Carr, Purcell, Meiboom, and Gill (CPMG) sequence of radiofrequency pulses with 2 signal acquisitions at 8 echo times, 100, 200, 300, 400, 500, 600, 700, and 800 ms, with interpulse delay of 100 ms and TR of 20 s. We used a classic model equation¹⁰ to calculate T_2 .

Simulations

After obtaining the experimental data, we numerically simulated the effect of multiple coexisting components of fatty acids to examine the degree of error induced by estimating temperature from a mixed signal of CH₂ and CH₃ based on a calibration result obtained for a CH₂ signal. Assuming temperature imaging for breast fat at 3T using an IR sequence, we determined T_1 values for CH₂ and CH₃ at 30°C. A previous work reported that the T_1 value for “bulk” breast fat *in vivo* at 3T is 366.78 ms.¹⁰ Our results yielded signal intensity fractions relative to the total fat in bovine samples of 0.596 ± 0.013 for CH₂ and 0.108 ± 0.007 for CH₃ see caption, Fig. 1b. Based on the results shown in Table 1, the average T_1 values of the same sample group at normal body temperature (37°C) would be obtained as 726.8 ms for CH₂ and 1263.4 ms for CH₃ at 11T, demonstrating the T_1 value of CH₃ as 1.738 times longer than that of CH₂. By ignoring the contribution from components other than CH₂ and CH₃ for simplification and assuming that the signal and T_1 ratios between CH₂ and CH₃ did not change with the static magnetic field strength and that the T_1 of the bulk fat is a weighted sum of T_1 values for the components, we estimated a T_1 value of 330 ms for CH₂ and 573 ms for CH₃ in the breast fat at 37°C at 3T. Moreover, we assumed the same temperature coefficients of T_1 for the components at 3 and 11T (1.79%/°C for CH₂ and 2.98%/°C for CH₃ at 30°C).

Based on the above parameter settings, we performed the simulation as follows. First, we as-

Fig. 1. Examples of proton spectra of a CDCl_3 -resolved olive oil sample (tetramethylsilane [TMS]-standard) (a) and a bovine fat sample *in vitro* (b). The intensity fractions (obtained as spectral heights) of Components 1 through 9 relative to the total fat in the 5 bovine samples were: 0.047 ± 0.004 (Component 1); 0.023 ± 0.013 (2); 0.018 ± 0.001 (3); 0.017 ± 0.001 (4); 0.064 ± 0.004 (5); 0.087 ± 0.006 (6); 0.067 ± 0.005 (7); 0.596 ± 0.013 (8); and 0.108 ± 0.007 (9).

Table 1. Temperature dependence of the spin-lattice relaxation time (T_1) of methylene chain (CH_2) and terminal methyl (CH_3)

	CH_2					CH_3				
	at 30°C [ms]	Temperature coefficient [ms/°C]	Correlation coefficient [%/°C]	Correlation coefficient	P-value	at 30°C [ms]	Temperature coefficient [ms/°C]	Correlation coefficient [%/°C]	Correlation coefficient	P-value
Olive oil + CDCl_3 + TMS	1366.5 ± 18.5	33.9 ± 1.06	2.48 ± 0.08	0.997 ± 0.001	<0.001	3160.6 ± 38.2	73.9 ± 3.01	2.34 ± 0.10	0.997 ± 0.002	<0.001
Olive oil + TMS	663.2	10.06	1.52	0.980		955.6	22.52	2.36	0.932	
Mayonnaise	630.4	10.96	1.74	0.996		1053.0	27.15	2.58	0.992	
Bovine fat	645.6 ± 18.9	11.6 ± 0.42	1.79 ± 0.07	0.991 ± 0.002		1045.0 ± 85.3	31.2 ± 3.92	2.98 ± 0.38	0.994 ± 0.001	
Porcine fat	637.6 ± 17.2	10.7 ± 0.53	1.69 ± 0.08	0.984 ± 0.002		990.4 ± 57.7	30.3 ± 2.08	3.06 ± 0.21	0.994 ± 0.002	

summed an arbitrary CH_2 fraction, f_{CH_2} , and calculated a signal series with 10 different inversion times (TI) as the sum of 2 different signal components, CH_2 and CH_3 , with a ratio of f_{CH_2} and $(1 - f_{\text{CH}_2})$. We then calculated an apparent T_1 of the summed signal using a linear least-mean-square method for simplicity, based on the equations:

$$\ln(1 - S(T)) = -\frac{1}{T_{\text{lapp}}(T)} TI + \ln 2, \quad (2)$$

$$S(T) = S_{\text{CH}_2}(T) + S_{\text{CH}_3}(T), \quad (3)$$

$$S_{\text{CH}_2}(T) = f_{\text{CH}_2} \cdot \left(1 - 2 \exp\left(-\left(\frac{TI}{T_{1\text{CH}_2}(T)}\right)\right)\right), \quad (4)$$

and

$$S_{\text{CH}_3}(T) = (1 - f_{\text{CH}_2}) \cdot \left(1 - 2 \exp\left(-\left(\frac{TI}{T_{1\text{CH}_3}(T)}\right)\right)\right), \quad (5)$$

where T [°C] is “true” temperature; $S_{\text{CH}_2}(T)$ is the normalized intensity of the CH_2 signal at temperature T ; $S_{\text{CH}_3}(T)$ is that of the CH_3 signal; $S(T)$ is that of the total signal; $T_{\text{lapp}}(T)$ is the “apparent” T_1 of the total signal at temperature T ; $T_{1\text{CH}_2}(T)$ is

the T_1 of CH_2 ; and $T_{1\text{CH}_3}(T)$ is that of CH_3 . Based on model function (2), linear regression will yield $T_{\text{lapp}}(T)$, which then produces an apparent temperature, $T_{\text{app}}(T)$ [°C], based on prior knowledge of the relative temperature sensitivity, α_{CH_2} ($=0.0179/^\circ\text{C}$), for CH_2 at 30°C as follows:

$$T_{\text{app}}(T) = [T_{\text{lapp}}(T) - T_{\text{lapp}}(30)] / (\alpha_{\text{CH}_2} \cdot T_{1\text{CH}_2}(30)). \quad (6)$$

Thus, we estimated the error in temperature measurement between 30 and 60°C. These sets of procedures were programmed on MATLAB® (2007b or later released versions, The MathWorks, Inc., Natick, MA, USA).

Results

Figure 1 shows the typical proton spectra⁵ obtained from the CDCl_3 -resolved olive oil sample (a) and a bovine fat sample *in vitro* (b). Nine components were recognized in all samples. In the bovine samples, the signal intensity fractions obtained as spectral heights were highest for CH_2 and second highest for CH_3 (see caption, Fig. 1b).

Figure 2 demonstrates the relationships between

Fig. 2. Relationships between the spin-lattice relaxation time (T_1) values of methylene chain (CH_2) and terminal methyl (CH_3) signals in bovine fat samples *in vitro*. The means and range of standard deviations for ascending and descending plots are shown with the original scatter plots. The standard deviations for room temperatures are also shown. Table 1 shows the temperature coefficients of these plots.

temperature and the T_1 values of 2 major components, CH_2 and CH_3 , in the samples of bovine fat. The T_1 values at 30°C were 645.6 ± 18.9 ms for CH_2 and 1045.0 ± 85.3 for CH_3 . The temperature relationships were linear for both components. We observed consistent regression lines for ascending and descending data for CH_2 with no hysteresis in the temperature range. As anticipated from signal intensities, the plots and, hence, regression lines deviated more significantly for CH_3 than CH_2 . The temperature coefficients for CH_2 and CH_3 differed significantly; in these bovine samples, the coefficient for CH_2 was 11.6 ± 0.42 ms/ $^\circ\text{C}$ and for CH_3 , 31.2 ± 3.92 ms/ $^\circ\text{C}$. Such temperature dependences and their differences were consistent in all samples (Fig. 3). Figure 3a, b shows the T_1 values and correlation coefficients between T_1 and temperature. Figure 3c, d shows the temperature coefficients (ms/ $^\circ\text{C}$) and their relative values (%/ $^\circ\text{C}$) as a ratio to the T_1 values at 30°C . For example, the above-mentioned temperature coefficient for the T_1 of CH_2 in bovine fat was 1.79 ± 0.07 (%) at 30°C . Table 1 summarizes the temperature dependence of T_1 . Temperature and relaxation time correlated significantly in all samples ($P < 0.001$).

Fig. 3. Spin-lattice relaxation time (T_1) properties in various samples. T_1 values at 30°C (a), correlation coefficients between T_1 and temperature (b), temperature coefficients (c), and temperature coefficients relative to the T_1 values shown in (a). Note that methylene (CH_2) peak split was observed for the CDCl_3 -resolved olive oil samples and only the data for the peak on the upward frequency are shown.

Fig. 4. Relationships between the spin-spin relaxation time (T_2) values of methylene (CH_2) and methyl (CH_3) signals in bovine fat samples *in vitro*. The means and range of standard deviations for ascending and descending plots are shown with the original scatter plots. The standard deviations for room temperatures are also shown. Table 2 shows the temperature coefficients of these plots.

Figures 4 and 5 show the results for T_2 similarly to Figs. 2 and 3 for T_1 . The inherently lower signal-to-noise ratio (SNR) in T_2 than T_1 measurement produces generally larger deviations in data. The T_2 values for bovine samples at 30°C were 249.7 ± 13.7 (ms) for CH_2 and 506.9 ± 21.1 (ms) for CH_3 , shorter than the T_1 values for those components. Air bubbles in the mayonnaise sample made the T_2 measurement seem unreliable. Table 2 summarizes the temperature dependence of T_2 . Temperature and relaxation time correlated significantly ($P < 0.001$) in most samples, except for CH_3 in the mayonnaise sample.

Figure 6 shows the temperature error evaluated when we used the temperature calibration of T_1 of CH_2 to estimate the temperature in tissue with a different mixing ratio of CH_2 and CH_3 . The error is zero when the actual signal contains only CH_2 , but if the signal contains 18% of CH_3 , mismatch in calibration causes the temperature error to reach 3.3°C .

Discussion

In this study, we examined the feasibility and re-

Fig. 5. Spin-spin relaxation time (T_2) properties in various samples. T_2 values at 30°C (a), correlation coefficients between T_2 and temperature (b), temperature coefficients (c), and temperature coefficients relative to the T_2 values shown in (a). Note that methylene (CH_2) peak split was observed for the CDCl_3 -resolved olive oil samples and only the data for the peak on the upward frequency are shown.

Table 2. Temperature dependence of the spin-spin relaxation time (T_2) of methylene chain (CH_2) and terminal methyl (CH_3) protons

	CH_2				P -value	CH_3				P -value
	at 30°C [ms]	Temperature coefficient [ms/°C]	coefficient [%/°C]	Correlation coefficient		at 30°C [ms]	Temperature coefficient [ms/°C]	coefficient [%/°C]	Correlation coefficient	
Olive oil + CDCl_3 + TMS	1039.5 ± 21.0	24.4 ± 0.95	2.34 ± 0.09	0.998 ± 0.001	<0.001	1937.9 ± 26.7	44.2 ± 1.97	2.28 ± 0.10	0.995 ± 0.003	<0.001
Olive oil + TMS	202.8	9.74	4.80	0.997		375.4	16.58	4.42	0.978	
Mayonnaise*	150.1	0.81	0.54	0.900		251.7	−0.47	−0.19	0.559	
Bovine fat	249.7 ± 13.7	9.99 ± 0.93	4.00 ± 0.37	0.994 ± 0.003		506.9 ± 21.1	16.9 ± 1.39	3.33 ± 0.28	0.991 ± 0.004	
Porcine fat	212.9 ± 26.7	11.0 ± 0.87	5.16 ± 0.41	0.991 ± 0.005		394.5 ± 81.5	22.3 ± 1.45	5.66 ± 0.37	0.981 ± 0.002	

* The data may have contained considerable error from susceptibility effect caused by air bubbles in the sample.

Fig. 6. Temperature estimation error in the case of using the temperature coefficient of spin-lattice relaxation time (T_1) of pure methylene (CH_2) for tissues with different fractions of terminal methyl (CH_3).

liability of using the temperature dependence of the relaxation times of the fatty acid protons of tissues with high fat content undergoing thermal therapies as a means to measure temperature visually and quantitatively. We used a spectrometer with 11T magnetic field for precise experiments and expect that use of relatively lower magnetic fields, such as 3T, should yield similar results.

Low viscosity produced the markedly longer T_1 value of the olive oil in the deuterated chloroform solution (Fig. 3a). The T_1 values of the other samples were similar within the CH_2 and CH_3 groups. In all samples, the T_1 of CH_2 and CH_3 were linearly dependent on temperature in both vegetable oil and animal fat with high correlation coefficients, as shown in Fig. 3b and Table 1. The temperature coefficients (ms/°C) of the T_1 of CH_2 and CH_3 differed by a factor of 2 to 3 in each sample, whereas those for each component were close among all samples except the oil in deuterated chloroform solvent. Standard deviations of the temper-

ature coefficients of CH_2 were 0.07% in bovine samples and 0.08%, in porcine samples, demonstrating that the coefficients were sufficiently reproducible.

T_2 results seemed relatively more dispersive from the lower SNRs in measurements. However, similarity with T_1 results was recognized in the values at 30°C, the correlation coefficients, and the temperature coefficients in samples of olive oil and animal fat. The T_2 values and their temperature coefficients in ms/°C were larger for CH_3 than for CH_2 .

Figure 6 shows that the inconsistency in CH_2 versus CH_3 ratio causes considerable deviation, such as the 3.3°C for 15°C of temperature elevation with signal contamination by 18% of CH_3 , which results from the difference in temperature dependences of the 2 components.

These findings demonstrate the need to separate the methylene and methyl proton signals of fatty acids and use the temperature coefficient of the T_1 of a particular component, like CH_2 , to image temperature quantitatively. Based on the reproducibility and common features of the temperature dependence of the T_1 of CH_2 , our results may yield a new technique for monitoring the temperature of tissues comprising both fat and water, such as those in breast.

Although it would be beneficial to interpret our results from the viewpoint of molecular science, we did not do this because our focus was to design an appropriate technique for imaging the temperature of fat. We also neglected the contributions from components other than CH_2 and CH_3 for simplicity and clarity and because we felt their use would be less practical for temperature imaging at clinically available field strength ($\sim 3\text{T}$). However, as we observed in earlier spectrometer experiments (results not shown), the components other than CH_2 and CH_3 have different temperature sensitivities in their relaxation times. To consider the effect from these components in imaging temperature, it would be preferable to separate signal using, for example, a multipoint Dixon-type technique, such as iterative

decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL),¹¹ which requires a specific signal model with a number of components with certain phase angles among them. Moreover, investigation of the temperature dependence of such components is an interesting topic that requires examination.

Conclusion

Separating the components of fatty acids would significantly improve the accuracy of quantitative imaging of fat temperature based on relaxation times. Use of the T_1 of CH_2 seems promising in terms of reliability and reproducibility for measuring temperature. Selective measurement of the relaxation time of the particular fatty acid component using multipoint Dixon technique with multiple flip-angle acquisitions¹² may be effective for extending this measurement technique to map temperature distribution. Calibrating the T_1 using temperature in the breast fat would be necessary for applying the imaging strategy in clinical practice.

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