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Proton Magnetic Resonance Relaxation Behavior of Whole Muscle with Fatty Inclusions[1]

This in vitro proton study of spin lattice (T1) and spin spin (T2) relaxation of muscle with storage-fat inclusions demonstrates slow exchange and lack of cross-relaxation between fat and water. Slow exchange causes biphasic T1 relaxation, but T2 relaxation is paradoxically uniphasic due to the nearly equal T2 values for both fractions. By careful dehydration and fat extraction, the relaxation information was deconvolved into water, fat, and protein contributions. The biphasic T1 decay has a short component due to lipid and a long component due to the water-protein combination. The fat content of muscle can be measured from the relative amplitude of the two T1 components or directly from the T2 relaxation time.

Index terms: Magnetic resonance, tissue characterization • Muscles, magnetic resonance studies, 40.1299 • Fat, magnetic resonance studies

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HIGH soft-tissue contrast in magnetic resonance (MR) images is a direct result of differences in the T1 and/or T2 relaxation times of the different tissues. Changes in contrast where there is disease are known to exist, but the factors causing these differences are understood only imperfectly. In addition, some diseases cause different changes in the T1 and T2 relaxation times, at times causing one to increase while the other simultaneously decreases. To optimize the contrast available for MR images of a specific disease systematically, we must better understand the processes involved.

In this study we evaluated the impact on proton MR relaxation behavior of including fatty tissue in host tissues such as muscle. It is well known that the protons in both water and fat contribute to the proton MR signal of tissues in cases where there is considerable fat content (1-4). However, the MR relaxation behavior is paradoxical.

Most nonfatty tissues have a uniexponential T1 decay and a multiexponential T2 decay (5), a behavior that is consistent with the explanations of the Zimmerman-Brittin exchange model (6). The standard explanation for the uniexponential decay curve is that the T1 relaxation times of water protons in separate environments are very long in comparison with the exchange time of protons or spins between compartments. The exchange between compartments is so fast that a single proton sees many environments before relaxation occurs. This yields a single, average relaxation time independent of the number of compartments present. In addition, however, it is now known that uniphasic behavior is also due to fast exchanges between the hydrophilic solids (e.g., proteins) and water by cross-relaxation (7-9).

The characteristic T2 relaxation times of these same compartments are typically much shorter, with the result that the exchange time is no longer fast in some instances with respect to the relaxation times but may be either intermediate between the relaxation times or slow with respect to them. This results in multicomponent T2 curves, each compartment relaxing with its own characteristic rate.

Our observations and those of others regarding tissues with large amounts of fatty inclusions (1-4) appear to contradict the accepted view; in these instances we observed a multicomponent T1 relaxation in conjunction with a single component T2 relaxation. We reasoned that the biexponential T1 behavior of tissues with fatty deposits occurred probably because of the hydrophobic character of large amounts of nonpolar lipids, mainly triglycerides (10). Fat deposits consist almost exclusively of nonpolar lipid, which is excluded by the water solvent because it lacks sites for water to form hydrogen bonds with the lipid molecule. Thus, water molecules do not have the opportunity to bind and exchange spins with these triglyceride molecules as they do with polar macromolecules such as proteins (7-9). Thus, if the exchange is so slow that T1 is biphasic, then the even faster T2 decays should also be nonexchanging. Under these conditions and assumptions, uniexponential T2 decay could result only from the instance where the T2 relaxation of

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the lipid and the water fractions are fortuitously nearly identical.

We have shown that these hypotheses are correct. By tissue dehydration and lipid extraction we showed that the amplitude of the short T1 component is directly related to the relative mass of lipid extract. The T2 relaxation time of the lipid extract was nearly identical with the T2 relaxation time of water on muscle with no fat, so much so that separate T2 relaxation curves cannot be deconvolved even when muscle includes large amounts of fatty deposits.

METHOD

The bovine tissues for these experiments were lean sirloin steak with a rim of subcutaneous fat (adipose tissue) purchased from a meat market. The fat and lean muscle tissue were divided into cubes of approximately 10 mm^3. No differences in relaxation times were observed for diced and whole samples. Samples were prepared with approximately 100, 75, 50, 25 and 0 per cent lean tissue, with the remainder consisting of adipose tissue from the same steak. Each sample was mixed by hand in a pretared MR tube and consisted of 5.0 g net tissue weight. The actual per cent lean tissue mass for each sample was determined from the mass measurements following addition of the lean and adipose fractions. In addition, samples of commercial ground beef, ground beef-lean, and ground beef-extra lean were evaluated for comparison.

Pulsed proton MR relaxation measurements were made on a Praxis Model II instrument (Praxis Corp., San Antonio) that has a 0.25 tesla permanent magnet, sample coil, and RF pulser tuned to 10.7 MHz. The instrument is interfaced with a microcomputer for fast acquisition of data and has built-in data-analysis software. The T1 or spin lattice relaxation time was measured using the saturation recovery pulse sequence, 90°-τ-90°, with results shown in Figure 1. This shows $[S_o - S(\tau)]$ versus delay time τ, where S_o is the signal with $\tau > 5$ T1 and $S(\tau)$ is the signal observed at a variable delay time τ. The resulting analysis of 30 free-induction-decay (FID) peak heights with a sequence of increasing interpulse delay times yields the T1 decay curve. Typically a biexponential T1 decay curve was observed for samples with fatty inclusions, and each component was fit sequentially by regression analysis with a curve peeling sequence supplied with the Praxis software. This analysis yielded short and long relaxation times ($T1_S$ and $T1_L$) as well as associated amplitude information (A_S and A_L). The T2 recovery was measured with the Hahn spin-echo sequence (90°-τ-180°-τ-echo) after demonstrating that diffusion effects were not significant. As shown in Figure 1, the T2 decay of muscle with fatty inclusions was uniexponential, which allowed the determination of the relaxation time from the least squares fit to all 30 data points.

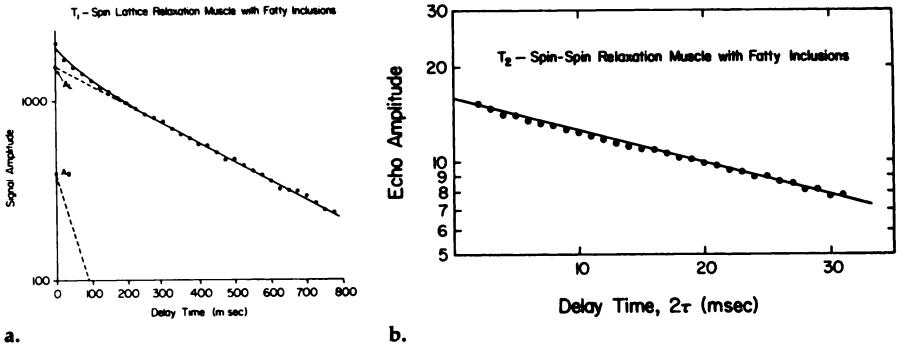
Following the initial MR measurements, the samples were dehydrated in a vacuum oven at 90°C over a period of several days until a stable mass was achieved. The T1 and T2 relaxation times of each lipid-solid residue was then measured. Following these measurements, the lipid was removed from each sample with a sequence of methanol, chloroform, and ether washes. Samples were washed twice with 25 ml of chloroform-methanol (1:1 by volume), once with 50 ml of chloroform-methanol (2:1 by volume), and finally with 50 ml of ether. The extracts from each sample were pooled, transferred, and dried in a single pretared MR tube. The relaxation times of the lipid extract and the solid residue from each sample were measured separately.

The T2 relaxation time of the solid (protein) residue was so short that the T2 could no longer be measured with the spin echo sequence and it was determined directly from the shape of the FID curve. As $1/T2' = 1/T2 + 1/T2_i$, where $T2_i$ is the characteristic decay time of the signal because of magnet inhomogeneity, $1/T2' = 1/T2$ when $T2 \ll T2_i$. The decay of the FID under these circumstances is exponential, and a semilog plot of signal as a function of time is a straight line. The inverse of the slope of this line is then $T2' = T2$. This is true only for a solid, in which the T2 time is very short. The $T2_i$ for our magnet over the sample volume used is approximately 150 μsec.

RESULTS

The results of T1 measurements are demonstrated graphically in Figure 2a, which shows the T1 relaxation

Figure 1

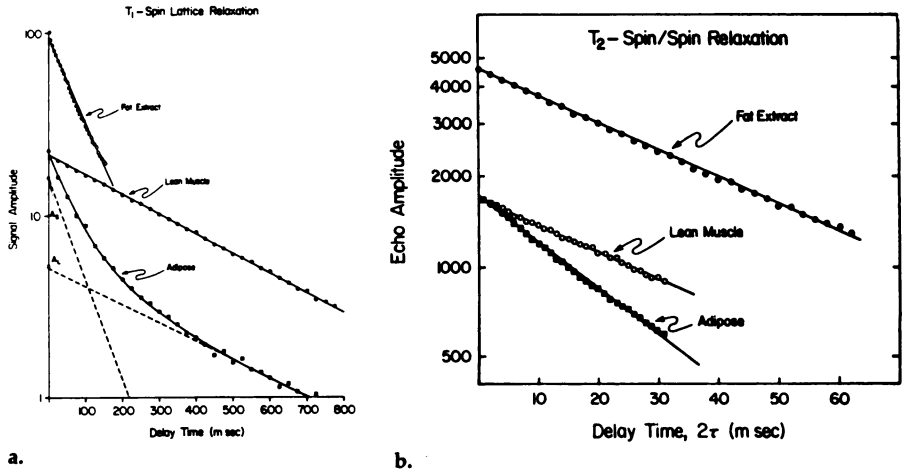


a. b.

a. Typical plot of T1 decay curve for muscle with fatty inclusions, obtained with the Praxis Model II using the saturation recovery (90°-τ-90°) pulse sequence with signal amplitude of $S_o - S(\tau)$. The decay is typically biphasic, with long and short relaxation times ($T1_L$ and $T1_S$) and gives associated amplitudes (A_L and A_S). T1 values are determined from the inverse of the slopes of the best fit lines (dashes) as determined by linear regression analysis.

b. Typical T2 relaxation curve for the same sample, obtained with the Hahn spin-echo sequence, is uniexponential. T2 value is determined as the inverse of the slope of the best fit line to all 30 data points.

Figure 2



a. b.

Plots of T1 and T2 relaxation curves for lean muscle, adipose tissue, and fat extract from adipose.

a. T1 relaxation curves of $[S_o - S(\tau)]$ versus delay time show lean muscle is uniexponential (water signal only) and long fraction on adipose has nearly the same slope and T1 value as lean muscle. The slope of the short adipose component is the same as the slope of the lipid extract.

b. T2 relaxation curves for the same samples show nearly identical slope for lean muscle (water only) and fat extract, implying equal T2 values. It can be argued that adipose curve begins to show signs of biexponential decay.

curves for lean muscle, adipose, and fat extract. Lean muscle with minimal fatty inclusions has only a single T1 component similar to most other tissues. The adipose tissue from this same muscle has biexponential decay, and the long component on adipose tissue has nearly the same slope (equivalent T1 value) as the water on lean muscle. The short component on adipose tissue, on the other hand, has nearly the same slope as that measured for the fat extract. The T2 measurements on the same three samples are presented in Figure 2b, which shows that the slopes (T2 values) of lean muscle and fat extract are nearly identical. These measurements as well as data from all other samples are summarized in Table 1.

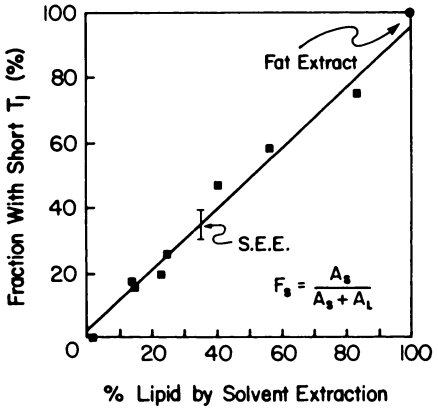
The mean T1 of the long fraction on all eight fresh samples is $T1_L$ = 404 ± 9 msec SEM, while the short component is $T1_S$ = 70 ± 6 msec. Both values are independent of the fat content of the sample. The long T1 fraction disappears during dehydration, which substantiates our identification of this fraction as being due to water. The T1 of the short component, however, increases significantly with dehydration: from 70 ± 6 msec to 93.6 ± 1.1 msec. This latter value does not change significantly, however, when the lipid is removed from the solid by extraction, where T1 = 95.6 ± 1.4 msec. This confirms that the short component is due to lipid. It is also noted that the T1 relaxation time of the solid residue, T1 = 108.0 ± 4.3 msec, is only slightly longer than the lipid value.

In Figure 3 we have plotted the relative fraction represented in the short compartment of fresh tissue, $F_S = A_S/(A_S + A_L)$, against the per cent of mass that is lipid as determined by weighing the lipid extract. This plot shows a direct relationship between the amplitude of the short compartment and the per cent lipid present in the tissue. The linear best fit to this data gives the relation $F_S = 2.32 + 93.41\ F_m$ per cent, where F_m is the ratio of the lipid mass to the original tissue mass. The correlation coefficient is 0.987 and the standard error of estimate is 4.34%.

The T2 relaxation times of fresh tissues show only a single component with contributions from both lipid and water. The signal from the lean sample is due to water; T2 = 48.8, which is significantly longer than the average value of 39.3 msec where varying amounts of lipid are included. In Figure 4 we have plotted the T2 relaxation time as a function of the per cent lipid by extraction. The linear regression analysis of this data gave the relation, T2 (msec) = 47.5 − 25.5 F_m, with a correlation coefficient of 0.962 and standard error of estimate of 2.08 msec. From these analyses it is obvious that the T2 relaxation time of the lipid extract and the water on muscle are not significantly different, and the T2 time of fat with water (T2 = 22 msec) is shorter than either value. The fat-water value was determined by extrapolation of the graph to 100% lipid. In addition, the T2 relaxation time of lipid on dehydrated samples, T2 = 46.7 ± 1.3, which includes both lipid and protein solids, does not differ significantly from the mean T2 relaxation times of the lipid extracts, T2 = 48.8 ± 0.8.

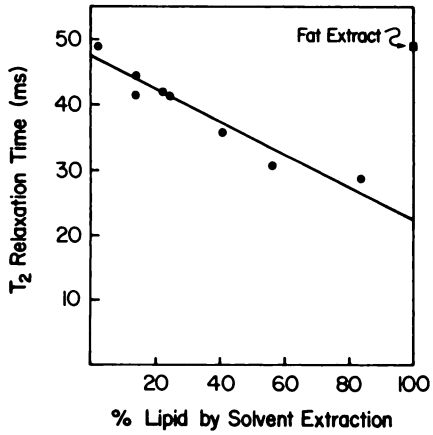
The T2 relaxation time for the solid residue or protein component of muscle, T2 = 7.8 ± 0.12 μsec, was much shorter than the relaxation time of the residue from the adipose tissue, T2 = 30 μsec. Recall that these values are determined from the shape of the FID.

Figure 3



Plot shows direct relationship between per cent short components, $F_S = A_S/(A_S + A_L)$ versus per cent lipid as determined by lipid extraction. Equation of best fit line is F_S = 2.32 + 93.41 F_m, where F_m is ratio of lipid mass to original tissue mass. Correlation coefficient is 0.987.

Figure 4



Plot of T2 as a function of per cent lipid content by solvent extraction demonstrates negative correlation. Equation of best fit line is T2 = 47.5 − 25.5 F_m, with correlation coefficient of 0.962.

DISCUSSION

From these results we are reasonably confident that the hydrophobic character of the nonpolar lipid comprising adipose tissue prevents cross-relaxation and rapid exchange of spins. This results in biphasic decay in both T1 andT2 relaxation. The T2 relaxation times of the lipid phase is so close to the relaxation time of the water phase that two distinct components cannot be detected in most experiments. The reduction of both T1 and T2 relaxation times of lipid in the presence of water does, however, im-

Table 1
Relaxation Times (msec)

	Fresh			Dehydrated		Lipid Extract/Solid			
Sample	T1 (Long)	T1 (Short)	T2	T1	T2	T1 (Lipid)	T2 (Lipid)	T1 (Solid)	T2 (Solid)
Lean	402	—	48.8	109.1	—	94.5	—	107.9	0.0080
Adipose	432	78.6	28.4	99.3	49.7	89.9	48.6	105.0	0.0300
50L/50F	421	90.4	36.0	93.1	45.6	96.4	47.0	106.3	—
75L/25F	421	79.3	41.5	91.1	42.4	95.3	47.1	120.0	—
25L/75F	397	76.9	30.6	92.3	42.6	93.7	46.9	133.5	—
GB	410	66.4	43.5	96.3	46.0	93.3	48.2	99.1	—
GB lean	401	58.9	44.4	93.7	50.2	103.5	50.9	96.2	0.0077
GBX-lean	348	40.0	41.2	89.5	50.2	97.9	52.6	95.8	0.0076
Means ± S.E.	404 ± 9	70 ± 6	39 ± 2.5	93.6 ± 1.1	46.7 ± 1.3	95.6 ± 1.4	48.4 ± 0.8	108.0 ± 4.3	0.0078 ± .0001*

Note.—L = lean, F = fat; GB = ground beef; X = extra.
* Muscle only.

ply that rapidly moving protons of water are influenced by the presence of hydrophobic surfaces. Others have already shown that cross-relaxation between water and protein is an important factor (7–9). The lack of change in both T1 and T2 relaxation times of lipid on the protein when extracted as pure lipid shows that direct interchange between lipid and protein molecules is negligible.

The fundamental source of the difference between lipid and protein solutes is thought to be increased separation of a water molecule from a polar site (2.8 A^0) versus a nonpolar site (3.7 A^0) (11). When dipole–dipole interaction falls off as $1/r^6$, where r is the dipole separation, we can expect little interchange between water and nonpolar materials. Polar materials such as proteins cross-relax readily.

It must be cautioned that in our investigations we have dealt primarily with tissue enriched in triglyceride lipids. Polar lipid such as the phospholipids found in membranes are less present in most tissues and may be expected to behave differently. Such phospholipids and other polar lipids can form hydrogen bonds with water molecules and because of this will likely exchange more rapidly with the water fraction.

CONCLUSIONS

It is clear from these results that the rate of chemical and spin exchange between various fractions of the protons making up a tissue plays an important role in the relaxation behavior of that tissue. There is slow exchange between the water proton and the proximate nonpolar storage lipid protons, probably resulting from the large positional separation. This causes T1 and T2 relaxations of both fractions to proceed independently of one another. In some instances—as with hydrophobic lipid and water—a single phasic T2 decay may be observed because of the inability to distinguish between two nearly equal values. The relaxation time of the fat does, however, reflect the presence or absence of water, which implies that the two compartments are not completely isolated from one another. This is probably caused by the so-called hydrophobic bonding effects on water (10). Muscle lipid and protein interchange is also slow, and no change is observed in lipid relaxation in the presence of protein. It is likely that the interaction of water with proteins involves complex mixtures of hydrophilic (near polar sites) and hydrophobic (near nonpolar sites) properties. We are currently addressing these issues.

It is worth noting that either the T1 relation shown in Figure 3 or the T2 relation shown in Figure 4 could be used to measure the fat content of a muscle sample nondestructively. These concepts may be useful in either in vivo imaging or in in vitro food processing applications. ■

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