Hydration Behavior of Heart Muscle Studies by Nuclear Magnetic Relaxation: Deuterium Exchange of Water Protons in the Myocardium

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SUMMARY. Proton transverse relaxation, deuterium and oxygen-17 NMR measurements on functional animal heart muscle were employed to study the distribution and exchange of water protons in the heart. Our nonlinear regression analysis of such data showed the presence of three proton transverse relaxation components that are likely to correspond, respectively, to two major types of water compartments in the heart muscle and the heart muscle matrix. A deuterium exchange study was undertaken to obtain additional information concerning the chemical exchange of water protons/deuterium within these two water compartments, and the effects of proton intermolecular dipolar interactions on the transverse relaxation of water protons. Our results are likely to influence the analysis and interpretation of MRI data for myocardium since it provides details of the microscopic water distribution in the myocardium which is important to the heart function.

Introduction

Cardiac muscle has cross-striations and has a functionally syncytial character that allows it to contract rhythmically in the absence of external innervation. The cardiac muscle fibers are arranged in a latticework, the fibers dividing, then recombining, and then spreading again. Cardiac muscle cells are 50-120 μm in length and have an intercalated disc that connects adjacent cardiac cells. The intercalated discs of cardiac muscle are located on the opposing ends of cardiac muscle cells and have complex interdigitating structures that maintain cell-cell cohesion. Since cross-striated muscle including cardiac muscle contains about 75% fluid, the trapping and binding of water in muscle are especially important[1]. The fluid consists of blood

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plasma, interstitial fluid and intracellular fluid. Despite the importance of the fluid distribution in muscle, the understanding of its distribution is still incomplete.

NMR techniques are non-destructive, extremely "gentle", and provide some of the best means available for investigating binding of water, chemical structure of biological systems and physicochemical changes[2]. Pulsed NMR relaxation times have been measured both for isolated muscle proteins in solution[3,4,5,6] and intact skeletal muscle[7,8,9,10,11]. There is little doubt that myofibrillar proteins are primarily responsible for the binding of water and ions in muscle. It is also conceivable that different types of water binding exist in various parts of the tissue. The phenomenon of multi-phase behavior in water proton relaxation has been observed in several biological systems, such as striated muscle[12,13] and myosin suspensions [14], or hydrated wheat flour doughs[15]. The nonexponential transverse magnetization decay of the spin echo signal as a function of the interpulse spacing (2τ) is caused by the existence of two or more water populations that do not exchange, or are only in slow exchange, on the NMR time scale compared to the relaxation times of bound water[16].

Recently, nuclear magnetic resonance imaging (NMRI) techniques were employed for the noninvasive characterization of tissues by noting changes in image intensity and NMR relaxation times of the diseased myocardium[17,18,19]. The prolongation of proton relaxation times (T_1, spin-lattice relaxation time, and T_2, spin-spin relaxation time) appears to be related to water and lipid accumulation in the region of myocardial infarction. Some studies were able to evaluate quantitatively the relationship between the NMR relaxation times and water, tissue fat and collagen content[20,21,22], or developmental growth changes[23]. However, most of the MRI and relaxation studies measured only weighted averages of T_2's of the muscle and water protons. This paper presents the existence and analysis of the distinct T_2 values in relation to several water compartments in the heart muscle. Several advantages are expected from the consideration of the existence of several water populations in the MRI analysis of muscle.

Our aim is to analyze the transverse ^1H NMR relaxation behavior of the heart muscle from chicken and pork. This study would provide a more precise assignment of the water compartments in ^1H transverse relaxation and confirm the multiple behavior via deuterium exchange study on muscle. The ^1H transverse relaxation analysis of heart muscle is relatively easy to perform and can be used routinely, giving much valuable information. The proton relaxation analysis alone, however, does not give precise results, because it is influenced by cross-relaxation or proton intermolecular dipolar interactions and chemical exchange.

Therefore, the effects on relaxation, such as proton intermolecular dipolar interactions, are removed by deuterium exchange allowing a more accurate analysis and confirming the results from the previous proton relaxation studies. The analysis of our NMR relaxation results for heart muscle is likely to be of both medical and pharmaceutical interest in relation to the myocardium as, for example, the interpretation/analysis of MRI results for the heart[24,25,26]. We anticipate that qualitatively similar results would be obtained for water proton magnetic relaxation in the human heart.

Methodology

Sample Preparation. Pork and chicken hearts were obtained from the Muscle Biology Laboratory, Department of Animal Science, University of Illinois at Urbana. The whole chicken heart was transferred into a one-inch diameter tube and NMR measurements were carried out. Pork and chicken hearts were also carefully trimmed and lean portions were ground. The moisture, fat, and protein contents were determined by AOAC methods[27] and are presented in Table 1.

Table 1. The chemical composition of pork and chicken heart muscle

	Moisture	Fat	Protein
Pork Heart	79.2 ± 0.5%	2.7 ± 0.1%	16.0 ± 2.1%
Chicken Heart	80.7 ± 0.5%	4.5 ± 0.1%	15.3 ± 0.6%

Such samples were put into standard 10 mm NMR tubes and allowed to equilibrate at 20°C before ^{1}H NMR measurements. The ground muscle was immersed into ten volumes of D_2O-Ringer solution, to allow for the sufficient exchange of protons with deuterium for 30 min and the excess heavy water solution was carefully removed. This exchange step was repeated several times for extensive deuterium exchange of water protons in such samples, and finally the excess water was filtered.

Pulsed ^{1}H NMR Measurements. Pulsed ^{1}H NMR measurements were carried out at 20 ±1°C and 10 MHz with a 0.235 Tesla magnet with a PC-10 NMR process analyzer (Bruker / IBM Instruments, Danbury CT). The multipulse Carr-Purcell-Meiboom-Gill (CPMG) sequence[28,29] was employed for measuring the decay of the transverse magnetization. The decay of the CPMG spin-echo maxima was monitored with a 30 MHz bandwidth Tektronix

storage oscilloscope (Model 5113, dual beam). The reproducibility of such measurements was within 5% and the fitting was usually better than 2% of T_2 values.

Relaxation Component Analysis. The transverse magnetization decay was monitored through the dependence of the CPMG spin-echo maxima on the interpulse spacing (2τ). T_2 values for the three proton populations in muscle were estimated by the nonlinear regression analysis with equation (1) where M(t) is the amplitude at the spin-echo maxima at $t=2\tau$, M_{0i} is the transverse magnetization at $2\tau = 0.0$, and T_{2i} are the T_2 values for each of the three components.

$$M(t) = \Sigma \{M_{0i} \cdot \exp(-t / T_{2i}) \} \quad i=1, 2 \text{ or } 3, \qquad (1)$$

The curve fitting was carried out with an iterating nonlinear regression program (SYSTAT (R) versions 3.1 & 5.0) on an Apple Macintosh II microcomputer equipped with a 68882 mathematical co-processor and 5 Mb RAM, and involved the use of both Simplex and Quasi-Newton algorithms[30]. These programs determine values of the parameters that minimize the sum of the squares of the distances of the experimental data points to the theoretical curve. The goal of the least squares method is to minimize the residual sum of squares (SS):

$$SS = \Sigma \{ (M_{experimental} - M_{estimate})^2 \} \qquad (2)$$

The fractional populations of the proton magnetization components can be calculated from M_{0i}, the initial spin echo signal extrapolated at $2\tau = 0.0$, which is proportional to the number of protons in each of the three components[31].

Results

Proton nuclear magnetic relaxation measurements have been used to study the states of tissue water. Proton spin-spin or transverse relaxation time (T_2) of cardiac muscle was measured to understand the water distribution in this biological tissue system. The CPMG sequence was employed to measure T_2 values. The transverse magnetization decay of water protons was represented by a plot of amplitude of magnetization versus time. Figure 1(a) shows the magnetization decay of H_20 and Figure 1(b) represents the magnetization decay of chicken heart. Since the proton NMR spectra of chicken hearts were dominated by the intense water resonance, the water properties in chicken heart can be characterized by T_2 values calculated from the proton transverse magnetization decay.

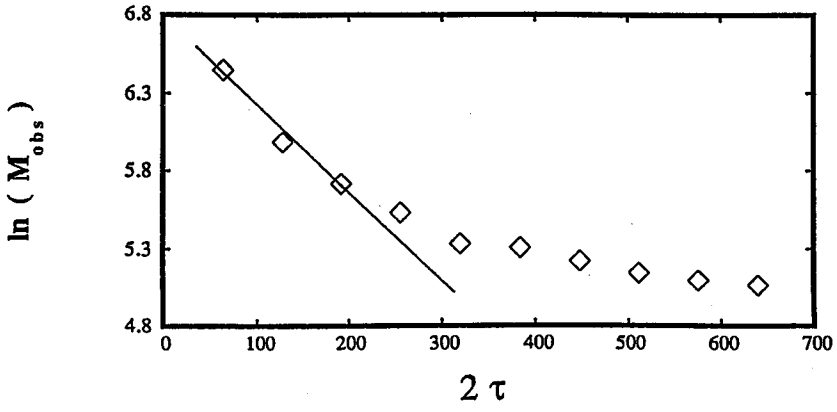

Figure 1. Semilogarithmic plot of the proton transverse magnetization decay of intact chicken heart. Data points were obtained with the multipulse, CPMG pulse sequence applied at the water proton resonance frequency. The bandwidth of the pulsed ^{1}H NMR spectrometer (PC-10) employed for these relaxation measurements was 2 MHz.

In order to calculate T_2 values from a semi-logarithmic plot of the magnetization decay, we employed

$$M(t) = M_{0i} \cdot \exp(-t / T_{2i}) \qquad (3)$$

Taking the natural logarithm on both sides,

$$\ln(M(t)) = \ln(M_{0i}) - t / T_{2i} \qquad (4)$$

Therefore, the slope of the semilogarithmic plot is $-1/T_2$. However, the semi-logarithmic plot of chicken heart did not have a simple linear dependence on time. This indicates the presence of several slowly exchanging components in chicken heart. The nonexponential decay of the CPMG spin-echoes as a function of the pulse spacing (2τ) can be resolved into at least two exponential components. The T_2 values corresponding to such exponential components were obtained by a nonlinear regression analysis of equation (1). The magnetization decay was analyzed, respectively, with a sum of one, two or three exponential terms. This multiexponential relaxation behavior of protons in the chicken heart suggests the existence of at least two or possibly three distinct water compartments, since most of the proton NMR signal originated from the water and little arose from fat, protein or carbohydrate. Each distinct water compartment was defined by each T_2 value. These results suggest that the water in chicken heart muscle was not distributed uniformly, but exists in separate

compartments with distinct characteristics; each water compartment does not exchange, or only slowly exchanges, with other water compartments, whereas the "bound" and free water populations within each compartment exchange fast in comparison with the transverse relaxation rate of the bound water protons.

Table 2(a) includes the T_2 values calculated by a nonlinear analysis of the multiexponential ^1H NMR transverse relaxation carried out on the intact chicken heart was 64 ms. The transverse relaxation of chicken heart can be analyzed with two exponential components in a first approximation. The value of the shorter T_2 equals 70 ms; the fractional population of each T_2 component accounts for 13% and 87%, respectively. Also, the transverse relaxation of chicken heart in a buffer solution was measured and shown in Table 2(b). The average T_2 value was 578 ms. The reason why the T_2 value increased remarkably

Table 2. Components of the proton tranvserve relaxation (T_2) and their fractional populations of chicken and pork heart muscle

	Two-Component Relaxation		Three-Component Relaxation	
	T_2	F_p	T_2	F_p
a) Intact chicken heart				
	70 ± 2 ms	(87 ± 0.8%)		-*
	3.9 ± 0.7 ms	(13 ± 0.5%)		-
b) Chicken heart in buffer				
	1191 ± 24 ms	(32 ± 1.1%)		-
	50 ± 1.3 ms	(68 ± 2.3%)		-
c) Chicken heart, ground				
	183 ± 8 ms	(21 ± 1.6%)	226 ± 38 ms	(9 ± 1.9%)
	50 ± 9 ms	(73 ± 0.2%)	54 ± 1.8 ms	(65 ± 1.5%)
			4.9 ± 0.4 ms	(26 ± 1.1%)
d) Chicken heart in D_2O for 11 min.				
	709 ± 0.2 ms	(27 ± 0.2%)	1243 ± 43 ms	(19 ± 0.2%)
	58 ± 9 ms	(73 ± 0.2%)	77 ± 0.7 ms	(65 ± 0.2%)
			8.5 ± 0.2 ms	(16 ± 0.3%)
e) Chicken heart, ground, exchanged with D_2O, and drained				
	113 ± 0.8 ms	(89 ± 0.3%)	159 ± 8 ms	(36 ± 8.6%)
	3.7 ± 0.2 ms	(11 ± 0.4%)	54 ± 6.8 ms	(54 ± 8.5%)
			3.0 ± 0.3 ms	(10 ± 0.4%)

was the strong free water signal that arose from the buffer solution. This can be confirmed by two-component analysis. The shorter T_2 value of chicken heart in the buffer solution was 50 ms and the longer T_2 value was 1191 ms. The shorter T_2 value seems to arise from water proton signal in the chicken heart itself and the longer T_2 value originates from the water proton signal of the buffer solution.

The ^1H transverse relaxation of ground chicken heart was also considered. The proton NMR magnetization decay of the heart was also multiexponential, and the T_2 values were calculated from the nonlinear regression. Two-and three-exponential component models were applied; the three-component model gives the best fit for the magnetization decay. Table 2(c) presents the results of two- and three-component analysis. The ground chicken heart had a value of the long T_2 relaxation component of 226±38 ms; the intermediate T_2 relaxation component value is 54±2 ms and is predominantly populated. The short T_2 relaxation component is 4.9 ± 0.4 ms (these error ranges are not representing experimental errors but the fitting errors from the nonlinear regression). By analogy with previous reported results for other types of muscle (for example, ref. 10), the long T_2 value can be assigned to an extracellular water proton component, and the intermediate T_2 value to the intracellular water protons. The ground pork heart muscle had a similar transverse relaxation tendency to that in the ground chicken heart (data are not shown here for simplicity). Its short T_2 component value (4.6 ms) is similar to that for the chicken heart (4.9 ms). The long T_2 component for pork heart muscle (335 ms) is higher than that for the ground chicken heart (226 ms). This can be interpreted as evidence that the pore sizes corresponding to the long T_2 component of pork heart are larger than those in the chicken heart and the presence of a larger fraction of loosely trapped water in the pork heart muscle; this observation could be further interpreted as suggesting that the extracellular space of pork heart muscle is wider than that of the chicken heart, or that the ratio of the free water population to that of the "bound" water population in the compartment with long T_2 values is higher in pork heart muscle than in the chicken muscle. The correct value of the long T_2 component may also be influenced by diffusion of water during the longer time intervals between pulses. The intermediate T_2 component (71 ms) dominates the relaxation in pork heart, as in the case of the chicken heart.

The transverse relaxation of chicken heart in D_2O-Ringer solution was also measured. The analysis of the multiexponential ^1H NMR transverse relaxation of chicken heart in D_2O

Table 3. Components of the proton transverse relaxation (T_2) and their fractional populations of chicken heart after immersing in D_2O

D_2O exchange time	11 min.		26 min.	
	T_2 component (ms)	fractional population F_p (%)	T_2 component (ms)	Fractional Population, F_p (%)
shorter T_2 component	58 ± 0.3	73 ± 0.2	73 ± 0.6	75 ± 0.4
longer T_2 component	709 ± 9.3	27 ± 0.2	1073 ± 17.7	25 ± 0.2

is summarized in Table 3; two of the transverse relaxation components of chicken heart in D_2O are shown.

The shorter T_2 component value, measured at 11 min. after immersing the chicken heart in D_2O-Ringer solution was 58 ms, and increased to 72 ms after 26 min. The value of the longer T_2 component also increased with time. As the exchange of the protons with deuterium proceeds in the heart muscle with time, H_2O is converted to the predominant form of HDO. The exchange of certain hydrogens in the chicken heart with deuterium diminishes the contribution of the proton intermolecular dipolar interactions (PIDI) to the NMR relaxation of water protons in the chicken heart; this exchange is, therefore, expected to increase T_2 values. The experimental results presented above show that proton intermolecular dipolar interactions do not dominate the relaxation in the intracellular water compartment (intermediate component, at approx. 26% of total magnetization). The extracellular free water is more affected by proton intermolecular dipolar interactions than intercellular water (approx. 51%). Note that the fractional population of the shorter T_2 component increased slightly upon exchange, in agreement with the expected effect of exchange on proton relaxation.

The transverse magnetization decay of chicken heart exchanged in D_2O for 11 min. was analyzed with both two- and three-relaxation components (Table 2d). The two-component relaxation analysis gave T_2 values of 58 ms and 709 ms, respectively, with the shorter T_2 component dominating. The analysis with three relaxation components yields 1243 ms for the long T_2 component, 77 ms as the intermediate T_2 component, and 8.5 ms for the short T_2 component. The intermediate T_2 component predominates (65% population). This multiexponential relaxation behavior of water protons in chicken heart suggests the existence of three distinct water compartments. In attempting to determine how many water

compartments are in the heart muscle, the root mean square (RMS) values were calculated for two- and three-compartment models[32]. The RMS is calculated as

$$RMS = \sqrt{\frac{SS}{(\text{Number of data points}) - (\text{Number of parameter})}} \quad (3.3)$$

The lower the RMS value is, the better the fit. The RMS value for the three component analysis (32.1 ms) is significantly lower than that for the two component analysis (34.2 ms) and for the four component analysis (34.9 ms), indicating that the three component model gives the best fit for the data. Water distribution in the heart (in D_2O) could be described in principle by either two, three or four compartments; we find that the choice of three compartments is the best among them.

The magnetization decays of water protons in the ground chicken heart are compared in Figure 2 with those of the ground chicken heart muscle after deuterium exchange. The chicken heart was ground, deuterium exchanged for one hour and measured after the removal of excess water. The relaxation decay of the deuterium exchanged heart muscle was faster than that of the ground heart muscle at very short times (2τ up to 10 ms). For longer times, the relaxation decay of the deuterium exchanged heart muscle was slower than that of the ground heart muscle in the absence of exchange.

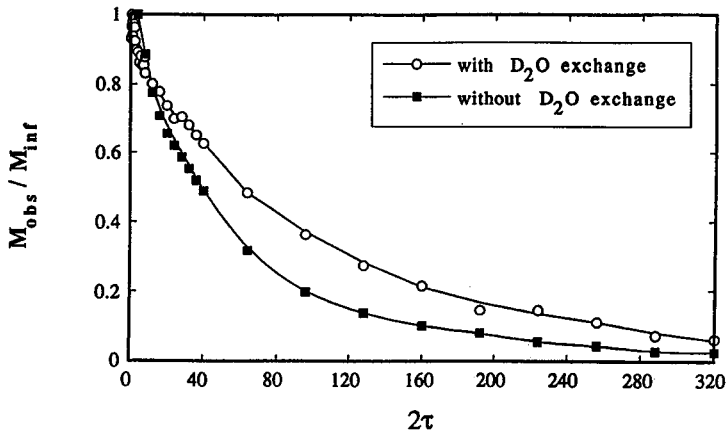

Figure 2. Proton transverse magnetization decay of ground chicken heart muscle before (-0-) and after (- þ -) deuterium exchange for 30 min at 20øC.

The T_2 relaxation components corresponding to the ground chicken heart after deuterium exchange are presented in table 3d. In this case, the three-component model fitted the experimental curves significantly better than the one- or two-component models. Herein, the validity of three-component analysis in deuterium exchanged heart muscle, as well as in ground heart muscle, is shown. A schematic of the three component analysis is shown in Figure 3.

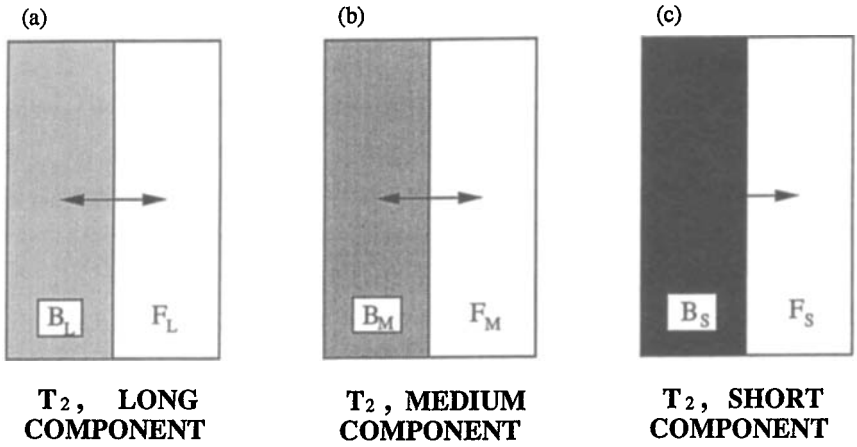

Figure 3. Schematic representation of water proton compartments and the corresponding T_2 values in the heart muscle.

The ground chicken heart following the exchange in D_2O had a shorter relaxation time for the long T_2 relaxation component than before the exchange and its fractional population was significantly higher. The decrease of T_2 values following deuterium exchange may be explained by the increased aggregation of myofibrillar proteins due to washing of salts by D_2O. The intermediate T_2 relaxation component had a value of 54 ms and it had the highest fractional population in the deuterium exchanged, ground chicken heart. When T_2 values of the three-components are compared between ground heart muscle with deuterium exchange and without exchange, the short T_2 value does not change much, whereas the intermediate and long T_2 values change considerably after D_2O exchange. This reveals that the proton signal

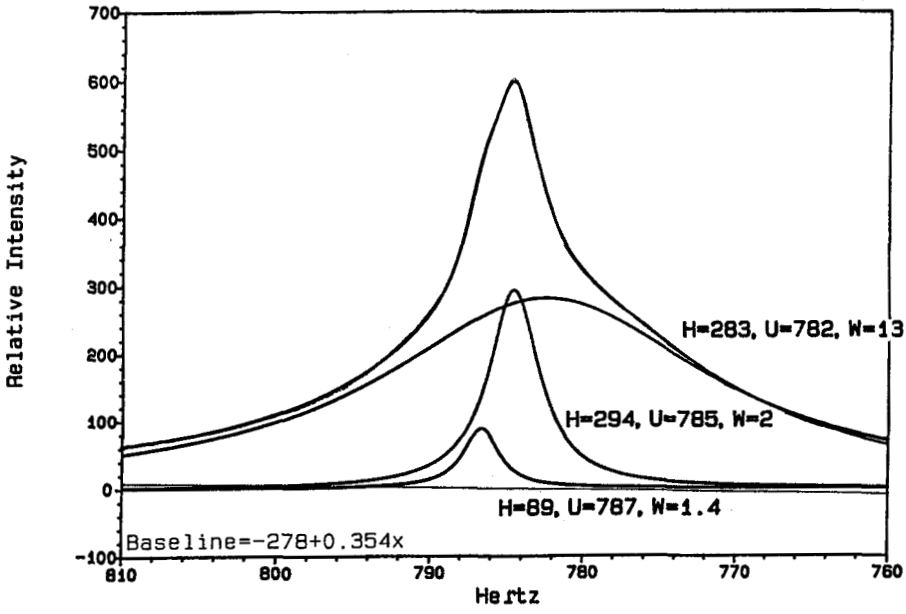

Figure 4. Deuterium NMR spectrum (46.13 MHz ^2H resonance frequency) of deuterium exchanged chicken hearts in D_2O. T_2 values and fractional populations of the water compartments were estimated by a deconvolution method. H_i is relative intensity of spectrum height, W_i is linewidth at half-height and U_i is the peak position in the spectrum, expressed in Hz.

which gives short T_2 values corresponds to the protons in the heart muscle matrix itself rather than to a water compartment (such as trapped water between myofibrils). The pork heart exchanged in D_2O also has three-relaxation components (data not shown here for simplicity). Whereas the three component analysis is proven to be also the best for deuterium exchanged ground heart muscle, the long T_2 component seemed to be affected by the degree of compression during the preparation process of deuterium exchanged ground heart samples (the intermediate and short T_2 values remained consistent). ^2H (46.13 MHz) and ^{17}O (40.74 MHz) NMR measurements were also carried out to provide additional information on water

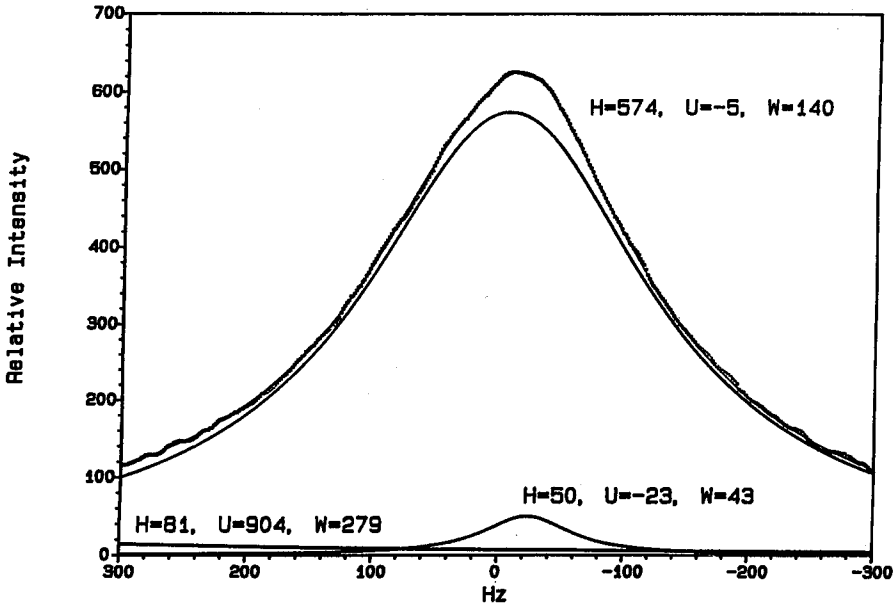

Figure 5. The ^{17}O NMR spectrum (40.74 MHz resonance frequency of deuterium exchanged chicken hearts in D_2O. T_2 values and fractional populations of the water compartments were estimated by a deconvolution method. H_i is relative intensity of spectrum height W_i is linewidth at half-height and U_i is the peak position in the spectrum, expressed in Hz.

compartments in the heart muscle. Unlike the proton, both ^{2}H and ^{17}O are quadrupolar nuclei, and therefore, their relaxation is not affected by cross-relaxation, but it is dominated by the electric field gradients at the bound water nucleus. The ^{2}H and ^{17}O NMR spectra of water in the muscle showed partially resolved peaks (Figs. 4 and 5, respectively).

The composite band could be analyzed into two or three peaks, separated by a deconvolution method. T_2^* values and fractional populations of water compartments were calculated. Deuterium NMR results for deuterium exchanged chicken heart indicated that there were at least two water compartments in the chicken heart; this agrees with the multicompartment relaxation analysis of proton transverse relaxation in the heart muscle. The

^{2}H and ^{17}O NMR relaxation times were comparable to the proton relaxation results in the sense that they are equivalent to the scaled water proton relaxation times; ^{17}O NMR also resolved two water populations. The water compartment corresponding to the short T_2 component or proton relaxation could not be found in either ^{2}H or ^{17}O NMR relaxation measurements. The relaxation time of this component is presumably too short to be measured for the ^{17}O nucleus. This result also supports the interpretation that the short T_2 component of the proton relaxation curve is likely to be due to the heart muscle matrix rather than trapped water between myofibrils.

Discussion

Noninvasive tissue characterization using NMRI can only be improved through a thorough understanding of the spin-lattice and spin-spin relaxation behaviors in such complex systems. Cardiac NMRI techniques have been employed to identify several pathological conditions of the myocardium. T_2 measurements have the potential of characterizing severely ischemic tissue which may have diagnostic and therapeutic implications. Previous studies have demonstrated a correlation between increased tissue water content and longer T_1 and T_2 proton relaxation times. The prolongation of proton relaxation times is also related to water and lipid accumulation in regions of myocardial infarction. A complete understanding of the relationships between water distributions and relaxation times of normal and abnormal myocardium might lead to improved diagnosis and early treatment of cardiac diseases.

In this paper, ^{1}H NMR transverse relaxation measurements of water in several animal hearts were presented and a three-component analysis was proposed. Each T_2 component is considered as an average T_2 value of the "bound" and free water populations that exchange fast within each water compartment. This average T_2 value is calculated according to a two-"state" model with fast exchange[16]; such T_2 values are population-weighted averages between the "bound" and free water populations[33]. For example, the long T_2 component represents an average over a free water population (F_L) and water at binding sites (B_L), that exchange fast with each other. However, the free water population in the compartment which exhibits a long T_2 component (F_L) does not exchange fast with the free water populations in the intermediate or short T_2 compartments (F_M or F_S). Therefore, this model with slow exchange between three types of compartments gives the total water proton magnetization as

the weighted sum over the three water compartments. We also found the existence of an additional water compartment with very long T_2 values while measuring chicken heart in D_2O. This fourth water compartment is probably assigned to the D_2O buffer added.

Several authors have discussed the origin of the multiexponential transverse decay in skeletal muscle and the current consensus is in favor of the existence of physically separated water compartments as the cause for the multiple exponential decay[34]. Figure 3 represents a simplified schematic that we propose for the water distribution in the heart muscle matrix. T_{2i} values are related in this model to pore sizes[35], whereas a certain M_{0i} value corresponds to the fraction of water molecules in a domain of equivalent pore size. There are three kinds of pore sizes in the heart muscle matrix, and summing areas of an equivalent pore size gives the fractional population of that water compartment. The long T_2 component would correspond in this model to the water compartment with large size pores in which 'bound' and free water populations exchange fast with each other. This schematic representation can be related to the existence of extracellular water (that would give the long T_2 component) and intracellular water (that would give the medium T2 component) in the intact muscle. Furthermore, the short T_2 water compartment may correspond either to the trapped water between myofibrillar proteins, or the proton relaxation of the heart muscle matrix itself. This schematic representation (Figure 3) is helpful in analyzing quantitatively the multiexponential relaxation behavior of various types of muscle. After deuterium exchange, the short T_2 component relaxation time was not changed, which means a water molecule corresponding to the short T_2 component is very rigid, or this proton signal is not from a water molecule but from a solid matrix. These non-exchangeable protons can be assigned to the heart muscle matrix itself. Furthermore, the ^{17}O and ^{2}H NMR results did **not** show the short T_2 component.

The long T_2 value increases further after D_2O treatment, and this increase shows the effect of proton intermolecular dipolar interactions on T_2 relaxation. The short T_2 seems to be due to the protons in the heart muscle matrix because it does not change after treatment. The cross-relaxation effect on T_1 in muscle, and for globular proteins, have been reported, but the effect of proton intermolecular dipolar interaction on T_2 has not been studied. D_2O exchange was here employed to analyze the effect of proton intermolecular dipolar interactions on the proton transverse relaxation, and proves the existence of multiple

transverse relaxation even after D_2O exchange. The relative contribution of proton intermolecular dipolar interactions, as well as new possibilities for varying contrast in MRI studies of animal hearts through deuterium exchange and multinuclear relaxation measurements were here considered. The increase in T_2 values after deuterium exchange suggests that the MRI study of deuterium exchanged animal heart muscle will lead to improvements in the contrast in MRI and to a better observation of the fat position, especially of the arteriohemolitic heart muscle in animals because fat protons do not exchange with deuterium. This will contribute to a better understanding of heart diseases.

Our main conclusion is that the ^1H NMR transverse relaxation of water in the hearts from chicken and pork is adequately described by a three-component model with a slow exchange between water compartments. This multicomponent analysis and the assignment of water compartments are also confirmed by deuterium exchange and multinuclear relaxation studies. The analysis of our NMR relaxation results for heart muscle is likely to be of both medical and pharmaceutical interest since it provides details of the water distribution in the myocardium which is known to be important for its function.

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