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Magnetic Resonance Spectroscopic Studies of the Maturation and Degeneration of Skeletal Muscle

Takeo KUWABARA, Takao OHNO*, Tatsuhiko YUASA and Tadashi MIYATAKE

Department of Neurology, Brain Research Institute, and *Analytical Instruments Laboratory, Niigata University, Asahimachi 1, Niigata 951, Japan

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Summary. **This paper describes the changes in proton (^{1}H) and sodium-23 (^{23}Na) magnetic resonance spectroscopy (MRS) occuring with the maturation and degeneration of skeletal muscle.**

Proton and ^{23}Na spin-lattice relaxation time (T_1) of the muscles of embryos and chicks were measured at 89.6 MHz and 23.7 MHz, respectively. As the tissue matured, the values of water proton T_1 decreased along with water content.

^{23}Na-T_1 shortened exponentially with maturation. Sodium content measured by ^{23}Na-MRS showed a similar decrease with maturation.

The sodium content measured by flame photometry was always higher than that observed by ^{23}Na-MRS at any stage of maturation, and this magnetic resonance (MR) visibility decreased exponentially as the tissue matured.

Proton T_1 of the hamster hindleg muscle with muscular dystrophy (DMP) was measured at 270 MHz using surface coil. The values lengthened until 11 weeks, and then shortened until 15 weeks. After that point, they remained unchanged. On the other hand, those of the controls shortened with age until 11 weeks, there reaching an "adult level" and remaining unchanged thereafter.

Not only changes in water content accompanying skeletal muscle maturation, but also several factors such as functional development of cell membrane and structural macromolecule were suggested to have important effects on the relaxation times and MR visibility.

INTRODUCTION

Magnetic resonance (MR) has been known among chemists for its ability to provide detailed information about the structure of molecules in solution. In recent years, however, MR has become a novel method of studying the metabolism and anatomical structure of intact biological systems in the field of biochemistry and clinical medicine.[1]

MR relaxation times originally played an important role in the understanding of the molecular organization of biological systems in analytical biochemistry. Recently, however, using these variations from tissue to tissue, magnetic resonance imaging (MRI) has provided information quite different from that obtained from X-ray computed tomography (CT) or radionucleide examination both qualitatively and quantitatively.[2] It has enabled us to achieve a biochemical analysis of living systems non-invasively. Thus, MR has developed from a standard tool of chemists into a very promising modality for clinical and analytical evaluation of human pathophysiology.

Differences in relaxation times between normal and pathological tissues are key to MRI contrast and the discrimination of diseases. Recently MRI has been used extensively for the diagnosis of muscular disorders[3] such as Duchenne muscular dystrophy[4] and its carrier,[5] metabolic myopathy,[6] myositis[7] and muscle injury.[8] Although newly developed techniques have contributed to an enhanced diagnostic capability, the application of MRI to the field of myopathy is relatively new and accumulated data from basic experiments are needed. Thus the evaluation of the relaxation data obtained from these MRI studies should be of value.

We here report how the proton and ^{23}Na spin-lattice relaxation time (T_1) change during the maturation of skeletal muscles by using proton (^{1}H) and sodium-23 (^{23}Na)-MRS. We also show our findings on the chronological changes of proton-T_1 of muscles in

muscular dystrophy, representing muscular degeneration and regeneration.

MATERIALS AND METHODS

Normal skeletal muscles

Eggs were chosen from the white leghorn breed and were incubated at 37°C in a closed incubator provided with humidity control.

The specimens were biopsied from the pectoral muscles of 12 (n=10) and 19 (n=10) day chick embryos and 6 (n=5), 5 (n=5, only in proton-T_1 experiments) and 20 (n=5) days after hatching.

Macroscopically, fat tissue was removed from the biopsied specimen. The muscle was gently packed in an MR tube 10 mm in height so that the specimen remained within the sensitive volume of the radio-frequency coil.

The specimens were used for the MR measurements immediately after biopsy, though in some experiments, they were stored once in a sterile humid chamber at 4°C and used for measurement within 2 h of the biopsy.

The 5 mm and 10 mm-diameter MR tubes were used for ^{1}H- and ^{23}Na-MRS experiments, respectively.

The MR experiments were carried out on a JEOL FX90Q FT-NMR spectrometer (2.1 Tesla, JEOL Ltd., Tokyo, Japan) and performed at room temperature. The resonance frequency of ^{1}H and ^{23}Na was set to 89.6 and 23.7 MHz, respectively.

T_1 of water proton and ^{23}Na was measured by an inversion recovery method using $180° \sim \tau \sim 90°$ pulse series. The pulse width of 180° pulse was set with each specimen.

Using a non-linear least square method, the T_1 were calculated by plotting the peak height of 8 free induction decays (FIDs) which were obtained by changing the inter-pulse delay τ.

After performing MR measurements, muscles were weighed on a microanalytical balance. Subsequently, the specimens were desiccated at 80°C for 12 h. Water content was then determined as a percentage of wet weight.

A quantitative measurement of one ^{23}Na-MR spectrum was performed by accumulating 1200 FIDs with a repetition time of 2 sec. This peak area was compared with a spectrum obtained from the same volume of physiological saline.

The specimens used for ^{23}Na-MRS were desiccated in the same way with those of ^{1}H-MR experiments, and were extracted with 30% nitric acid. Subsequently, Na content was measured by flame photometry. The results obtained by ^{23}Na-MRS and flame photometry were compared, and ^{23}Na visibility was determined.

Dystrophic skeletal muscle

Muscular dystrophy (DMP) hamsters (UM-X 7.1, male) were used as a DMP group. As controls, male golden hamsters, matched by age were used. They were maintained in a room at constant temperature and allowed free access to water and food.

NMR experiments were performed at 5 (control n=9, DMP n=5), 11 (control n=11, DMP n=9), 15 (control n=7, DMP n=12), 24 (control n=11, DMP n=9) and 31 (control n=5, DMP n=5) weeks after birth.

^{1}H-MRS were recorded *in vivo* at 270 MHz on a JNM GX 270 FT-NMR spectrometer (6.4 Tesla, JEOL Ltd., Tokyo, Japan) using surface coil.

All the animals were anesthetized with an intraperitoneal injection of pentobarbital (35 mg/kg). A 8 mm diameter, two-turn round-shaped surface coil was placed on the sural surface of the right hindleg, and the animal was held in the center of a magnet.

T_1 of water proton was measured by a pulse-burst saturation recovery method.[9] A pulse width of 90° pulse was set to 2 μsec so that the "point" sample volume at the center of the coil was obtained from between 2 and 4 mm in depth. Relaxation delay was set to 25 sec. Using a non-linear least square method, T_1 was calculated by plotting the peak height of 10 FIDs which were obtained by changing the inter-pulse delay τ from 0.3 to 10.1 sec.

Statistical analysis

Statistical significance was determined by an analysis of variance with Student's t test. Data are given as mean $\pm$ standard deviation (SD).

RESULTS

Normal skeletal muscle

As shown in Fig. 1, proton T_1 of the normal skeletal muscle shortened exponentially with maturation; T_1 values were 2.08 ± 0.09 and 1.61 ± 0.11 for 12 and 19 day chick embryos, and 1.32 ± 0.11, 1.11 ± 0.04 and 1.09 ± 0.05 sec at 6, 15 and 22 days after hatching, respectively.

The relation between proton T_1 and water content

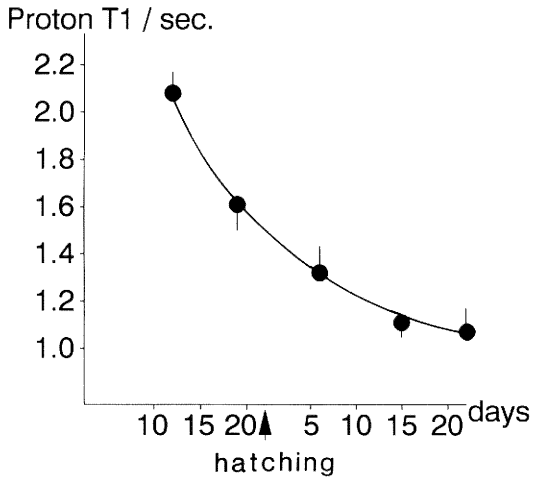

Fig. 1. Changes in proton T_1 accompanying maturation. Proton T_1 of skeletal muscle has shortened exponentially. Values are expressed as mean ± SD.

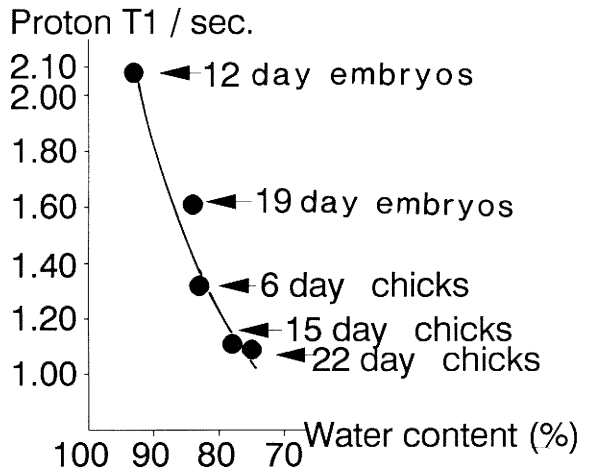

Fig. 2. Relation between proton T_1 and water content. Proton T_1 of skeletal muscle has shortened exponentially as the water content decreases.

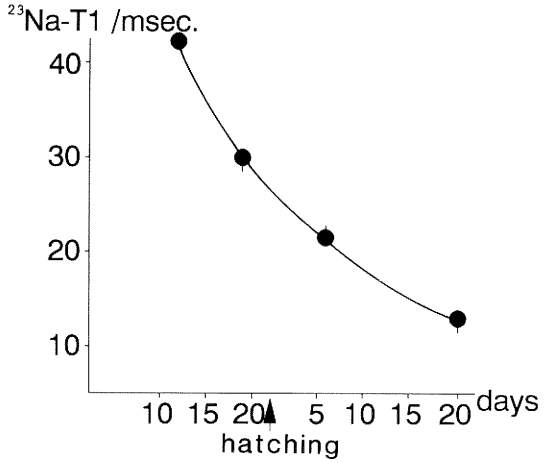

Fig. 3. Changes in ^{23}Na-T_1 accompanying maturation. ^{23}Na-T_1 of skeletal muscle has shortened exponentially with maturation. Values are expressed as mean ± SD.

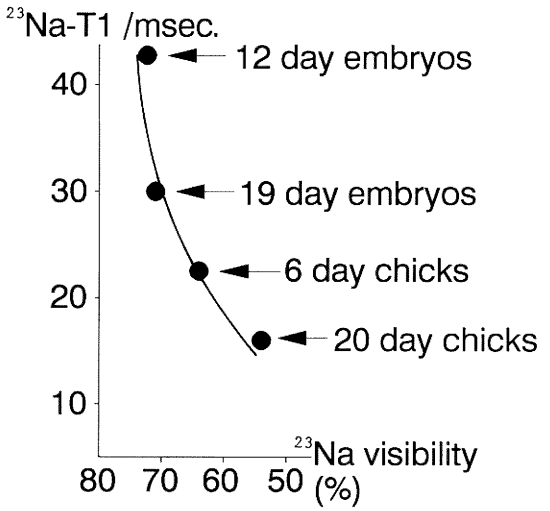

Fig. 4. Relation between ^{23}Na-T_1 and ^{23}Na visibility. ^{23}Na-T_1 of skeletal muscle has shortened exponentially as the NMR visibility of ^{23}Na decreased.

Table Changes of in Na contents

	Embryo		Chick (after hatching)	
Age (days)	12	19	6	20
^{23}N-NMR (uEq/g)	59.5±3.51	49.0±4.95	47.5±3.87	10.3±2.13
Flame (uEq/g)	81.9±4.74	68.3±4.22	71.0±3.63	18.8±1.61
NMR visibilty (%)	72.6±1.76	71.7±1.17	66.9±1.07	54.7±7.35

All values are expressed as mean±SD
Flame: Flame photometry

accompanying maturation is shown in Fig. 2. The water content decreased exponentially as the tissue matured. The mean water contents for 12, 19 day embryos and 6, 15, 22 days after hatch were 93, 86, 83, 78 and 76%, respectively. Proton T_1 shortened exponentially as the water contents decreased.

As shown in the Table, Na contents showed a decrease with maturation both by MRS and flame photometry. The values obtained by flame photometry always showed higher values than those obtained by MRS.

As shown in Fig. 3, ^{23}Na-T_1 shortened exponentially with maturation. The values were 42.9 ± 0.68 and 29.9 ± 0.67 for 12 and 19 day chick embryo, and 22.8 ± 0.66 and 1.63 ± 0.93 msec for 6 and 20 days after hatching, respectively. ^{23}Na-T_1 shortened exponentially as the MR visibility of ^{23}Na decreased. (Fig. 4)

Dystrophic sekeletal muscle

Chronological changes of proton T_1 of DMP muscle are shown in Fig. 5.

Proton T_1 of the controls were 1.67 ± 0.06, 1.41 ± 0.02, 1.44 ± 0.03, 1.43 ± 0.03 and 1.35 ± 0.03 sec for 5, 11, 15, 24 and 31 weeks after birth, respectively; the values decreased as the tissue matured until 11 weeks after birth ($p<0.01$), at which point "adult levels" were reached and remained unchanged.

On the other hand, the values of proton T_1 of DMP at the same ages were 1.64 ± 0.08, 1.80 ± 0.11, 1.57 ± 0.09, 1.54 ± 0.08 and 1.57 ± 0.03 sec, respectively; they showed an increase until 15 weeks ($p<0.01$); after that point, they remained unchanged.

After 5 weeks of age, proton T_1 of DMP were significantly longer than those the of controls. ($p<0.01$)

DISCUSSION

We demonstrated in this paper the decrease in water content, shortening of proton T_1, and decrease in ^{23}Na-MRS and its visibility along with the shortening of ^{23}Na-T_1 occurring with the maturation of chick skeletal muscle. On the other hand, we also demonstrated that values of proton T_1 of DMP muscle were higher than those of controls at any stages after 5 weeks of age and, above all, values increased progressively until 11 weeks.

Applying NMR to a biological material is the same with holding it as a group of atomic nuclei. The findings that ^{1}H- and ^{23}Na-MRS changed as the muscle matured are interpreted as there being different modes of existence for water proton and sodium ions in the tissue with each stage of maturation.

The process of maturation includes the formation and production of macromolecules in cells and tissues. Accompanying the maturation, tissue bound water increases, and the relative content of free water decreases. The observed proton relaxation rate ($1/T_1$) is essentially equal to the sum of the free and tissue-bound water relaxation time.[10] Therefore, the fact that the proton-T_1 changes with the stage of muscle maturation means that the mode of existence of the water proton, namely, the physical property of

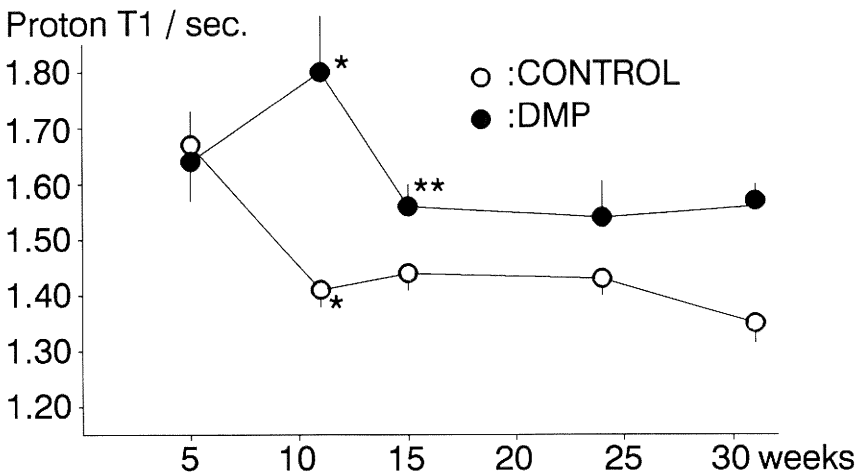

Fig. 5. Chronological changes in proton T_1 of the DMP muscle. The values of controls (○) decrease until 11 weeks ($p<0.01$), but remain unchanged thereafter. On the other hand, values of DMP (●) show an increase until 11 weeks ($p<0.01$), and then decrease until 15 weeks ($p<0.01$), but after that point, remain unchanged. After 5 weeks, proton T_1 of DMP are significantly longer than those of controls ($p<0.01$). Values are expressed as mean ± SD. * $p<0.01$ verses 5 weeks. ** $p<0.01$ versus 11 weeks.

biological water, varies with each stage. That is, our results reflect the characteristic properties of aging such as the decrease in water content, the increase in bound water and the decrease in free water fraction, the conformational changes of structural protein, and the diminution of the physiological functions.

In our experiment, there were exponential correlations between proton T_1 and water content. This suggests, therefore, that the tissue proton T_1 is predominantly determined by the tissue water content. The major cause of the differences between the proton T_1 of the immature and mature stage is the higher water content in the former.[11]

However, the changes in water content with respect to the proton T_1 are so marginal that the physical significance of the correlation between proton T_1 and water content alone is doubted.[10] Macromolecular composition and structure are important for the relaxation times as well as for the tissue function.[12] The differences in macromolecular composition and structure, which could well influence the amount and motion of absorbed water molecules, and the total tissue water content as well, thus probably constitute the primary source of variations in T_1 among different stages of the maturation.[10]

For immature tissue, as mentioned by Bottomley et al.,[10] the elongation of proton T_1 does correlate with the increased water content, but the proton T_1 is also determined predominantly by intermolecular interactions between macromolecules and the single bound hydration layer. Therefore, the possible role of the macromolecular development and the first hydration layer development as the principal determining factor cannot be excluded.

Jasmin et al.[13] studied the chronology of pathologic events in the skeletal muscle of dystrophic hamster (UM-X 7.1) and demonstrated that the initial necrotic changes appear around 3 weeks of age, and, with the progression of the disease, the necrotizing process as well as the regeneration extends progressively to the whole musculature, reaching its maximum severity at 13 weeks and decreasing thereafter until 23 weeks.

Therefore, the progressive elongation of proton T_1 between 5 and 11 weeks, which we showed in this report, is well reflected in their findings that the necrotizing process, that is, the degradation of macromolecules such as structural protein, reaches its maximum severity during this period.

The ^{23}Na-T_1 shortened exponentially as the tissue matured, and an exponential correlation was found between the ^{23}Na visibility and the relaxation times T_1 of muscle tissue ^{23}Na. This suggests that ^{23}Na-T_1 is mainly dominated by the free sodium content. But its intracellular concentration is considerably too low to determine the ^{23}Na-T_1.

We showed that sodium contents detected by the flame photometry were always higher than those observed with ^{23}Na-MRS and the MR visibility of ^{23}Na varied with the stage of maturation.

This discrepancy of ^{23}Na visibility is explained by the quadruple effect.[14] ^{23}Na ions bound to macromolecules are divided into two components, short and long spin-spin relaxation time (T_2) components. The visible signal of tissue ^{23}Na represents the transition $1/2 \leftrightarrow -1/2$ of all the ^{23}Na in the tissue, and it has long T_2 and narrower components. Consequently, the components of long T_2 comprise about 40% of the total intensity in the fully matured tissue.[14,15] Thus, about 40% of ^{23}Na is reported to be visible with the fully matured skeletal muscles,[15,16] and the result which shows the visibility of 54.7% at the age of 20 days is interpreted as showing that the chick skeletal muscle at this stage is not yet fully mature.

The ratio of visible ^{23}Na during the embryonic period remained unchanged, but once hatched, it decreased rapidly to 65 and 55% at 6 and 20 days after hatching, respectively. There is no considerable difference in the rate of decrease in tissue water content between the two periods, before and after hatching. Therefore, it may be inappropriate to explain this rapid increase in the ^{23}Na visibility only by the changes in free ^{23}Na ions. It is likely that the relaxation time T_1 of muscle tissue ^{23}Na is dominantly determined by the interaction with the macromolecules.

The changes in proton T_1, ^{23}Na-T_1 and ^{23}Na visibility support the environmental changes of the free water proton and ^{23}Na ions, that is, the functional development of the cell membrane and structural protein accompanying muscle maturation.

As shown in this paper, relaxation time varies with aging as well as tissue differences. Therefore, when using the relaxation times as a diagnostic parameter in muscle disease, the data obtained from an MR study should be re-evaluated after due consideration of the factors which might influence the relaxation time.

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