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Elucidation of Membrane Destabilization in Post-mortem Muscles Using an Extracellular Paramagnetic Agent (Gd-DTPA): An NMR Study

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The effect of Gd-DTPA on the development in NMR relaxation of skeletal rabbit muscles post-mortem was investigated by dynamic low-field (0.47 T) relaxation measurements from 4 min post-mortem and until 23 h post-mortem. Twelve rabbits were included in the study, and half of the animals were administered 0.2 mmol of Gd-DTPA iv 15 min before sacrifice, while the other half was administered an isotonic salt solution. A significant effect of Gd-DTPA treatment corresponding to a 25% reduction in the T_1 relaxation time was observed. T_2 relaxation was decomposed into two components reflecting intra- and extracellular components (T_{2_α} and T_{2_β}, respectively), and Gd-DTPA treatment was found to affect both components. However, around 150 min post-mortem a dramatic increase in the difference between control and Gd-DTPA-treated rabbits was observed in the relaxation time of the intracellular water population (T_{2_α}). Electrical stimulation of the muscles resulted in a significantly earlier onset of the increased effect of Gd-DTPA on the T_{2_α} population. The increased effect of Gd-DTPA treatment on the T_{2_α} component is believed to reflect leakage of water from the muscle cells due to membrane destabilization, known to be promoted by electrical stimulation. Accordingly, the present study demonstrates how Gd-DTPA can be used for probing membrane integrity in post-mortem muscles known to be of importance for subsequent water distribution and final water-holding capacity.

KEYWORDS: NMR; T_2 relaxation; T_1 relaxation; water; membrane disintegration; leakage

INTRODUCTION

Muscles are highly organized with their arrangement of individual muscle fibers as building blocks and interconnections through the connective tissue. Likewise, the individual muscle fiber is extremely structured with its surrounding sarcolemma, which contains the plasma membrane that forms the T system extending the plasma membrane into the interior of the muscle cell and thereby allowing a fast and efficient signaling to the entire muscle cell. Water diffusion across the plasma membrane is controlled and regulated in response to osmotic gradients, and the plasma membrane is expected to have a key role in maintaining appropriate homeostasis in this actively contracting cellular system (*1*). Accordingly, the integrity of the plasma membrane and its water-transporting channels is significant for maintenance of normal cell functioning, and reports exist on how abnormalities in muscle cell physiology (e.g., muscular dystrophy) are associated with alterations or defects in these structures (*2*, *3*). When muscles are exposed to high levels of stress, the intracellular production of osmotically active solutes increases markedly, causing osmotic regulation. However, if the regulation potential of the cell is exceeded, for example, upon long-term exercise, ion homeostasis is disturbed and membrane barriers cannot be sustained, and leakage of intracellular compounds and osmolytes takes place (*4*−*7*). The post-mortem situation, where the disruption of blood supply results in anoxia, can be considered an extreme stress condition for the muscle cell, and leakage of intracellular compounds has been demonstrated in post-mortem muscles (*8*). Moreover, an earlier study has shown that disruption of membrane barriers post-mortem is involved in the post-mortem redistribution of water and subsequent water-holding properties of the meat (*9*). Accordingly, measurement of commencing membrane disintegration is of substantial importance in further understanding the role of membrane disintegration for the progression in post-mortem processes.

Gadolinium(III) chelate paramagnetic contrast agents, for example, Gd- diethylenetriaminepentaacetic acid (GD-DTPA), are commonly used to enhance contrast in MR imaging (*10*, *11*). Gd-DTPA does not penetrate cell membranes but remains in the extracellular space (*12*, *13*), and the compound has been used as a tool to characterize the extracellular space in muscles (*14*, *15*) and other tissues (*16*). Transverse relaxation (T_2) in muscle tissue is often reported to contain two or more

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exponential components (*17*−*26*), which has been ascribed to intra- and extracellular water compartments in slow exchange (*17*−*19*, *21*−*22*, *25*). Because Gd-DTPA remains extracellular, it is expected mainly to affect the T_2 component reflecting the extracellular compartment (*25*, *26*). Moreover, Gd-DTPA may enable measurement of membrane disintegration, as this would imply a transition from Gd-DTPA only affecting the T_2 of extracellular water to Gd-DTPA affecting the T_2 of both intra- and extracellular water. In the present study, the effect of a paramagnetic agent, Gd-DTPA, on NMR relaxation characteristics in rabbit muscles post-mortem was studied to explore the potential use of Gd-DTPA in the elucidation of membrane disintegration in post-mortem muscles.

MATERIALS AND METHODS

Animals and Tissue Preparation. A total of 12 California rabbits with a live weight of 3−5 kg were included in the present study. After sedation with ketamine/imidazolam (Ketaminol, Veterinaria AG, Switzerland; Dormicum, Alpharma, Norway), six animals were injected with 0.2 mmol/kg Gd-DTPA (Omniscan, Nycomed, Denmark) into an ear vein, which corresponds to the same concentration applied in a previous study (*25*). Six control animals were treated similar except for iv injection with an isotonic salt solution (Nycomed, Denmark) instead of Gd-DTPA. After 15 min, the rabbits were sacrificed by cervical dislocation and exsanguinated. The right m. longissimus dorsi was excised, and muscles from four rabbits (two Gd-DTPA-treated and two controls) were electrically stimulated (DC, 80 V, ∼15 mA) for 2.5 min. Thereafter, a sample (approximately 3.5 cm long) was cut out and inserted into a 10-mm-diameter NMR tube used for the NMR measurements (see below).

The experiment was approved by the Danish Inspectorate of Animal Experimentation, and the rabbits were treated in accordance with the guidelines by the same authority.

pH Measurements. pH was measured at the posterior and the center of the left m. longissimus dorsi in all rabbits and at the posterior of the right m. longissimus dorsi in all electrically stimulated muscles. The pH measurements were performed 30 min post-mortem with a pH meter (Metrohm AG CH 9101 Herisau, Switzerland) equipped with an insertion glass electrode (LL glass electrode, Methrom). The electrode was calibrated at 35 °C.

NMR Relaxation Measurements. The NMR measurements were performed on a 0.47 T Maran benchtop pulsed NMR Analyzer (Resonance Instruments, Witney, U.K.) operating at 20 MHz and equipped with an 18 mm variable temperature probe head. All measurements were carried out at a constant temperature of 30 °C. The experimental protocol consisted of repeatedly measuring the following in sequence: (1) Transverse (T_2) relaxation measurements were obtained using spin−echoes and the Meiboom−Gill modification of the Carr−Purcell pulse sequence (CPMG) (*27*). (2) Longitudinal (T_1) relaxation was measured using an inversion−recovery pulse sequence. The CPMG experiments were performed with a π-spacing (time between subsequent 180° pulses) of 300 μs, using a relaxation delay of 3 s. Data were acquired as the amplitude of every second echo (to avoid influence of imperfect pulse settings) in a train of 4096 echoes as an average of 16 repetitions. In the inversion−recovery pulse sequence (π-t_i-π/2-T_{eq}) where T_{eq} is the equilibrium recovery time, the FID was sampled following the π/2 pulse after a dead time of 15 μs. The experiment was repeated with 12 values of t_i between 1 ms and 16 s. The measurements were started 4 min post-mortem for the nonstimulated muscles and 9 min post-mortem for the electrically stimulated muscles. The acquisition time of each cycle with a CPMG and an inversion−recovery experiment was 12 min, and subsequent measurements were started immediately and repeated until 23 h post-mortem.

Analysis of Relaxation Data. Discrete exponential fitting of relaxation data was performed with MatLab version 6.5 (The MathWorks Inc., Natick, MA) using in-house scripts. The NMR relaxation signal can be expressed mathematically as a sum of exponential decays,

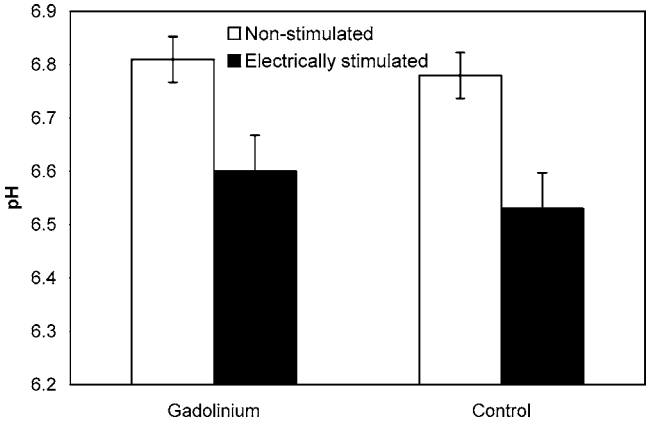

Figure 1. pH in nonstimulated (n = 8) and electrically stimulated (n = 4) muscles for the Gd-DTPA-treated group and for the control group. The pH was measured in *m. longissimus* 30 min post-mortem using a glass electrode. LSMeans are given. Bars show standard errors.

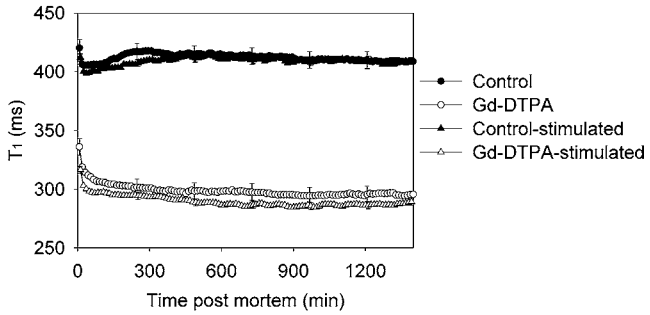

Figure 2. Development in longitudinal (T_1) relaxation in m. longissimus (n = 12). LSMeans and standard errors for the four combinations of nonstimulation/electrical stimulation, Gd-DTPA-treatment/control (saline solution) are given.

and the following equations are valid for T_1 and T_2 relaxation, respectively [eqs 1 and 2]:

$$I(t) = \sum_{n=1}^{N} M_{0,n}\left\{1 - 2^{*}\exp\left(\frac{-t}{T_{1,n}}\right)\right\} \quad (1)$$

$$I(t) = \sum_{n=1}^{N} M_{0,n}\exp\left(\frac{-t}{T_{2,n}}\right) \quad (2)$$

$I(t)$ is the measured signal amplitude at time t, N is the number of exponentials, and M_0 is the magnitude. In discrete exponential fitting, the relaxation profile is decomposed into a limited number of exponentials by least-squares fitting. T_1 and T_2 data were decomposed into one and two exponential components, respectively.

Statistical Analysis. Statistical analysis was carried out with the Statistical Analysis System (SAS Institute Inc.). Analysis of variance was performed using PROC GLM. The statistical models in analysis of variance included the fixed effects of treatment (Gd-DTPA treatment vs control) and electrical stimulation (electrically stimulated vs non-stimulated).

RESULTS

Figure 1 displays pH values 30 min post-mortem in muscles from Gd-DTPA-treated and controls (0.9%-NaCl-treated) in both electrically stimulated and nonstimulated muscles. Electrical stimulation decreased the pH of the muscles significantly.

The development in T_1 relaxation in nonstimulated and stimulated muscles of controls and Gd-DTPA-treated rabbits is shown in **Figure 2**. A strong significant effect of Gd-DTPA

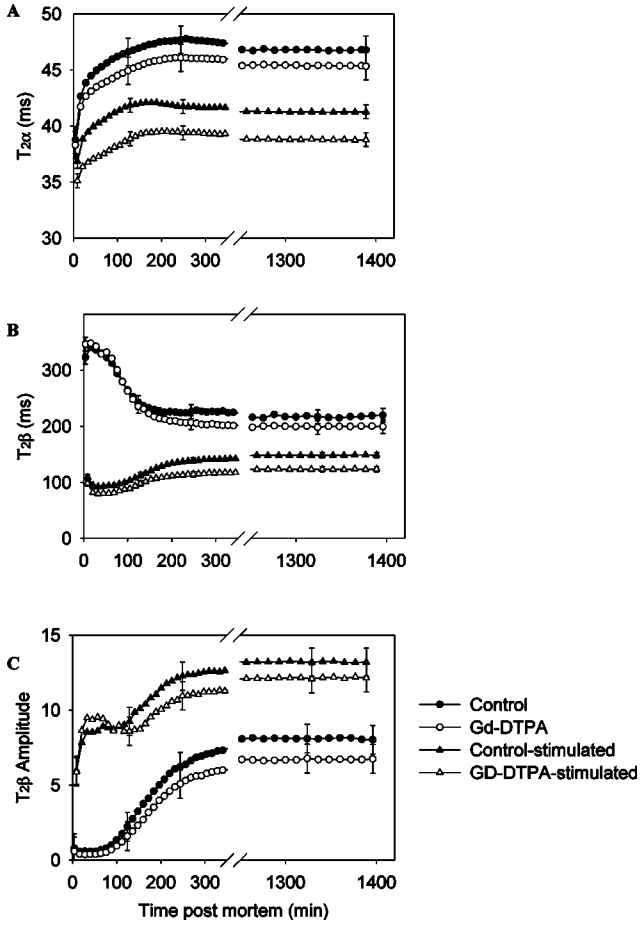

Figure 3. Development in transverse (T_2) relaxation parameters measured in m. longissimus: (**A**) T_{2_α} time constant, (**B**) T_{2_β} time constant, and (**C**) proportion of T_{2_β} population. LSMeans and standard errors for the four combinations of nonstimulation/electrical stimulation, Gd-DTPA-treatment/control (saline solution) are given.

treatment was observed during the entire period for both stimulated and nonstimulated muscles, as Gd-DTPA-treatment caused a decrease in the T_1 relaxation time relative to the control of approximately 100 ms, from 400 to 300 ms.

Figure 3 presents the development in the relaxation time of the two T_2 components (T_{2_α} and T_{2_β}) and the relative proportion of the slowest relaxing component (T_{2_β}). In all samples, the fastest T_2 component (T_{2_α}) increased initially post-mortem and was characterized by a time constant around 35−45 ms (**Figure 3A**). Initially post-mortem the T_{2_α} time constant had similar values in muscles from Gd-DTPA-treated rabbits and the control-group; however, instantaneously the T_{2_α} time constant increased less in muscles from the Gd-DTPA-treated group as compared to muscles from the control-group. In addition, electrical stimulation lowered the T_{2_α} time constant. The T_{2_β} component was characterized by a time constant around 100−300 ms (**Figure 3B**). In nonstimulated muscles, the T_{2_β} time constant decreased initially post-mortem, whereas in electrically stimulated muscles the T_{2_β} time constant only slightly increased initially post-mortem. Moreover, in electrically stimulated muscles, a lower T_{2_β} time constant in muscles from Gd-DTPA-treated animals as compared to those from control animals was evident already in the first measurements, whereas a similar difference developed in nonstimulated muscles only after 150 min post-mortem. **Figure 3C** shows that the proportion of the T_{2_β} component was very small initially post-mortem; however, with time the component increased in intensity.

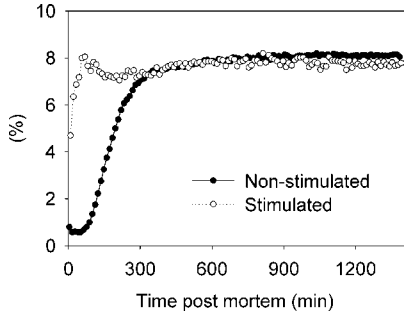

Figure 4. Percentage difference between the relaxation time of the T_{2_α} time constant measured in muscles from control (saline solution) and in muscles of Gd-DTPA-treated rabbits. Mean values for nonstimulated and electrically stimulated muscles are given.

Electrical stimulation of the muscles significantly increased the proportion of the T_{2_β} component during the entire period, and while the T_{2_β} component made up about 1% and 7% of the total magnitude in nonstimulated muscles at 4 min and 24 h post-mortem, respectively, the corresponding amounts were about 5% and 12%, respectively, in electrically stimulated muscles.

The effect of Gd-DTPA-treatment on the T_{2_α} time constant was calculated as the percentage difference in the relaxation time of T_{2_α} in muscles of the control-group and the Gd-DTPA-treated for the nonstimulated and stimulated group (**Figure 4**). For the nonstimulated muscles, a pronounced increase in the effect of Gd-DTPA-treatment on the T_{2_α} time constant took place around 150 min post-mortem. In contrast, in electrically stimulated muscles, this increase in the effect Gd-DTPA-treatment had on the T_{2_α} time constant took place already within the first 30 min. For both electrically stimulated and nonstimulated muscles, the percentage difference ended up at about 8%.

DISCUSSION

Cell membranes are susceptible to destabilization and disintegration when the muscle cell is exposed to stress conditions such as intense, long-term exercise (*4*−*7*) or anoxia, induced during post-mortem conditions. Employing an extracellular paramagnetic contrast agent (Gd-DTPA), the present study investigated the use of ^{1}H NMR relaxation to detect membrane destabilization in rabbit muscles post-mortem. A strong, significant effect of Gd-DTPA treatment on the longitudinal (T_1) relaxation in the muscles post-mortem was observed (**Figure 2**). Gd-DTPA-treatment reduced T_1 from about 400 to 300 ms, which quantitatively corresponds to earlier reported effects of Gd-DTPA on T_1 in skeletal muscles (*20*, *25*, *28*). The comparison of effects of Gd-DTPA in electrically stimulated and nonstimulated muscles revealed small differences between the two treatments. For control groups, the T_1 relaxation times were identical, while for the Gd-DTPA-treated groups the T_1 relaxation time was lower in stimulated muscles than nonstimulated muscles (**Figure 2**). This might be a result of the lower pH in stimulated muscles (**Figure 1**), as the relaxivity of paramagnetic agents is pH-dependent (*29*).

T_1 relaxation in muscle is most often decomposed into a single exponential (*14*, *30*−*32*), and previous data have strongly indicated that the T_1 relaxation is monoexponential (*32*). This may be ascribed to the few number of data points acquired in common inversion−recovery experiments, making it difficult to identify more components. Likewise, our data also indicated only one T_1 population, and accordingly data were decomposed into a single exponential. Within the first hour post-mortem, a slight decrease was observed in the T_1 relaxation time. It has

been argued that the T_1 relaxation time depends on water−protein interfacial interactions (*33*). Moreover, changes in the morphology of proteins caused by thermal denaturation affect the T_1 relaxation of tissue (*34*). Accordingly, the decrease in T_1 observed initially post-mortem should most probably be ascribed to structural changes in the muscle proteins during this period.

T_2 relaxation of muscles is often reported to be multiexponential (*17*−*26*), and the individual components are believed to reflect different anatomically separated physical states of water within the muscle that are in slow exchange (*17*−*19*, *21*−*23*, *25*). Two T_2 relaxation components with a relaxation time around 30−50 ms (T_{2_α}) and 100−300 ms (T_{2_β}) have been ascribed to protons in intra- and extracellular water, respectively (*21*, *25*). Accordingly, in the absence of water exchange between the two compartments, only the T_{2_β} component should be affected by Gd-DTPA, which remains extracellular (*12*−*13*). However, water exchange affects the net effect of Gd-DTPA on water relaxation in compartmentalized systems (*35*), and Gd-DTPA has previously been found to affect both T_{2_α} and T_{2_β} in muscles (*25*, *26*). Likewise, Gd-DTPA treatment resulted in a reduction in the relaxation time of both T_{2_α} and T_{2_β} in the present study (**Figure 3**). Ideally, Gd-DTPA treatment could have been expected to have a strong effect on the T_{2_β} time constant early post-mortem, where membranes are intact with a succeeding increasing effect on the T_{2_α} time constant concomitant with the progression of membrane destabilization. However, early post-mortem the effect of Gd-DTPA treatment on the T_{2_β} time constant was minor, which most probably can be ascribed to the fact that the proportion of the T_{2_β} component is negligible early post-mortem. Difficulties in detecting an effect of Gd-DTPA due to the insignificant size of the T_{2_β} component are supported by the fact that the effect of Gd-DTPA treatment on the T_{2_β} time constant early post-mortem was much more pronounced in electrically stimulated muscles where the proportion of the T_{2_β} component was significantly higher as compared to that in nonstimulated muscles. Moreover, it has been reported that the relaxivity of Gd-DTPA depends on macromolecular concentration (*36*), which is higher in the intracellular compartments containing myofibrillar proteins, implying that the T_{2_α} time constant is more susceptible to be affected by Gd-DTPA. To obtain a measure of the effect of Gd-DTPA on the T_{2_α} component, which must be expected to increase when barriers between intra- and extracelluar compartments are disrupted, the difference in the relaxation time of the T_{2_α} component between the control-group and the Gd-DTPA-treated group was calculated. For nonstimulated muscles, a pronounced increase in the difference in the T_{2_α} time constant between the control-group and the Gd-DTPA-treated group took place around 150 min post-mortem (**Figure 4**), implying that a severe destabilization of the cell membranes took place at this point in time.

Electrical stimulation of muscles provokes a cascade of processes involving increased contractions, increased rate of glycolysis, disruption of the integrity of muscle cell structures, and leakage of Ca^{2+} ions into the cytosol (*37*). An increased Ca^{2+} level is one of the factors that propagates activation of phospholipases, known to mediate hydrolysis of membrane structures (*38*). Accordingly, a much faster destabilization and disintegration of the cell membranes is expected upon electrical stimulation. In the present study, electrical stimulation was performed on a group of muscles, and lower pH values 30 min post-mortem point toward accelerated glycolysis in the stimulated muscles (**Figure 1**). In agreement with this, the difference in the relaxation time of the T_{2_α} component between the control-group (0.9%-NaCl-treated) and the Gd-DTPA-treated group of stimulated muscles increased significantly earlier (around 30 min post-mortem) than in nonstimulated muscles (**Figure 4**), implying an earlier on-set of membrane destabilization and leakage of Gd-DTPA into the intracellular space. Consequently, comparison of nonstimulated and electrically stimulated muscles in the present study confirms that the observed increase in difference in relaxation time of the T_{2_α} component between the control and the Gd-DTPA-treatment is reflecting loss of membrane integrity. Consequently, the present data demonstrate that Gd-DTPA can be used for probing membrane integrity in post-mortem muscles. The technique may have future potential for in vivo studies on membrane integrity upon exposure to stressors such as long-term exercise.

During the post-mortem period, clear changes were observed in the T_2 relaxation characteristics, which furthermore were affected by electrical stimulation (**Figure 3**). However, independent of treatment, an increase in the T_{2_α} time constant was observed early post-mortem corresponding to a decreased relaxation rate. This is in agreement with previous findings on post-mortem muscles (*9*, *39*). Interestingly, a similar decrease in the relaxation rate of T_2 has also been reported in vivo upon exercise (*40*, *41*), which has been suggested to be a result of intracellular acidification and volume increase (*42*). Early post-mortem, conditions may be compared to exercise-induced anoxia in the muscle, and most probably intracellular acidification and volume increase also contribute to the observed increase in the T_{2_α} time constant post-mortem. However, as acidification is more pronounced in electrically stimulated muscles (**Figure 1**), other factors must also contribute to the relaxation time of T_{2_α}. In muscles, the T_{2_α} time constant has been suggested to reflect water located within the highly organized myofibrillar protein matrix structures (*23*, *24*). Accordingly, the lower T_{2_α} time constant in electrically stimulated muscles most probably reflects a more pronounced protein denaturation (*42*), promoted by the more acidic environment, and resulting in local shrinkage of the myofibrillar spaces (*43*). This also agrees with the significantly higher proportion of the T_{2_β} population in electrically stimulated muscles (**Figure 3C**), resembling less water within these structures in these muscles. Noticeably, following the initial increase in the T_{2_β} population, a lag period was observed in electrically stimulated muscles. This most probably reflects the end of the electrical stimulation-induced membrane disruption followed by the normally occurring progression in disintegration of the remaining membrane structures. The relaxation time of the T_{2_β} population, which has been suggested to reflect extracellular/extramyofibrillar water (*21*, *23*−*25*), decreased initially post-mortem, however, only in the nonstimulated muscles (**Figure 3**). This decrease in the relaxation time of the T_{2_β} population most probably reflects an increase in extracellular osmolytes, which already had occurred in stimulated muscles. During the entire measuring period, the relaxation time of the T_{2_β} population was found to be significantly lower in electrically stimulated muscles than in nonstimulated muscles (**Figure 3B**). In the electrically stimulated muscles, an increased release of Ca^{2+} is taking place (*37*), which stimulates proteolytic enzymes (*44*). Accordingly, the lower relaxation time of T_{2_β} in electrically stimulated muscles may arise as a consequence of a higher content of smaller, soluble peptides. Further studies are needed to confirm this.

In conclusion, the present dynamic study investigated the effects of the extracellular paramagnetic agent Gd-DTPA on the development in NMR relaxation characteristics of skeletal rabbit muscles post-mortem. Around 150 min post-mortem, a dramatic increase in the difference between control and Gd-

DTPA-treated rabbits in the relaxation time of the T_2 component that reflects intracellular water (T_{2_α}) took place. This increased effect of Gd-DTPA on the relaxation of intracellular water is believed to reflect leakage of water due to membrane disintegration. This hypothesis was supported by a significantly earlier onset of the increased effect of Gd-DTPA on the T_{2_α} time constant in muscles where membrane destabilization was promoted by electrical stimulation. Accordingly, the present study has demonstrated that Gd-DTPA can be used for probing membrane integrity in post-mortem muscles.

LITERATURE CITED

(1) Watkins, S. C.; Cullen, M. J.; Hoffman, E. P.; Billington, L. Plasma membrane cytoskeleton of muscle: A fine structural analysis. *Microsc. Res. Tech*. **2000**, *48*, 131−141.

(2) Shibuya, S.; Wakayama, Y. Freeze-fracture studies of myofiber plasma membrane in X-chromosome-linked muscular dystrophy (mdx) mice. *Acta Neuropathol*. **1988**, *76*, 189−184.

(3) Wakayama, Y.; Jimi, T.; Misugi, N.; Kumagai, T.; Miyake, S.; Shibuya, S.; Miike, T. Dystrophin immunostaining and freeze-fracture studies of muscles of patients with early stage amyotrophic lateral sclerosis and Duhenne muscular dystrophy. *J. Neurol. Sci.* **1989**, *1*, 191−205.

(4) Hansen, K. N.; Bjerre-Knudsen, J.; Brodthagen, U.; Jordal, R.; Paulev, P. E. Muscle cell leakage due to long distance training. *Eur. J. Appl. Physiol. Occup. Physiol*. **1982**, *48*, 177−188.

(5) Roxin, L. E.; Hedin, G.; Venge, P. Muscle cell leakage of myoglobin after long-term exercise and relation to individual performance. *Int. J. Sports Med*. **1986**, *7*, 259−263.

(6) Lijnen, P.; Hespel, P.; Fagard, R.; Lysens, R.; Vanden Eynde, E.; Goris, M.; Goossens, W.; Lissens, W.; Amery, A. Indicators of cell breakdown in plasma of men during and after a marathon race. *Int. J. Sports Med*. **1988**, *9*, 108−113.

(7) Gastmann, U.; Dimeo, F.; Huonker, M.; Bocker, J.; Steinacker, J. M.; Petersen, K. G.; Wieland, H.; Keul, J.; Lehmann, M. Ultra-triathlon-related blood-chemical and endocrinological responses in nine athletes. *J. Sports Med. Phys. Fitness* **1998**, *38*, 18−23.

(8) Sandercock, D. A.; Hunter, R. R.; Nute, G. R.; Mitchell, M. A.; Hockin, P. M. Acute heat-stress induced alterations in blood acid−base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. *Poult. Sci*. **2001**, *80*, 418−425.

(9) Bertram, H. C.; Rosenvold, K.; Schäfer, A.; Andersen, H. J. Physical changes of significance on early post mortem water distribution in porcine M. longissimus. *Meat Sci.* **2004**, *66*, 915−924.

(10) Schima, W.; Murkejee, A.; Saini, S. Contrast enhanced MR imaging. *Clin. Radiol*. **1996**, *51*, 235−244.

(11) Wallis, F.; Gilbert, F. J. Magnetic resonance in oncology: an overview. *J. R. Coll. Surg. Edinb*. **1999**, *44*, 117−125.

(12) Goldstein, E. J.; Burnett, K. R.; Hansell, J. R.; Casaia, J.; Farrar, B.; Gelblum, D.; Wolf, G. L. Gadolinium DTPA (an NMR proton imaging contrast agent): chemical structure, paramagnetic properties and pharmokinetics. *Physiol. Chem. Phys. Med. NMR* **1984**, *16*, 97−104.

(13) Weinmann, H. J.; Brasch, R. C.; Press, W.-R.; Wesbey, G. E. Characteristics of gadolinium-DTPA complex: A potential NMR contrast agent. *Am. J. Roentgenol*. **1984**, *142*, 619−624.

(14) Dedieu, V.; Finat-Duclos, F.; Joffre, F.; Vincensini, D. In vivo tissue extracellular volume fraction measurement by dynamic spin−lattice MRI relaxometry: application to characterization of muscle fiber types. *Invest. Radiol*. **1999**, *34*, 185−189.

(15) Landis, C. S.; Li, X.; Telang, F. W.; Molina, P. E.; Palyka, I.; Vetek, G.; Springer, C. S. Equilibrium transcytolemmal water-exchange kinetics in skeletal muscle in vivo. *Magn. Reson. Med*. **1999**, *42*, 467−478.

(16) Larcombe-McDouall, J. B.; Seo, Y.; Steward, M. C. Continuous measurement of cell volume changes in perfused rat salivary glands by proton NMR. *Magn. Reson. Med*. **1994**, *31*, 131−138.

(17) Belton, P. S.; Jackson, R. R.; Packer, K. J. Pulsed NMR studies of water in striated muscle. I. Transverse nuclear spin relaxation times and freezing effects. *Biochim. Biophys. Acta* **1972**, *286*, 16−25.

(18) Pearson, R. T.; Duff, I. D.; Derbyshire, W.; Blanshard, J. M. V. An NMR investigation of rigor in porcine muscle. *Biochim. Biophys. Acta* **1974**, *362*, 188−200.

(19) Hazlewood, C. F.; Chang, D. C.; Nichols, B. L.; Woessner, D. E. Nuclear magnetic resonance transverse relaxation times of water protons in skeletal muscle. *Biophys. J*. **1974**, *14*, 583−606.

(20) Sobol, W. T.; Jackels, S. C.; Cothran, R. L.; Hinson, W. H. NMR spin−lattice relaxation in tissues with high concentration of paramagnetic contrast media: evaluation of water exchange in intact rat muscle. *Med. Phys*. **1991**, *18*, 243−250.

(21) Cole, W. C.; LeBlanC, A. D.; Jhingran, S. G. The origin of biexponential T_2 relaxation in muscle water. *Magn. Reson. Med*. **1993**, *29*, 19−24.

(22) Saab, G.; Thompson, R. T.; Marsh, G. D. Multicomponent T_2 relaxation of in vivo skeletal muscle. *Magn. Reson. Med*. **1999**, *42*, 150−157.

(23) Bertram, H. C.; Karlsson, A. H.; Rasmussen, M.; Pedersen, O. D.; Dønstrup, S.; Andersen, H. J. The origin of multi-exponential T_2 relaxation in muscle myowater. *J. Agric. Food Chem*. **2001**, *49*, 3092−3100.

(24) Bertram, H. C.; Purslow, P. P.; Andersen, H. J. Relationship between meat structure, water mobility and distribution−a low field NMR study. *J. Agric. Food Chem*. **2000**, *50*, 824−829.

(25) Adzamli, K.; Jolesz, F. A.; Bleier, A. R.; Mulkern, R. V.; Sandor, T. The effect of gadolinium DTPA on tissue water compartments in slow- and fast-twitch rabbit muscles. *Magn. Reson. Med*. **1989**, *11*, 172−181.

(26) Le Rumear, E.; De Certaines, J.; Toulouse, P.; Rochcongar, P. Water phases in rat striated muscles as determined by T_2 proton NMR relaxation times. *Magn. Reson. Imaging* **1987**, *5*, 267−272.

(27) Meiboom, S.; Gill, D. Modified spin−echo method for measuring nuclear times. *Rev. Sci. Instrum*. **1958**, *29*, 688−691.

(28) Wolf, G. L.; Fobben, E. The tissue proton T_1 and T_2 response to gadolinium DTPA injection in rabbits. A potential renal contrast agent for NMR imaging. *Invest. Radiol*. **1984**, *19*, 324−328.

(29) Kalish, H.; Arbab, A. S.; Miller, B. R.; Lewis, B. K.; Zywicke, H. A.; Bulte, J. W. M.; Bryant, L. H., Jr.; Frank, J. A. Combination of transfection agents and magnetic resonance contrast agents for cellular imaging: relationship between relaxivities, electrostatic forces, and chemical com position. *Magn. Reson. Med*. **2003**, *50*, 275−282.

(30) Currie, R. W.; Jordan, R.; Wolfe, F. H. Changes in water structure in postmortem muscle as determined by NMR T1 Values. *J. Food Sci.* **1981**, *46*, 822−823.

(31) Cameron, I. L.; Finnie, M. F.; Fullerton, G. D. Post mortem changes in skeletal muscle proton spin−lattice relaxation times. *Physiol. Chem. Phys. Med. NMR* **1984**, *16*, 351−354.

(32) Yamada, T. ^{1}H NMR spectroscopy of the intracellular water of resting and rigor frog skeletal muscle. In *Mechanisms of Work Production and Work Absorption in Muscle*; Sugi, Pollack, Eds.; Plenum Press: New York, 1998; Chapter 18, pp 145−155.

(33) Koenig, S. H.; Brown, R. D. A molecular theory of relaxation and magnetization transfer: Application to cross linked BSA, a model for tissue. *Magn. Reson. Med*. **1993**, *30*, 685−695.

(34) Koenig, S. H.; Brown, R. D. The raw and the cooked or the importance of the motion of water for MRI revisited. *Invest. Radiol*. **1988**, *23*, 495−497.

(35) Donahue, K. M.; Weisskoff, R. M.; Burstein, D. Water diffusion and exchange as they influence contrast enhancement. *J. Magn. Reson. Imaging* **1997**, *7*, 102−110.

(36) Stanisz, G. J.; Henkelman, R. M. Gd-DTPA relaxivity depends on macromolecular content. *Magn. Reson. Med*. **2000**, *44*, 665−667.
(37) Hwang, I. H.; Devine, C. E.; Hopkins, D. L. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. *Meat Sci.* **2003**, *65*, 677−691.
(38) Lipton, P. Ischemic cell death in brain neurones. *Physiol. Rev*. **1999**, *79*, 1432−1568.
(39) Bertram, H. C.; Karlsson, A. H.; Andersen, H. J. The significance of cooling rate on water dynamics in porcine muscle from heterozygote carriers and noncarriers of the halothane gene—a low-field NMR relaxation study. *Meat Sci.* **2003**, *65*, 1281−1291.
(40) Jenner, G.; Foley, J. M.; Cooper, T. G.; Potchen, E. J.; Meyer, R. A. Changes in magnetic resonance image of muscle depend on exercise intensity and duration, not work. *J. Appl. Physiol*. **1994**, *76*, 2119−2124.
(41) Damon, B. M.; Gregory, C. D.; Hall, K. L.; Stark, H. J.; Gulani, V.; Dawson, M. J. Intracellular acidification and volume increases explain R2 decreases in exercising muscle. *Magn. Reson. Med*. **2002**, *47*, 14−23.
(42) Bertram, H. C.; Whittaker, A. K.; Andersen, H. J.; Karlsson, A. H. pH-dependence of the progression in NMR T_2 relaxation times in post mortem muscle. *J. Agric. Food Chem*. **2003**, *51*, 4072−4078.
(43) Offer, G. Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. *Meat Sci.* **1991**, *30*, 157−184.
(44) Suzuki, K.; Sorimachi, H. A novel aspect of calpain activation. *FEBS Lett.* **1998**, *433*, 1−4.

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