Magnetization Transfer and T2 Measurements of Isolated Muscle: Effect of pH

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Introduction

Exercise is well known to increase the transverse relaxation time constant (T_2) of contracting muscles [1,2]. The mechanism for the T_2 change remains unclear and may relate to increased intracellular free water and acidification. Previously, we have observed that the intracellular T_2 ($T_{2,I}$) change varies directly with field strength and that this dependence cannot be explained by magnetic susceptibility phenomena [2]. This finding suggests that increases in intracellular water and acidification may affect chemical exchange processes between intracellular free water and proteins. In this study, we test the hypothesis that pH changes affect T_2 by affecting the rate of proton exchange between free intracellular water and intracellular proteins.

Methods

Tissue Preparation The gastrocnemius muscles of 7 frogs (*Rana pipiens*) were excised and stored overnight at 4 ºC in a Ringer's solution (pH 7.0) or modified Ringer's solution in which 40 mM NH_4Cl replaced an equivalent amount of NaCl (pH 7.0). The muscle in modified Ringer's was rinsed with standard Ringer's solution for > 4 hours prior to use, reducing the intracellular pH to 6.5.

MRI/MRS The experiments were performed on a 33 cm, 4.7 T Varian Inova MR imager/spectrometer. The chemical shift of the carnosine peak was measured using a water-suppressed, modified PRESS sequence (TE/TR = 28ms/3000ms) and used to calculate the intracellular pH. Muscle volume was measured using a multi-slice FSE sequence (TE/TR 30ms/ 2000ms). T_2 was measured using non-localized CPMG acquisitions with echo spacings of 2 and 8 ms (TR = 5000s) and using multiple spin-echo images with echo spacings of 8 and 30 ms (TR = 3000s). Magnetization transfer (MT) rates (k) and pool size ratio (p) between free (f) and macromolecular (m) (immobile) proton pools were measured using an inversion-recovery FSE sequence with 25 inversion times, pseudo-log spaced between 3.5 ms and 8 sec, a 1000 µs hard inversion pulse and TE/TR = 10ms/3000ms. The MT results quantify the magnetization transfer between the macromolecules and free proton pools (k_{fm} and k_{mf}), the ratio of their pool sizes (p_m/p_f), and the observed longitudinal recovery rate (R_1) [3].

Results

Exposure to NH_4Cl significantly decreased pH and increased muscle mass. The T_2 results are shown in Table 1. Figure 1 shows the percent muscle water loss of the control and treated muscles. The control muscles had a pH value of 7.05 ± 0.07 and they underwent a small amount of water loss (8 ± 2%). The treated muscles had a pH of 6.53 ± 0.12 and underwent less water loss (4 ± 2%). The experimental results for the fast rate (k_{mf}) and pool size ratio (p_m/p_f) in Figure 2 show no statistical difference between the control and treated (acidic) muscles. However, the slow MT rate (k_{fm}) was significantly greater for the control than the acidic muscle ($p < 0.05$).

Table 1. Results of intracellular T_2.

Echo spacing (ms)	T_2 (ms) Control muscle	T_2 (ms) Acidic muscle
2†	33 (7)	40 (3) *
8†	32 (5)	47 (9) *
8§	25 (3)	37 (6) *
30§	26 (6)	45 (8) *

Mean (SE) is given. *Significant difference between acidic and control muscles (p<0.05).
† non-localized, § localized

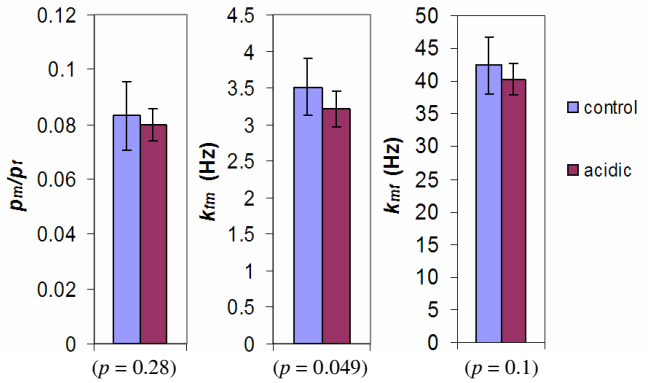

Figure 2. Magnetization transfer results of pool size ratio (p_m/p), slow MT rate (k_{fm}), and fast rate (k_{mf}). pH of muscles: control 7.05 ± 0.07 and acidic 6.53 ± 0.12.

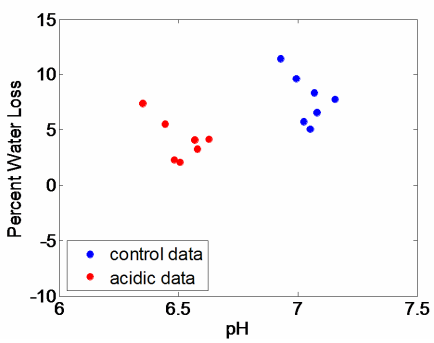

Figure 1. Percent water loss for control and treated muscles were 8 ± 2% and 4 ± 2%, respectively.

Discussion

As expected, the acidic muscle shows an increased $T_{2,I}$ value, regardless of how it was measured (localized or non-localized, high or low refocusing rate). The increase in mass for acidic muscle may be due to an increase in intracellular water secondary to the activation of Na^+/H^+ exchange. The fast magnetization transfer rate (k_{mf}) and pool size ratio (p_m/p_f) did not reveal a statistical difference among the pH's measured, which may be due to the limitation of the physiological pH range. However, the acidic muscles show a decrease in k_{mf} and p_m/p_f compared to the control muscles and the slow rate (k_{fm}) was reduced in the acidic muscle. Since muscle has a large histidine content [4], amide proton exchange may contribute to the slow rate (k_{fm}) measurement due to the effect of pH. Thus, more studies are needed and underway to explore the amide proton exchange as a function of pH in muscle to aide in determining the mechanism of the $T_{2,I}$ changes in exercised muscle to enhance our ability to characterize muscle function non-invasively.

References

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