Effect of pH on CEST in Muscle

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INTRODUCTION

Recently, researchers have developed the technique of chemical exchange saturation transfer (CEST) and applied it to imaging in kidney, brain, and liver tissue. CEST shows contrast from pH effects, protein content, and glycogen content due to the exchange of protons from proteins (-NH) or glycogen (-OH) to water protons [1,2,3]. Proton chemical exchange rate between molecules and water is pH sensitive [1,4,5]. Muscle has a high protein content, approximately 20% by weight [6], and these muscle proteins have a substantial histidine content [7]. In addition, glycogen, a major energy substrate is found in muscle. Histidines, as well as the backbone amide protons in proteins, and glycogen are molecules that have protons (-NH, -OH) that exchange with water. The purpose of this study was to use CEST imaging of muscle tissue to 1) indirectly detect proteins and/or glycogen in muscle via the water resonance by chemical exchange between exchangeable –NH and -OH protons and water protons, 2) provide a pH map of muscle to show active and non-active regions, and 3) quantify changes in protein or glycogen content in muscle due to pH changes or muscle stimulation, respectively. The ability to quantify pH and protein and/or glycogen changes, would provide a non-invasive means to monitor muscle metabolism in diseased and aging muscle.

METHODS

Sample preparation: The gastrocnemius muscle of 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 replaces an equivalent amount of NaCl (pH 7.0). The muscle in modified Ringer's provides a sample with a reduced intracellular pH of 6.5. Seven pairs of muscles were studied.

CEST measurements: The MRI measurement entailed using a saturation period composed of a train of 400 Gaussian pulses (flip angle 180°, length 6.6 ms, delay 3.4 ms), followed by an echo planar imaging acquisition with a repetition time (TR) of 10 s and the following parameters: FOV: 60 mm x 60 mm, 2 mm slice thickness, and 64 x 64 matrix. A Z-spectrum was collected over a range of ± 1800 Hz in steps of 50 Hz using a 7T 16 cm bore Varian Inova scanner and a 38 mm Doty ^{1}H RF coil. The Z-spectrum data was used to determine the magnetization transfer ratio asymmetry parameter, $MTR_{asym} = S_{sat}/S_0$ (negative offset) – S_{sat}/S_0 (positive offset) [2] where S_{sat} and S_0 are the signal intensity when the saturation pulse is on and off, respectively. Paired, two-tailed Student's *t*-tests were performed to compare the control and acidic muscles.

RESULTS

Figure 1 shows the magnetization transfer asymmetry (MTR_{asym}) plot of the acidic (pH 6.5) and control (pH 7.0) muscles (N=7). At 3.5 ppm, the MTR_{asym} plot reveals a 1 % difference (p = 0.2) between the control and acidic muscles. Between 0.5 and 1.2 ppm, there is a difference of 3-5% MTR_{asym} (p = 0.08).

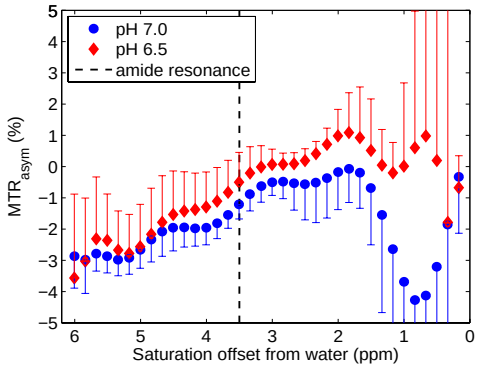

Figure 1. MTR_{asym} spectra of gastrocnemius muscle (N=7) at pH 6.5 and 7.0.

DISCUSSION

The preliminary data show a nonsignificant difference for the amide resonance at 3.5 ppm (offset from the water resonance) between acidic and control muscles. This result reveals the pH dependence of the CEST technique for an intracellular muscle pH difference of 0.5 was not detectable. Between 0.5 and 1.2 ppm, a larger difference in MTR_{asym} (3-5%) exists and may be due to muscle metabolites containing -OH groups, such as glycogen. Future studies include altering glycogen content in muscle by exercise or stimulation to test whether CEST is able to detect changes in glycogen content of muscle.

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