MRS Evaluation of Muscle Glycogen Content in a Mouse Model of Pompe Disease

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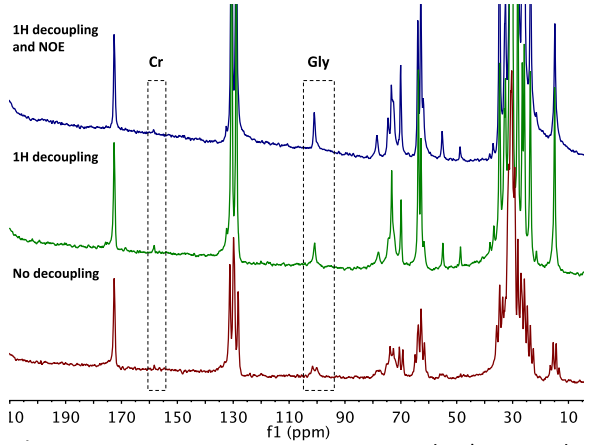
Introduction

Skeletal and smooth muscles of patient with Pompe disease are lacking the enzyme responsible for glycogen degradation, GAA, which leads to a progressive accumulation of undigested glycogen within the lysosome. This excess of glycogen coupled to the impaired autophagic function eventually induces the disruption of the lysosome, interruption of the myofibril, mitochondrial alteration, and muscle degeneration. This phenotype is closely reproduced by the engineered mouse model of the disease, knockout for the Gaa gene, which represents a unique opportunity to study the mechanisms of the disease and the preclinical implementation of therapeutic strategies. This study aims at implementing specific magnetic resonance spectroscopy (MRS) quantitative techniques to determine the time course of glycogen accumulation in skeletal and smooth muscle of GAA knockout mice. Our strategy is to take advantage of the high magnetic field systems available at the NHMFL to improve the sensitivity of the technique.

Experimental

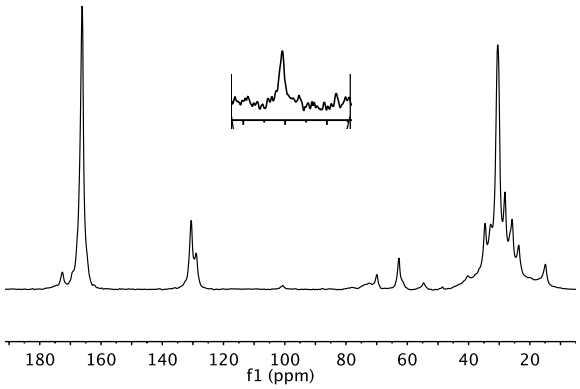
In vitro experiments were performed at 11T, 14T/600MHz, and 17.6/750MHz and in vivo experiments at 11T. In vivo, we used 2 orthogonal single turn surface transmit-receive coils for 1H and 13C. The first step of our work has been to establish a standard solution for the evaluation of coils efficacy and inter field strength comparison. The phantom was made of a 20% intra-lipids emulsion, 200mM oyster Glycogen, 50 mM Creatine, 50 mM Taurine, in order to mimic the basic components of muscle participating in the C13 spectroscopic signal (**Figure 1**). In vivo experiments were performed in the lower limb of 6-month old GAA knockout mice.



sion, glycogen, creatine, taurine) acquired at 11T.

Results and Discussion

In vitro experiments aimed at calibrating the optimal MRS pulse sequences in term of radiofrequency pulses length and power, appropriate repetition time, optimal 1H decoupling power, and number of accumulations required to achieve sufficient signal-to-noise ratio (SNR), to be used as reference parameters for the calibration of the ex vivo and in vivo measures. We calibrated the decoupling and nuclear overhauser effect (NOE) parameters. Our experiments showed that decoupling was possible with our set up at all fields including at 11T. Figure 1 shows the increase in SNR obtained with decoupling and NOE, all other parameters being identical.



$aa^{-/-}$ mouse hindlimb muscle at 11T using ^{1}H decoupling

A major objective was to develop the tools necessary to carry out a quantitative longitudinal study in vivo on wild type (WT) and Gaa-/- mice and monitor the accumulation of glycogen in lower limb skeletal muscles. **Figure 4** shows a typical example of 13C acquisition on a 6 month-old mouse leg. The acquisition time was 90 minutes and formic acid was used as an external standard for quantification.

Conclusions

These preliminary results showed the feasibility of glycogen detection in mice leg at high magnetic field. Further developments will aim at validating a quantification method in vivo, which will ultimately serve as a tool to assess the efficacy of preclinical gene therapy strategies in our model of Pompe disease.

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