In Vivo Metabolomics in Mice with Glycogen Storage 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. In this study we performed *in vivo* metabolomics in gaa$^{-/-}$ mice and wild type mice.

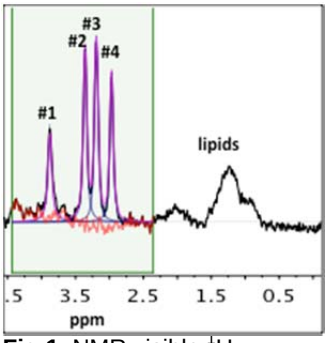


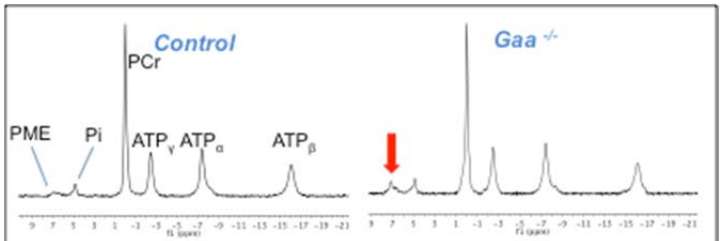
Fig 1. NMR visible ^{1}H metabolites in the mouse gastrocnemius muscle.

Experimental:

We compared resting muscle metabolites in gaa-/- and in control mice using both *in vivo* ^{1}H-MRS (Fig 1) and ^{31}P-MRS (Fig 2). We also measured *in vivo* metabolic fluxes (Fig 3) using electrical stimulation to elicit muscle contractions in the lower limb, and monitored the time course of changes in phosphorylated metabolites (primarily inorganic phosphate (Pi); phosphocreatine (PCr); and intracellular pH (pH$_i$).

Results and Discussion

In gaa$^{-/-}$ and control mice, we acquired water suppressed ^{1}H spectra from a 1x1x3mm^{3} voxel placed within the gastrocnemius muscle (Fig1). We also acquired unsuppressed spectra from the same region for quantitation purpose. Our spectral region of interest, between 2.5 and 4ppm, comprises Cr2 (#1), Taurine/TMA (#2, #3) and Cr3 (#4). We quantified total creatine content in two ways: 1) Cr3 relative to Taurine (#2), (2) Cr3 relative to total muscle water. Both measures showed no statistical difference between gaa$^{-/-}$ and controls, indicating that total levels of creatine are not depleted in gaa$^{-/-}$ muscle at 18 months. Our main finding based on *in vivo* ^{31}P-MRS was an elevation of the signal in the phoshpomonoesters (PME) resonances in the gaa$^{-/-}$ group, possibly due to the accumulation of glucose-6-P. We observed that PME levels were constant with age in control mice but significantly elevated in gaa$^{-/-}$ as early as 6 months of age and progressively increased throughout the lifespan of the animals, up to 18 months. Dynamic ^{31}P measurements revealed that gaa$^{-/-}$ muscles did not show impairment in the *in vivo* mitochondrial oxidative capacity, similar rates of PCr breakdown, and a end of exercise pH$_i$ which was less acidic in gaa$^{-/-}$.



group	pH	[Pi]/[PCr]	[PCr]/[total ATP]	[PME]/[total ATP]
control	7.04 ± 0.05	0.13 ± 0.04	0.67 ± 0.01	0.05 ± 0.02
gaa-/-	7.05 ± 0.02	0.13 ± 0.02	0.61 ± 0.05*	0.08 ± 0.01*
p value			(p=0.02)	(p=0.01)

Fig 2. Resting ^{31}P muscle Metabolites and Intracellular pH

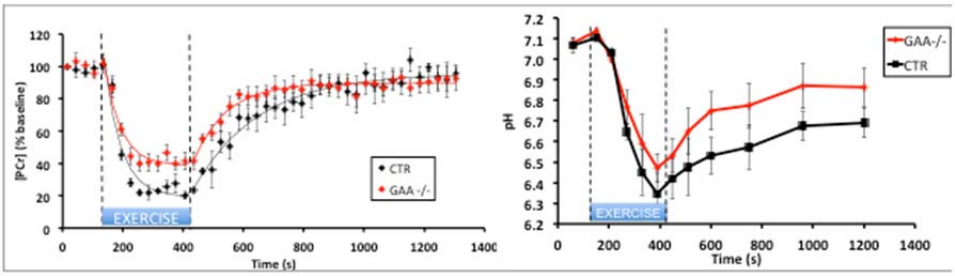


Fig 3. Dynamic changes in PCr and intracellular pH throughout exercise and recovery in control and gaa$^{-/-}$ mice.

Conclusions

In vivo ^{1}H and ^{31}P metabolomics can be used to study differences in a mouse model of Pompe disease.

Acknowledgements

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