

Poster Abstract: Diagnostic

Energy Status in Skeletal Muscle in a Mouse Model of Pompe Disease

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BACKGROUND

Pompe disease (PD) is a rare autosomal recessive disorder caused by the deficiency of the lysosomal enzyme acid alpha glucosidase and results in the accumulation of glycogen in multiple tissues, although the major clinical manifestations are seen in cardiac and skeletal muscle. Enzyme replacement therapy is at present the only approved treatment for PD, but shows limited efficacy in some patients, and does not completely correct the disease phenotype. We have used a mouse model of PD in order to focus on a better understanding of the cellular pathophysiologic mechanisms of the disease. We have studied the effect of glycogen accumulation on the energy status of skeletal muscle fibers.

MATERIALS AND METHODS

Gastrocnemius from Gaa KO mice and wild-type mice were used to analyse different parameters related to energy status in the cell. The content of adenosine-5'-triphosphate (ATP) was quantified by using the luciferin–luciferase reaction method (ATP Determination Kit). Creatine kinase protein expression was assessed by Western blot analysis. The NAD/NADH ratio was assessed using a colorimetric reaction method (NAD/NADH Assay Kit, Abcam). Isolated fibers from flexor digitorum brevis loaded with

Fluo-4 AM were used to analyze calcium signals in response to acetylcholine or caffeine stimulation. Statistical analysis was performed by ANOVA. Data are expressed as mean \pm S.E.M.

RESULTS

We found differences in the content of ATP in fibers isolated from Gaa KO and from wild-type mice. Gaa KO mice ATP content was significantly decreased ($p < 0.001$) vs wild-type mice. We did not find differences in the protein expression of creatine kinase in the gastrocnemius muscle from both genotypes. NAD/NADH ratio, NAD total, and NAD⁺ were significantly increased ($p < 0.05$) in Gaa KO vs wild-type mice. NADH content showed no differences. We analyzed calcium signals induced by acetylcholine or caffeine in isolated fibers and found a rapid increase in intracellular calcium concentration followed by a rapid decline to basal levels in wild-type mice. However, in Gaa KO mice, the results showed a slower increase in intracellular calcium concentration followed by a slower decline to basal levels, suggesting that mechanisms of release of calcium from sarcoplasmic reticulum and mechanisms of calcium removal from cytosol are affected in Gaa KO mice.

CONCLUSIONS

The lower content of ATP and altered ratios of NAD⁺/NADH, together with different calcium transients in fibers with glycogen accumulation, may help to explain the progressive skeletal muscle weakness of patients with PD.

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