Altered mitochondrial oxidative phosphorylation capacity in horses suffering from polysaccharide storage myopathy

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Introduction: Polysaccharide storage myopathy (PSSM) is a widely described cause of exertional rhabdomyolysis that has been found in more than 35 equine breeds. Recent studies identified a dominantly genetic defect in the skeletal muscle glycogen synthase (*GYS1*) enzyme at the basis of the Type-1 PSSM phenotype (McCue *et al*., 2008). The condition is characterized by increased skeletal muscle glycogen concentration, and abnormal polysaccharide accumulation in myofibers. Gene expression studies (Barrey *et al*., 2009) indicated a down-regulation of some mitochondrial genes and we hypothesized that, in type-1 PSSM-affected horses, the energetic production through the oxidative phosphorylation (OXPHOS) in the mitochondria of myofibres might be impaired.

Our aim was to explore the muscle mitochondrial function of PSSM-affected horses using the High Resolution Respirometry, a sensitive diagnostic of the mitochondrial activity recently validated in the equine species (Votion et al., 2010).

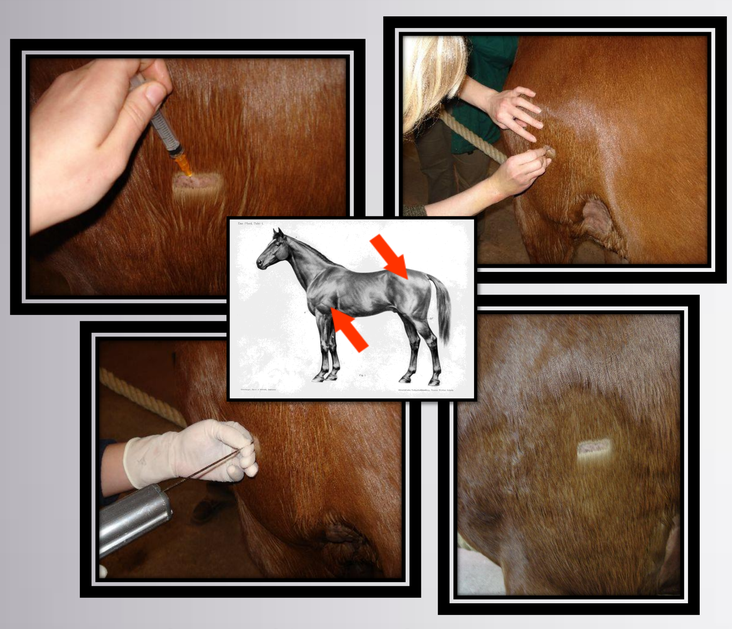


Fig.1. **Muscle microbiopsy technique:** An average 20 mg of muscle tissue was obtained from both the *m. triceps brachii* and *m. gluteus medius* using a 14G biopsy needle mounted on an automatic instrument (Pro-Mag™, Angiotech, USA). Skin was shaved, desensitized and aseptically prepared. Muscle samples were immediately transferred to BIOPS, a preservation solution.

Materials and Methods:

- Eight horses (mean age 8.1 - 4.4 y.o.), different breeds
- History of recurrent exertional rhabdomyolysis, exercise intolerance, increased serum CK/AST
- Specific complementary exams were performed to exclude other possible causes of exercise intolerance
- The presence of the *GYS1* mutation was tested on each horse (EDTA whole blood sent to the Royal Veterinary College of London, England)
- Muscular biopsies were collected on the m. *gluteus medius* and m. *triceps brachii* muscles of each horse using the microbiopsy technique
- Histological analysis and high resolution respirometry (HRR) were realised on the muscle samples

Results: Four horses were tested positive to type-1 PSSM (GYS1 mutation) at the genetic test and were included in the study. Histology revealed the presence of Periodic Acid Schiff (PAS)-positive accumulations of abnormal glycogen. A severe depression of the maximal oxidative phosphorylation capacity was observed (minus 38±14%) by HRR, therefore confirming altered mitochondrial function in type-1 PSSM horses. Surprisingly, one PSSM-positive horse showed very high levels of mitochondrial respiration, even higher than control sport horses.

Results: *m. triceps brachii*

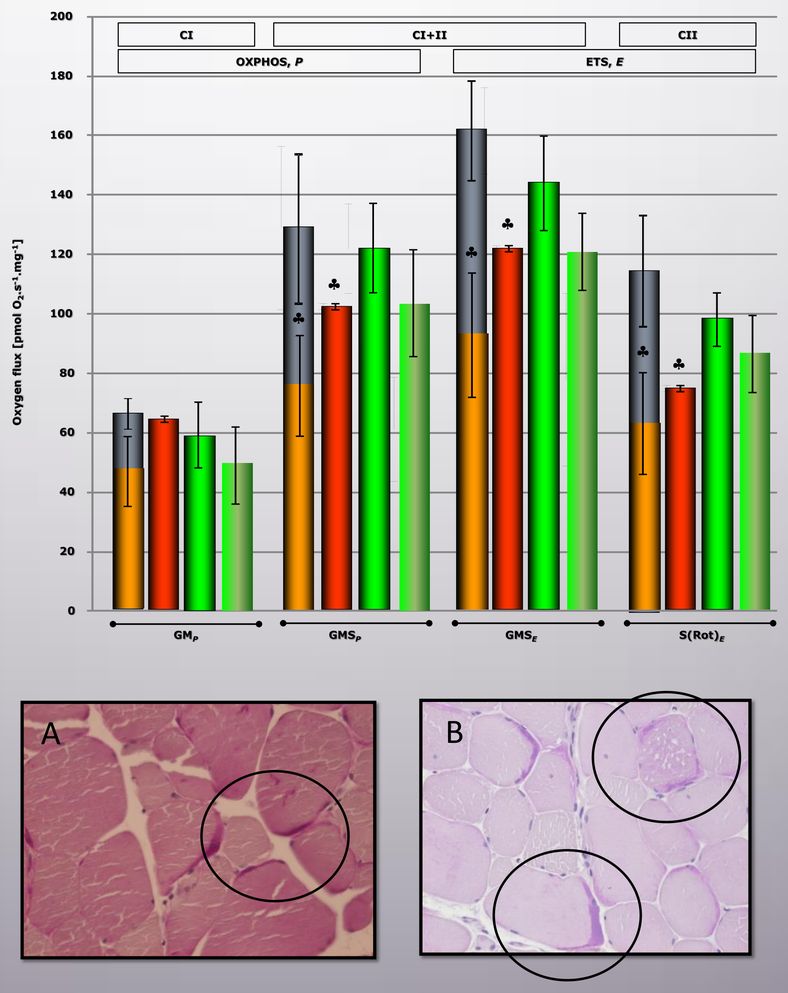
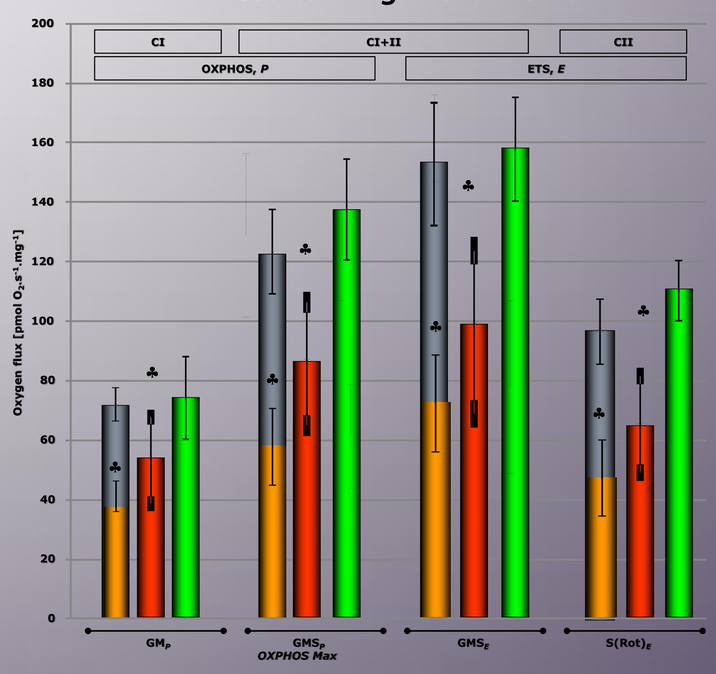


Fig.2. Results of HRR for the *m. triceps brachii* and *m.gluteus medius.*

Results: *m. gluteus medius*



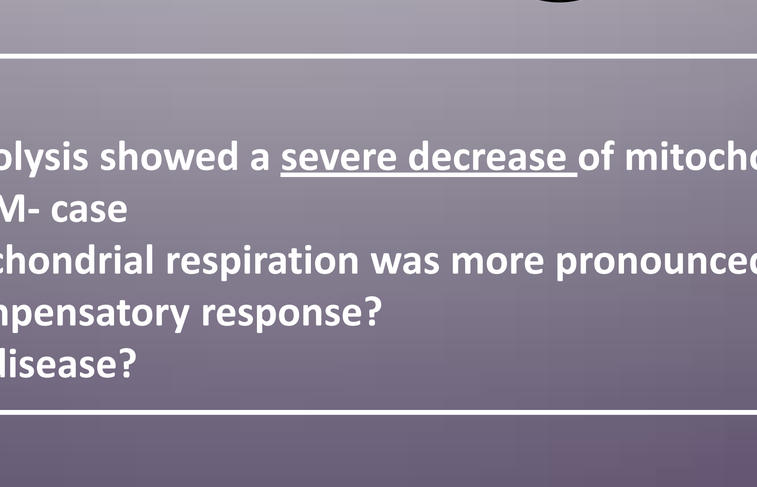


Fig.2. *Gluteus medius* muscle formalin fixed and paraffin embedded. Notice the presence of thick deposits of subsarcolemmal and intracytoplasmic glycogen (circles) with both the ematoxylin-eosin (A) and Periodic Acid Schiff staining (B)

Conclusions

- **All horses suffering from exertional rhabdomyolysis showed a severe decrease of mitochondrial respiration**
- **PSSM+ horses respiration was lower than PSSM- case**
- **In the *m. gluteus medius* the decrease of mitochondrial respiration was more pronounced than in the *m. triceps brachii***
- **Is there a possible mitochondrial recovery/compensatory response?**
- **Equine PSSM is more than a glycogen storage disease?**