Effects of muscle damage on P-31-MRS indices of energetic status and sarcolemma integrity in young mdx mice

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Received: 16 December 2021 | Revised: 27 December 2021 | Accepted: 29 December 2021

DOI: 10.1002/nbm.4688

EDITORIAL

NMR IN BIOMEDICINE WILEY

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The main function of skeletal muscle is to contract, thereby enabling us to, amongst other things, move, breathe and maintain our posture. In skeletal muscle, movement is generated during the so-called cross-bridge cycle, where the thick and thin filaments within the muscle fiber are pulled in opposite directions, and subsequently released due to the binding of adenosine triphosphate (ATP). Skeletal muscle fibers need a constant input of free energy to generate this movement and maintain homeostasis. In a reaction catalyzed by the enzyme creatine kinase (CK), the molecule phosphocreatine (PCr), which is the phosphorylated form of creatine (Cr), provides a fast reservoir for ATP. This reaction is mainly important in tissues with high and fluctuating energy demands, such as skeletal muscle and the brain.[1]

Healthy skeletal muscle typically recovers fully and quickly from minor muscle damage, due to the high regenerative capacity of the tissue. In neuromuscular diseases such as Duchenne muscular dystrophy (DMD), however, this regenerative capacity cannot keep up with the increased demand. In DMD, absence of the dystrophin protein is thought to leave the muscle fiber membrane, the sarcolemma, more vulnerable to damage upon contractions. The chronic nature of this progressive muscle damage induces inflammatory processes, altered cellular energetics[2] and eventual replacement of muscle fibers by fibrotic and fat tissues due to diminished regenerative capacity.[3] There are many pathogenic processes contributing to the chronic skeletal muscle disease cascade in DMD, including functional ischemia, free radical damage, calcium overloading and mitochondrial damage, and several of these overlap.[3] A better understanding of the timing, importance and interdependence of these processes can guide therapeutic approaches.

Quantitative MRI and MRS are playing an important role in understanding the pathophysiology and developing outcome measures in DMD and other neuromuscular diseases.[4] Chemical-shift based imaging techniques are being used to assess fat replacement as a measure for disease progression, and these methods show promise as a surrogate outcome measure or monitoring biomarker (Figure 1).[5] An increase in the water T_2 relaxation time is used as a proxy for the presence of edema and inflammation, while phosphorus MRS (^{31}P-MRS) methods are used to assess cellular energetics and sarcolemma integrity.[4] These latter two methods are therefore more reflective of the disease activity, rather than disease progression. Due to the multi-factorial etiology of the muscle damage in DMD, and in fact in many muscular dystrophies, it is especially important to use multi-parameter approaches to study the temporal order and co-occurrence of pathological features,[6,7] and to assess the origin of observed metabolic changes.[8,9]

In this article by Lopez et al, entitled "Effects of muscle damage on ^{31}P-MRS indices of energetic status and sarcolemma integrity in young *mdx* mice," the authors use a multi-parametric MR approach where they studied muscle T_2 relaxation times and ^{31}P-MRS indices of energetics and sarcolemma integrity in a mouse model of DMD, the *mdx* mouse.[10] They assessed the inorganic phosphate over PCr ratio (P_i/PCr) as a measure for energetic status, assessed the flux of PCr to ATP to determine CK reaction rates and used intracellular magnesium [Mg^{2+}] and tissue pH as a proxy for sarcolemma integrity. The authors used *mdx* and wild type mice at a young age, during the peak of the degeneration/regeneration phase, and quantified these indices in the gastrocnemius and soleus before and 24 h after a downhill running protocol. Compared with wild type mice, *mdx* mice showed an increased water T_2, P_i/PCr and CK reaction rate and reduced Mg^{2+} before downhill running. Water T_2 was highly correlated to the inflammatory marker CD45. One day after the running protocol, water T_2 relaxation times were increased in *mdx*, while they decreased in wild type mice. In the muscles with increased T_2 signal, indicative of muscle damage, localized ^{31}P-MRS measurements showed a clear reduction in Mg^{2+} and increase in tissue pH. Interestingly, these changes were absent when spectra were not specifically localized in damaged regions, and no changes were observed in wild type mice. When all data were combined in a correlation analysis, water T_2, tissue pH and intracellular [Mg^{2+}] were highly correlated in *mdx* mice, indicating that inflammation and compromised sarcolemma integrity co-occur.

The work of Lopez et al clearly indicates the added value of combining different MR measurements, where assessments are co-localized based on pathology, to assess downhill running induced exacerbation of pathology of dystrophic muscle. They very nicely show that the alterations measured by ^{31}P-MRS are indicative of sarcolemma integrity, as they are influenced by muscle damage. As they correctly acknowledge in the discussion, the *mdx* mouse represents a mild phenotype. In DMD patients, fat replacement is a prominent feature, and water T_2 elevations are milder due to the use of corticosteroids.[11] However, in general the ^{31}P-MRS findings align between *mdx* mice and DMD patients, even though

Abbreviations: ^{31}P-MRS, phosphorus MRS; ATP, adenosine triphosphate; CK, creatine kinase; Cr, creatine; DMD, Duchenne muscular dystrophy; Mg^{2+}, free intracellular magnesium; PCr, phosphocreatine; P_i, inorganic phosphate.

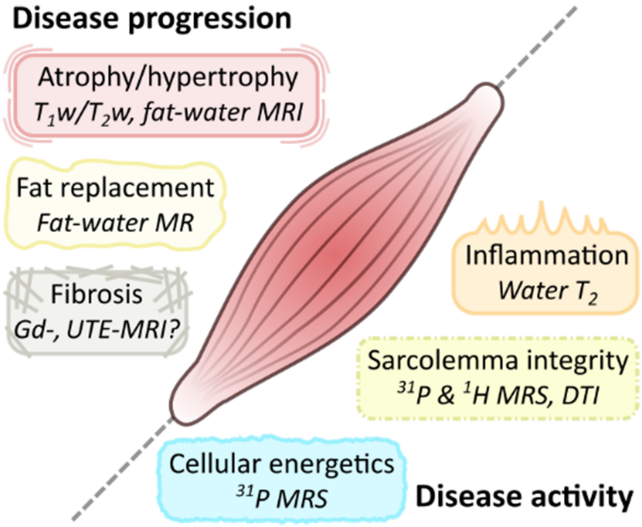

FIGURE 1 Overview of common aspects of the disease cascade in muscular dystrophies, and MR methods used to assess these. Aspects that reflect disease progression are outlined on the left, while features that are more related to disease activity are outlined on the right

the clear increase in phosphodiesters observed in patients[6,9,12] and separation between the P_i compartments[12] are absent from the mouse. As such, this study provides a very good starting point to disentangle the different pathogenic processes in mice, but also in patients, by including ^{31}P-MRS measurements in studies testing therapeutic interventions. A possible application could be to use this multi-modal approach for safety assessments in exercise studies, in combination with water T_2 measurements.[13]

ACKNOWLEDGEMENT

The author would like to thank Dr Maaike van Putten and Dr Donnie Cameron for critical reading of the manuscript and Dr Donnie Cameron for editing the figure.

CONFLICTS OF INTEREST

The author receives research support from Philips Healthcare, and trial support from ImagingDMD-UF outside the submitted work. All reimbursements were received by the LUMC. No personal financial benefits were received.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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