Investigating GSD type III patients with multi-parametric functional NMR imaging and spectroscopy

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INTRODUCTION

Debranching enzyme deficiency, or glycogen storage disorder III (GSD 3) is a rare autosomal recessive disorder, with over 50 identified mutations on the single AGL gene of chromosome 1p21, and has a clinically heterogeneous and "morphing" presentation. Often characterized by liver dysfunction in early childhood, with spontaneous remission at puberty, it then frequently evolves to a slowly progressive myopathy of distal muscles in adult life. Dietary therapy has proved efficient for children presenting liver cases of GSD3, but its effect on later muscle involvement is far from established. The mechanisms of the disease, and in particular of the transition from hepatic to muscular involvement are totally unknown. The present NMR study is included in a wider study aiming at improving characterization, understanding, and ultimately therapeutic approaches of GSD3.

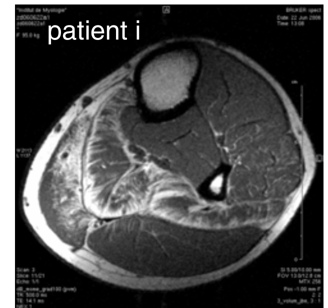


fig. 1

MATERIALS AND METHODS

Patients In all, 11 biochemically confirmed GSD 3 patients, (6F, 5M), aged 12 to 67 underwent NMR examinations. Of these 8 have been included in a prospective protocol (patients d-k) and have undergone as complete explorations as there condition would allow. Retrospective data from 3 other patients (a-c) are also included in the study.

NMR examinations All experiments were run with patients lying supine in a 4T- 46cm free bore magnet, interfaced to a Bruker Biospec Console. When possible, patients underwent three successive experiments:

A Glycogen (Gly) was quantified relative to total creatine (Cr) by static ^{13}C NMRS in all patients, with a 7x10cm surface coil, using a dedicated interleaved T1-weighted sequence to ensure co-localized referencing of both molecules in residual healthy muscle [1].

B Imaging to evaluate degeneration and fatty infiltration of calf muscles was performed with a discrete cosine volume coil covering 18cm of calf muscle with fast spin echo NMRI (TE14/TR500), FOV 13cm^2, mtx 256^2, in 5/11 patients. Positioning images were obtained with the surface coil in all patients, providing some indication of muscle trophicity in all cases.

C Phosphorus metabolites were measured at rest and during recovery from a bout of aerobic plantar flexion exercise by ^{31}P NMRS in 8/11 patients. **Exercise**, was performed on a pneumatic amagnetic ergometer connected to a computer used to program workload and to collect force output, and monitored by online reconstructed ^{31}P NMRS until the subject reached 40% PCr consumption. In 4/11 patients, this was interleaved with a measurement of **perfusion** by ASL-NMRI, as routinely carried out in the laboratory [2]. Aerobic exercise recovery perfusion curves (fig.2) were analysed with a model of 2 sequential exponentials. Three parameters were analysed: value of peak perfusion, delay to peak perfusion (T), and time-constant of perfusion recovery.

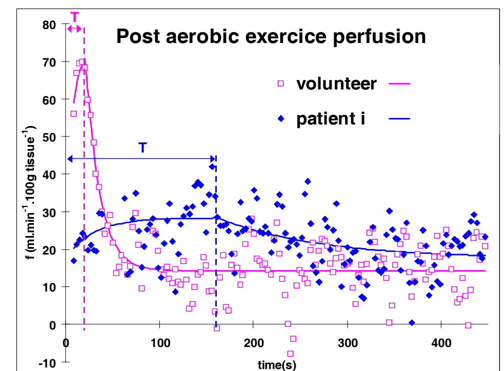


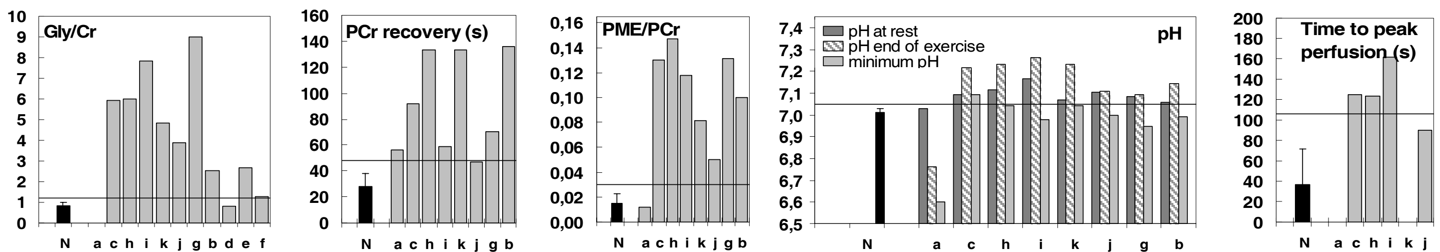
fig. 2

RESULTS

Images demonstrated fatty infiltrations and muscle remodeling in all 10 patients. Muscles were altered in the following decreasing order: soleus, vastus medialis, then lateralis of gastrocnemius, peroneus and tibialis anterior. We graded each muscle between 0 for no visible alteration, to 3.

patient	a	b	c	d	e	f	g	h	i	j	k
age/gender	67/F	40/F	43/F	48/M	36/F	33/M	12/M	19/F	14/M	29/M	21/F
muscle destruction : calf (0-9) / leg (0-15)	- / -	4 / -	1 / 1	5 / -	6 / 6	7 / 11	3 / 5	1 / 3	1 / 3	3 / 4	1 / 3

Parameters which were altered in a majority of patients are charted below, together with laboratory norms and 95% confidence limit. Patients (except a) are sorted according to increasing calf muscle destruction. Work output during exercise was low, ranging from 128 J to 1808 J (N= 1454±617J), but all patients reached at least 38% PCr depletion. Glycogen for (a) was ~4-fold the norm, but not referenced to creatine. Glycogen, though normalised via creatine to residual muscle, correlated inversely with age (R^2=0.62; P=0.007), more than with the defined muscle wasting index (R^2=0.48; P=0.02).



DISCUSSION AND CONCLUSION

As expected for this glycogen storage disorder, and documented on isolated patients, absence of acidosis at exercise [5] and glycogen excess [3, 4] were observed by NMRS, though quantification of glycogen showed a reduction with disease progression. Muscle wasting was also characterized by NMRI, and quantified image analysis could yet improve this index. More unpredictably, the combination of various NMR modalities identified yet unknown abnormalities, such as accumulation of metabolites –possibly glycolytic intermediates– in the PME region at rest (but did not significantly increase at exercise as in GSD VII [6]); impaired oxidative phosphorylation and muscle perfusion which might contribute to symptoms of exercise intolerance. The relation between retarded perfusion responses and altered mitochondrial energetics deserves further investigation.

The intrinsic potential of NMR for multi-parametric functional, biochemical and anatomical investigations is rarely exploited. We show here the wealth of information which NMR offers when suitably tailored to address the question of metabolic dysfunction in muscle. It provides several quantitative indices which improve characterization of this rare disorder, might help evaluating the natural progression of GSD3, and potentially future therapy.

1. Wary C et al., Neuromuscul Disord, 2003, **13**:545-53; 2. Raynaud JS et al., Magn Reson Med, 2001, **46**:305-11; 3. Beckmann N et al., Magn Reson Med, 1990, **16**:150-60; 4. Jehenson P et al., Neuromuscul.Disord, 1991, **1**:99-101. 5. Duboc D et al., Neurology, 1987, **37**:663-71; 6. Argov Z et al., Ann Neurol, 1987, **22**: 46-51.