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

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ABSTRACT

Debranching enzyme deficiency (Glycogen storage disease (GSD) type III) causes progressive muscle wasting myopathy. A comprehensive nuclear magnetic resonance study involving spectroscopy (NMRS) and imaging (NMRI) evaluated status and function of calf muscles in 18 GSDIII patients. At rest, ^{31}P NMRS showed elevated pH and accumulation of anomalous phosphomonoesters, ^{13}C NMRS quantified excess glycogen accumulation and NMRI demonstrated progressive fat replacement that paralleled muscle weakness. Multi-parametric functional NMR, performed at recovery from a single bout of aerobic exercise, simultaneously assessed oxidative phosphorylation from ^{31}P NMRS, muscle perfusion and BOLD, a marker of blood oxygenation, from arterial spin labeled NMRI, and oxygen uptake from deoxymyoglobin proton NMRS. While blocked glycogenolysis caused inadequate substrate supply to the mitochondria, combined measurements suggested that altered perfusion was also responsible for impaired post-exercise phosphocreatine recovery and could contribute to exercise intolerance in GSDIII. These non-invasive investigations provide new indices to quantify the progression of GSDIII.

1. Introduction

Glycogen storage disease type III (GSDIII, Cori – Forbes disease), is a rare autosomal recessive disorder characterized by debranching enzyme deficiency [1,2]. GSDIII patients often suffer from liver dysfunction in early childhood, with hepatomegaly and hypoglycaemic crisis necessitating dietary therapy, generally followed by spontaneous partial remission at puberty. Most commonly (subtype IIIa, 78% patients), the dual enzymatic activity of the debranching enzyme is deficient in both muscle and liver. Myopathy is often present since childhood, possibly overshadowed by the liver disease and not recognized as a problem until the 3rd or 4th decade of life [3]. Phenotypes of GSDIII myopathy are extremely variable [4,5], and unlike myophosphorylase deficiency (GSDV) which precedes it directly in the glycogenolytic pathway, GSDIII is mainly characterized by muscle weakness while GSDV manifests clinically by "pure" exercise intolerance.

In the context of evaluating heterogeneous disease progression, and searching for effective therapies, supplementary indices of disease characterization are invaluable.

Increasingly, genetic and molecular studies are identifying factors which may influence phenotype (for instance [6,7]) and prognosis [8], yet may still not explain clinical variability nor mechanisms of disease evolution.

At the other end of the scope, *in vivo* nuclear magnetic resonance (NMR) offers quantitative assessment of status and function of diseased muscle. Here, we investigated metabolic dysfunction in calf muscle of 18 GSDIII patients by a variety of imaging (NMRI) and spectroscopy (NMRS) modalities, ranging from the more

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conventional anatomical NMRI, phosphorus (^{31}P) or carbon (^{13}C) NMRS, to state-of-the art multi-parametric functional NMRI–NMRS.

2. Materials and methods

2.1. Patients

Patients were followed either at the Institute of Myology (neuromuscular center of Pitié-Salpêtrière Hospital Paris, France) or in the Paediatrics Department of Beclere Hospital (Clamart, France). The NMR study was part of a wider investigation, performed to better characterise muscular involvement of GSDIII patients at all ages.

Eighteen biochemically confirmed GSD III patients, (12F, 6 M), aged 11–67 underwent as complete NMR examinations as their condition would allow. Of these, 17 were included in the clinical prospective evaluation (patients **b**–**r**), but only 15 patients (**d**–**r)** underwent new NMR examinations, while retrospective data from 4 patients (**a**–**c, p**) were included. Patient **a** died in 2006 at age 73 years from pneumonia, patient **b** was excluded from NMR because of increased BMI, patient **c** was claustrophobic and unwilling to be re-examined in NMR, and patient **p**'s condition had deteriorated and he could no longer perform exercise. Patients were unrelated, except for **g**, **h**, **i** who are siblings.

Diagnosis relied on biochemical analysis showing a deficiency in debrancher enzyme activity, by enzymatic assay in muscle (n = 1) or hepatic biopsy (n = 2), erythrocytes (n = 5) and by spectrophotometric assay on leucocytes (n = 10). Clinical evaluation included assessment by the Walton score [9], primarily evaluating lower limb muscle weakness on a scale from normal (grade 0) to bedridden (grade 10), through difficulties in walking (grade 2) and inability to climb the stairs (grade 4).

2.2. NMR examinations

Experiments were run with patients lying supine in a 4T–46 cm, Bruker Biospec system (Bruker BioSpin MRI GmbH, Ettlingen, Germany). The dynamic functional NMR acquisitions were interleaved through the manufacturer's multiscan control (MSC) tool [10].

2.2.1. Glycogen quantitation by ^{13}C NMRS

Glycogen was quantified by natural abundance ^{13}C NMRS in either calf (n = 15) or thigh (n = 3) of patients. Natural abundance ^{13}C signal was collected during 15–20 min, with a 7 × 10 cm surface coil facing muscle, using a dedicated interleaved sequence [11]. This approach ensured colocalized referencing of resonances from the C1 of glycogen (100.5 ppm, Gly) to the C═N resonance of creatine and phosphocreatine (157 ppm, Cr), which serves as a marker of residual muscle tissue [12]. Data are expressed as the ratio of Gly:Cr, measured on individual resonances after suppression of contaminating lipid signals in the time domain and compared to previously established laboratory norms (n = 23) [13].

2.2.2. Muscle degeneration by NMRI

Multi-slice gradient-echo positioning images provided some indication of calf muscle trophicity in all patients. Anatomical fast spin-echo images were acquired in 12 patients, covering 18 cm of the leg (5 mm slices every 10 mm). Additionally, 9 patients benefited from full lower-limb imaging on a recently installed 60 cm free-bore 3 Tesla Siemens TIM system (Siemens Medical, Erlangen, Germany). Images using an axial T1-weighted turbo-spin-echo sequence (TR/TE = 270/10-ms, slice thickness 5 mm every 10 mm, FOV 400 × 198 mm^2, in-plane resolution 0.9 × 0.9 mm^2) were obtained with 5 min acquisition.

Muscle appearance in terms of degeneration and fatty infiltration was graded from 1 (normal) to 4 (severe involvement) for each muscle head, according to Mercuri's classification [14]. Soleus and gastrocnemii scores were summed to give a total/12 for the calf (3 = normal), and added to scores for tibialis anterior and peroneous to give a score/20 for the leg (5 = normal).

2.2.3. Resting muscle energetics by ^{31}P NMRS

Phosphorus metabolites were determined at rest in the calf of 16 patients, with a repetition time TR = 1.5 s and NS = 64 averages. Phosphated metabolites were measured by integrating resonances of phosphocreatine (PCr), inorganic phosphate (Pi), adenosetriphosphate, phosphomonoesters (PME), phosphodiesters, and expressed as ratios. Resting pH was determined from the frequency interval between Pi and PCr [15]. Values were compared to laboratory norms (n = 30) [16].

2.2.4. Dynamic muscle energetic function by ^{31}P NMRS

Muscle acidification during aerobic plantar flexion exercise and efficiency of oxidative phosphorylation at recovery were measured by ^{31}P NMRS (TR = 1.5 s, NS = 4) in 15 patients (including patient **p** on a previous occasion). Exercise (1 plantar flexion/1.5 s) was performed in the magnet, using a computer driven pneumatic ergometer, and monitored by ^{31}P NMRS until the subject depleted the PCr resonance by at least 40%. Data of PCr recovery were measured for 10 min post-exercise and fitted by a mono exponential function to determine the PCr time constant of recovery (τ_{PCr}), which was normalised for end-exercise pH [17].

2.2.5. Muscle perfusion by arterial spin labeled (ASL) NMRI and blood oxygen level dependent (BOLD) signal intensity

Non-invasive measurement of perfusion by ASL-NMRI relies on the magnetic labeling by radiofrequency pulses of arterial water spins to generate time resolved maps of perfusion. We used the variant SATIR [18], to measure post-exercise perfusion recovery in the calf of 11 patients. TR between two images was 1.5 s, during which phosphorus and deoxymyoglobin data were interleaved using multi-parametric functional (*mpf*) NMR [10,19,20], in order to measure all variables during recovery from a single bout of aerobic exercise.

Perfusion images were 6-mm-thick axial views of the leg acquired with single-shot fast spin-echo, alternately following slice-selective (SS) or non selective (NS) labelling modules. Evolution time of magnetization between labelling and imaging was Tev 1.3 s.

Concurrently, normalised sums of signal intensity from SS and NS images procured a T2-weighted measure which depends on the combined effects of capillary blood oxygen level (BOLD), flow and volume [21,22]. Though complex to interpret [23,24], this contrast is a further indicator of muscle function [25].

Perfusion was calculated analytically [18] from signal intensity measured in regions of interest (ROIs) in gastrocnemii muscles. Perfusion curves constructed as a function of time were filtered using a Savitzky-Golay moving polynomial, (http://www.bowdoin.edu/∼rdelevie/excellaneous). Measured perfusion parameters at recovery were: time (T_{max}) to maximum peak perfusion (f_{max}) and the area under the curve (f.t) measured in 15 s-steps throughout 9 min of recovery.

The same ROIs were used for BOLD signal, temporal curves constructed, and parameters were Delta-BOLD and $T_{maxBOLD}$: the amplitude and time to maximum signal increase post-exercise.

2.2.6. Muscle oxygen uptake, from deoxymyoglobin (dMb) ^{1}H NMRS

Myoglobin, the intramyo-cellular oxygen carrier, de-oxygenates in exercising muscle [26]. ^{1}H NMRS measurement of dMb was interleaved into the same acquisition sequence as perfusion, BOLD and ^{31}P NMRS, [27]. In patients for whom dMb increased above