Metabolic alterations in exercising muscle of patients with Becker Muscular Dystophy assessed by 31P MRS

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Introduction

Phosphorous Magnetic Resonance Spectroscopy (31P MRS) is a non-invasive tool to investigate the skeletal muscle metabolism in different physiological conditions (rest, exercise and recovery) through the measure of high energy phosphate metabolites such as ATP, Phosphocreatine (PCr), inorganic Phosphate (Pi), Phosphodiesters (PDE) and Phosphomonoesters (PME) and cytosolic pH.

Becker Muscular Dystrophy (BMD) is an X-linked skeletal muscle disorder characterized by a reduced content and/or size of dystrophin in the muscle fibre. Dystrophin is a subsarcolemmal protein necessary for the muscle fibre resistance to mechanical stress. The main symptom of this disorder is a progressive reduction of muscle strength associated with structured contractures: the clinical spectrum of the muscle weakness is very wide, ranging from nearly asymptomatic to rapidly, in second decade of life, wheelchair bound patients.

Up to date in literature just few studies on the exploration of metabolic alterations in dystrophinopathic muscle measured by 31P MRS have been reported [1,2,3]. Furthermore the role of the lack of dystrophin in the determination of the metabolic effect revealed by 31P MRS is not yet clear. The aim of this study was to compare 31P MRS data obtained in a sample of BMD patients with those obtained in healthy age-matched controls at rest, at the end of a short-term isometric intermittent incremental exercise normalized to the Maximum Voluntary Contraction (MVC) and during the recovery from the effort. In particularly the goal was to investigate if the dystrophin deficit accounts for an impairment of the BMD skeletal muscle metabolism.

Materials and methods

31P MR spectra of 9 male BMD patients (median age 29 years, range 24-44) were compared with those obtained in 10 healthy male volunteers (median age 30 years, range 24-34). Data were acquired using a 1.5 T MR system (LX Signa Horizon 1.5 GE Healthcare, Milwaukee, WI, USA) operating at 25.86 MHz, with a transmit/receive spectroscopy coil centred in the middle of the gastrocnemius. A short TE slice selective spin echo sequence (8 cm slab) was used both for the spectrum at rest and for the data set sampled during the exercise-recovery protocol (Repetion Time TR of 4 sec, flip angle FA of 60°, phase cycling 180°, 2 NEX, 128 signals, acquisition time of about 8 min, spectral width of 2500 Hz, 2048 complex data points). Patients and volunteers performed a foot plantar flexor exercise using a MR compatible ergometer [4] that permits to register the flexion amplitude and to calculate the work spent from the calf in each exercise beat. The exercise beat was performed at a frequency of 0,75Hz with an isometric intermittent incremental workload starting from 20% of the MVC and gradually increased by 10% every 30 seconds until the exhaustion.

Signals were processed using jMRUI [5] and quantified in the time-domain by AMARES [6]. For the data at rest, the relative concentration of PME, Pi, PDE, PCr, were computed using the β-ATP signal as internal reference (supposed at a concentration of 8.2mM). The 64 couples of signals acquired during the exercise-recovery protocol were analyzed individually and the relative changes in the amplitude of the Pi and PCr were evaluated.

The pH was calculated by the tritation curve: $pH=6.75+\log_{10}[(3.27-\delta_{Pi-PCr})/(\delta_{Pi-PCr}-5.69)]$ were δ_{Pi-PCr} is the relative chemical shift of Pi and PCr.

The PCr recovery was fitted through the mono-exponential function $PCr(t) = PCr_{end-ex} + (PCr_{\infty}-PCr_{end-ex})(1-e^{-t/\tau})$ where PCr_{end-ex} and PCr_{∞} respectively are the PCr level at the end of the exercise and of the recovery and τ is the time constant of the function.

The validity of the mono-exponential model [7] was assessed through the *a posteriori* analysis of fit residuals. Model was rejected whenever the chi-square value exceeded the 95% threshold for the degree of freedom of the fit, or residuals were characterised by a strong non-random behaviour.

In those cases in which the mono-exponential model resulted valid, the initial rate of recovery V was calculated from the expression $V=PCr_{rest}[mM]\ (1 - PCr_{end-ex}/PCr_{\infty})/\tau$, assuming a PCr concentration at the end of the recovery equal to that measured at rest.

Results and Discussion

Resting muscle: our results showed a significant increase of the pH (pH_{rest}) in BMD patients as already described [1,2,3], but non-significant differences in Pi and PCr basal concentrations or in their ratio Pi/PCr_{rest} (see Table where * means that the two sample t-test gives $p<0.05$).

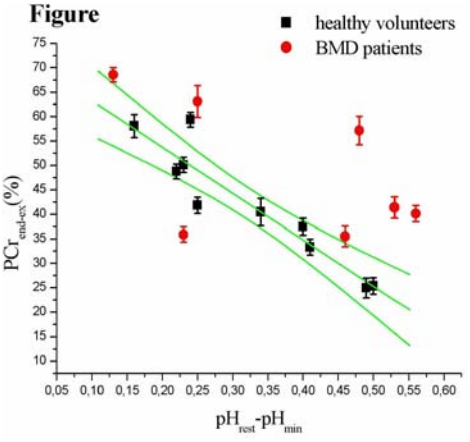
	Subjects	pH_{rest} *	PCr_{rest}[mM]	Pi_{rest}[mM]	Pi/PCr_{rest}	PME_{rest}[mM]	PDE_{rest}[mM]
Healthy Volunteers	10	7.03±0.03	37.5±4.9	6.4±1.0	0.171±0.027	2.4±1.1	6.0±2.1
BMD patients	9	7.07±0.02	43.3±7.9	7.0±1.4	0.162±0.024	3.6±1.9	7.7±1.9
Two samples t-test (p)		-3.39 (0.003)	-1.94 (0.069)	-1.14 (0.270)	0.754 (0.461)	-1.68 (0.112)	-1.86 (0.081)

Exercise: two out of the 9 BMD patients were unable to perform the exercise correctly. Clinical features of the remaining 7 BMD patients accounted for a mild phenotype. At the end of the exercise BMD patients showed a wide pattern of acidification (see Figure). A strong linear correlation ($R=0.961$, $p<0.000$) between the $PCr_{end-ex}(\%)$ (the PCr_{end-ex} in percentage respect to PCr_{∞}) and the acidification index (pH_{rest}-pH_{min}) was found in normal subjects, meanwhile all BMD patients lied outside the 95% confidence band of the linear fit. However 6 out of the 7 BMD patients showed a higher level of acidification index respect to the healthy subjects, under the same condition of PCr consumption. Only one patient exhibited a lower acidification degree as previously reported in literature [3].

Recovery from exercise: in the PCr recovery analysis no difference was observed in the values of V (mean±SD) between the 6 BMD patients and 7 controls for which the mono-exponential model was valid. These values were V_{BMD}= 34±8[mM]/min and $V_{controls}$=37±13[mM]/min.

Possible explanations for the metabolic pattern found in our BMD patients could be the presence of a defective consumption of PCr in concomitance of an excessive activation of the lactate production in mildly affected patients. An anticipated shift to anaerobic ATP synthesis during the incremental exercise, due to impaired blood flow in response to exercise, as previously reported in BMD [8], could have further contributed to our results.

Figure



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