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Mitochondrial dysfunction in myotonic dystrophy type 1

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Abstract

The pathophysiological mechanism linking the nucleotide expansion in the *DMPK* gene to the clinical manifestations of myotonic dystrophy type 1 (DM1) is still unclear. *In vitro* studies demonstrate DMPK involvement in the redox homeostasis of cells and the mitochondrial dysfunction in DM1, but *in vivo* investigations of oxidative metabolism in skeletal muscle have provided ambiguous results and have never been performed in the brain. Twenty-five DM1 patients (14M, 39 ± 11years) underwent brain proton MR spectroscopy (^{1}H-MRS), and sixteen cases (9M, 40 ± 13 years old) also calf muscle phosphorus MRS (^{31}P-MRS). Findings were compared to those of sex- and age-matched controls. Eight DM1 patients showed pathological increase of brain lactate and, compared to those without, had larger lateral ventricles ($p < 0.01$), smaller gray matter volumes ($p < 0.05$) and higher white matter lesion load ($p < 0.05$). A reduction of phosphocreatine/inorganic phosphate ($p < 0.001$) at rest and, at first minute of exercise, a lower [phosphocreatine] ($p = 0.003$) and greater [ADP] ($p = 0.004$) were found in DM1 patients compared to controls. The post-exercise indices of muscle oxidative metabolism were all impaired in DM1, including the increase of time constant of phosphocreatine resynthesis (TC PCr, $p = 0.038$) and the reduction of the maximum rate of mitochondrial ATP synthesis ($p = 0.033$). TC PCr values correlated with the myotonic area score ($\rho = 0.74$, $p = 0.01$) indicating higher impairment of muscle oxidative metabolism in clinically more affected patients. Our findings provide clear *in vivo* evidence of multisystem impairment of oxidative metabolism in DM1 patients, providing a rationale for targeted treatment enhancing energy metabolism.

Keywords: Myotonic dystrophy type 1; Magnetic resonance imaging (MRI); Muscle phosphorous MR spectroscopy; Brain proton MR spectroscopy

1. Introduction

Myotonic dystrophy type 1 (DM1) is the most common form of autosomal dominant muscular dystrophy due to an expansion of an unstable CTG-repeat in the 3'-untranslated region of the myotonic dystrophy protein kinase (*DMPK*) gene [1]. Based on triplet expansion, four categories (E1-E4) are distinguished, and related to the severity of clinical presentation [2].

To link the nucleotide expansion in the *DMPK* gene to the multisystem involvement characterizing the DM1 adult form, several pathophysiological hypotheses have been developed [3]. Among them, robust evidence suggests that mitochondrial dysfunction is crucial in the pathophysiology of DM1. *In vitro* studies attested the role of DMPK in the cell's redox homeostasis [4], and an increased susceptibility to oxidative stress in a model of CTG repeat in the myotonin protein kinase gene [5]. Moreover, signs of mitochondrial alteration in muscle biopsy and plasmatic markers of oxidative stress have been detected in DM1 patients [6].

Proton MR spectroscopy (^{1}H-MRS) is a non-invasive technique sensitive to *in vivo* brain oxidative metabolism, detecting pathological accumulation of lactate (Lac) in primary [7] or secondary mitochondrial oxidative impairment [8]. Similarly, phosphorous MRS (^{31}P-MRS) is able to detect *in vivo* skeletal muscle impairment of oxidative mitochondrial metabolism due to mitochondrial DNA mutations [9,10] or

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other genetic neurodegenerative disorders [11,12]. Results of previous skeletal muscle ^{31}P-MRS studies of DM patients without molecular confirmation were ambiguous, in that impairment of mitochondrial oxidative metabolism was detected in the forearm flexor digitorum muscles but not in the calf muscles [13].

We investigated the role of mitochondrial dysfunction in the pathogenesis of DM1 by assessing *in vivo* skeletal muscle and brain oxidative metabolism using proton and phosphorus MRS.

2. Materials and methods

2.1. Subjects

Twenty-five DM1 patients (14 males and 11 females, mean age ± SD = 39 ± 11 years, range = 22–71years), twenty-four part of a previous neuroimaging study [14], were recruited from the IRCCS Institute of Neurological Sciences of Bologna (Table 1). Genetic diagnosis was performed quantifying the size of CTG repeats in peripheral leucocytes [2].

Clinical evaluation, including the MRC calf muscle strength score [15], the Muscular Impairment Rating Scale (MIRS) score [16] and the DM1 functional scale [17] was performed by a neurologist expert in neuromuscular disorders (RLi).

All DM1 patients were scanned in a 1.5T *GE® Medical Systems Signa HDx 15* system at the Functional MR Unit (S. Orsola-Malpighi Hospital of Bologna), following a protocol including brain MRI and single voxel ^{1}H-MRS within the lateral ventricles, to maximize Lac detection [18]. We did not acquire proton MR spectra from healthy controls, as previous studies showed that under resting conditions the CSF Lac content is far below the threshold of detectability for ^{1}H-MRS at 1.5T, in both the intracellular and extracellular compartments [19].

Sixteen patients (9 males and 7 females, mean age ± SD = 40 ± 13years, range: 22–71years) also underwent ^{31}P-MRS examination, and 11 (7 males and 4 females, mean age ± SD = 36 ± 9 years, range: 22–49 years) of them were able to performed an aerobic exercise of adequate intensity. In Table 1 the demographic and clinical data of all sub-sets of patients are reported (Table 1).

For skeletal muscle ^{31}P-MRS, 14 healthy controls (7 males and 7 females, mean age = 34 ± 9 years, range = 22–52years) were recruited among Hospital and University workers. None was a trained athlete or had a personal or family history of neuromuscular disorders or other medical conditions that could influence muscle oxidative metabolism.

The study was approved by the Local Hospital Ethics Committee, and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

2.1.1. Skeletal muscle ^{31}P-MRS acquisition protocol

^{31}P-MRS investigations were performed using an 8-cm diameter surface coil placed under the right calf [20]. Muscle spectra were acquired, with a repetition time (TR) of 5 s, at rest (128 scan spectrum), during an aerobic incremental exercise (12 scan spectra), and the following recovery (32 two-scan spectra). The muscle was exercised by plantar flexion at 0.66 Hz against a pedal using a pneumatic ergometer. The force resistance of the pedal was 10% of lean body mass calculated from body weight and skin fold thickness [21]. After the acquisition of two spectra, corresponding to the first 2 min of exercise, the resistance was increased by 5% of lean body mass for each subsequent 1 min spectral acquisition. As soon as the last 12 scan exercise spectrum was collected the exercise was stopped and data collected for 5′20″

Calculation of lean body mass from body weight and skin fold thickness tends to overestimate it in patients with degenerative muscular disorder, as this method cannot allow for fat replacement of degenerated muscle fibers. Therefore, a relatively heavier load might be expected in our DM1 patients that may explain, at least partially, the shorter exercise duration compared to healthy controls. The effect of a relative heavier load on DM1 patients' exercise and recovery data is addressed in the Discussion.

2.1.2. Brain MRI/^{1}H-MRS acquisition protocol

Brain MR studies were performed in the same scanner, using a quadrature birdcage head coil. All patients underwent a standardized MR protocol [14] and ^{1}H spectra were acquired using the Point RESolved Spectroscopy (PRESS) sequence.

Table 1
Demographic and clinical data of DM1 patients.

		Brain ^{1}H MRS	Muscle ^{31}P MRS		
			All	*With exercise*	*Only rest*
No. of patients		25	16	11	5
M/F		14/11	9/7	7/4	2/3
Age (years)	mean (SD)	39 (11)	40 (13)	36 (9)	49 (13)
Age at onset (years)	mean (SD)	21 (13)	20 (12)	19 (12)	16 (20)
Disease duration (years)	mean (SD)	16 (11)	14 (10)	14 (11)	28 (9)
Calf muscle MRC score	median (IQR)	4 (1)	4 (1)	5 (1)	4 (0.5)
MIRS scale score	median (IQR)	4 (1)	3.5 (1.75)	3 (2)	4 (0.5)
DM1 functional scale score	median (IQR)	23 (13)	24 (17)	23 (12)	31 (5)
Neuropsychological area	median (IQR)	3 (4)	3 (5)	3 (5)	5 (5)
Motricity area	median (IQR)	11 (5)	11 (9)	10 (9)	12 (6)
Myotonia area	median (IQR)	7 (2)	7 (3)	7 (3)	7 (1)
Daily life area	median (IQR)	3 (4)	3 (3)	2 (1)	5 (0)

MRC: Medical Research Council Scale [15]; MIRS: muscle impairment ratings scale [16]; SD: standard deviation; IQR: interquartile range.