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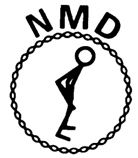
Neuromuscular Disorders 10 (2000) 430–435



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Mitochondrial impairment of human muscle in Friedreich ataxia in vivo ☆

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Received 6 October 1999; received in revised form 31 January 2000; accepted 14 February 2000

Abstract

Friedreich ataxia occurs due to mutations in the gene encoding the mitochondrial protein frataxin. This ^{31}P magnetic resonance spectroscopy study on the calf muscle of Friedreich ataxia patients provides in vivo evidence of a severe impairment of mitochondrial function. Mitochondrial adenosine triphosphate resynthesis was studied by means of the post-exercise recovery of phosphocreatine. After ischemic exercise in calf muscles of all patients, phosphocreatine recovery was dramatically delayed. Time constants of recovery correlated with mutations of the *frataxin* gene, the age of the patients, and disease duration. ^{31}P magnetic resonance spectroscopy represents the first expedient tool for monitoring therapeutic trials in Friedreich ataxia non-invasively. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Friedreich ataxia; Skeletal muscle; Oxidative phosphorylation; Mitochondrial function; Magnetic resonance spectroscopy

1. Introduction

Friedreich ataxia (FRDA) is the most common hereditary ataxia occurring with a frequency of about 1 in 50 000 in the Caucasian population. The disease onset is usually within the second decade of life. Major clinical findings include progressive ataxia of limbs and gait, dysarthria, loss of tendon reflexes, pyramidal weakness, extensor plantar responses, impaired position sense and predominantly sensory axonal neuropathy in association with hypertrophic cardiomyopathy, kyphoscoliosis and increased incidence of diabetes mellitus.

FRDA is an autosomal recessive degenerative disorder that primarily affects dorsal root ganglion cells, dorsal columns, spinocerebellar and pyramidal tracts of the spinal cord as well as the heart and pancreas. In about 98% of FRDA chromosomes the disease-causing mutations consist of an expanded $(GAA)_n$ trinucleotide repeat in intron 1 of the *frataxin* gene on chromosome 9q13. The repeat expansion causes reduced transcription of the *frataxin* gene and therefore decreased expression of its protein product. Frataxin comprises 210 amino acids and is associated with the inner mitochondrial membrane [1]. In adult human tissues, frataxin mRNA is predominantly expressed in tissues with high metabolic rates and it is most abundant in heart, liver, skeletal muscle and pancreas. In the central nervous system (CNS), it is highly expressed in the spinal cord and at lower levels in the cerebellum [2–4]. Frataxin is a highly conserved protein with homologues in mouse, *Caenorhabditis elegans* and *Saccharomyces cerevisiae*. Knockout of the yeast *frataxin* homologue (Δyfh1) leads to mitochondrial iron accumulation [5]. Increased iron is thought to stimulate toxic free radical generation causing mitochondrial damage. Moreover, mitochondrial dysfunction has been observed in heart biopsies from two FRDA patients when rations of respiratory chain complex activities were used [6]. This indicates that the pathomechanism in human FRDA may be quite similar as in the yeast-depleted *frataxin* homologue.

An important barrier to characterize disease causing mechanisms in human FRDA has been the quantification of mitochondrial dysfunction in vivo. ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) offers non-invasive measurement of muscle bioenergetics and permits the

☆ Presented in part at the Fifth Annual Meeting of the German Neurogenetics Society, Bonn, Germany, 17–18 September, 1999, and at the Fourth International Congress of the World Muscle Society, Antalya, Turkey, 14–16 October, 1999.

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PII: S0960-8966(00)00108-5

continuous monitoring of kinetic changes of phosphocreatine (PCr), inorganic phosphate (P_i), ADP, ATP, sugar phosphates and pH during exercise. Post-exercise PCr resynthesis directly depends on mitochondrial ATP synthesis and thus reflects mitochondrial functioning in vivo [7]. In this paper, we present in vivo experiments to test the hypothesis that the mitochondrial metabolism is impaired in human skeletal muscle of patients with FRDA.

2. Patients and methods

2.1. Subjects

Eight ambulatory FRDA patients from seven families (three male, five female; age 34 ± 11, range 19–54 years; Table 1) homozygous for the $(GAA)_n$ repeat expansion in the *frataxin* gene were included in this study. Genetic testing for FRDA was performed from peripheral blood lymphocytes as previously described [8]. The control group consisted of 15 healthy subjects (seven male, eight female, age 34 ± 11, range 16–63 years). Furthermore, four ambulatory male patients with genetically confirmed X-chromosomal recessive Becker's muscular dystrophy (BMD) with similar age (29 ± 4, range 24–32 years) and degree of motor handicap were examined as a disease control group. None of the patients and controls were taking medication at the time of investigation. All subjects gave their written informed consent. The study was approved by the ethics committee of the Ruhr University Bochum.

2.2. The standardized calf muscle metabolism test

The subjects lay supine with the upper body elevated by 25°. The foot was fixed on a pedal ergometer designed for isometric foot plantar flexion exercise. The angle between the pedal and the horizontal plane was 70°. Force was continuously recorded and displayed to the patient. The right calf was placed on a calf holder with integrated radio-frequency surface coil. Maximum voluntary test force (MTF) was determined as the best of three contractions of 5 s duration with 1 min recovery in between.

Energy metabolism was monitored during the following exercise protocol: 1 min rest, 3 min 30% MTF, 7 min rest, 3 min rest + arterial occlusion, 3 min 30% MFT + arterial occlusion, 10 min rest. Arterial occlusion was applied using an air cuff placed around the lower part of the right thigh. For the application of ischemia the cuff was rapidly inflated to 280–300 mmHg using compressed air. At the end of contraction or in case of emergency, the cuff could be deflated within seconds by the subject or the obligatorily accompanying person.

Table 1
Clinical characteristics of FRDA patients[a]

Parameter	Patients
Age (years)	34 ± 11 (19–54)
Age of disease onset (years)	20 ± 8 (12–36)
Disease duration (years)	13 ± 7 (6–25)
GAA1	424 ± 265 (139–808)
GAA2	861 ± 161(524–1011)

[a] Values are given as mean ± SD (range). GAA1, GAA repeat lengths of the smaller allele of each patient; GAA2, GAA repeat length of the larger alleles.

2.3. ^{31}P magnetic resonance spectroscopy and energy metabolism

The MR spectra of the calf were obtained in a 4.7 Tesla 40-cm horizontal bore spectrometer (Bruker-Biospec 47/40, Bruker-Medizintechnik, Karlsruhe, Germany) using a 5-cm diameter $^1H/^{31}P$ surface coil, placed under the right calf. The resonance frequencies were 200 MHz for ^1H and 81 MHz for ^{31}P. ^1H MR spectra were used to optimize magnetic field homogeneity (shimming). A line half-width lower than 48 Hz for the water signal was accepted. In most examinations a value of 39 Hz was reached. A pulse length of 100 μs was used for ^{31}P MR spectroscopy. The flip angle was 60° at the centre of the coil. For each spectrum, 32 free induction decays (FIDs) were acquired in 10 s. The vector size was 4096 data points. Sweep width was 10 kHz. In patients with BMD half-widths of the water signal were between 53 and 70 Hz because of large magnetic field inhomogeneities. Due to a poor signal-to-noise ratio received from these patients, 64 FIDs were accumulated within 20 s for each spectrum. Spectra were evaluated for PCr, P_i, the levels of the beta positions of the phosphate atoms in ATP, and for phosphomonoesters (PME). The area under each peak was corrected for partial spin saturation.

Metabolite concentrations were generally given in percent of [PCr] at initial rest (%$[PCr]_i$). The intracellular pH was determined by the chemical shift of the P_i-peak (δ in ppm) relative to PCr: $[pH = 6.75 + \log((\delta - 3.27)/(5.69 - \delta))]$ [9]. The resonance frequency of the PCr signal was defined as 0 ppm.

2.4. Statistical data evaluation

Data are expressed as mean values ± standard deviations (range minimum to maximum value). For statistical data evaluation the appropriate Student's *t*-test was used. In each analysis $P < 0.05$ was considered significant. If the minimum and maximum values in the patient and control cohort were non-overlapping, the mean values were called absolutely different. For correlation studies the Pearson correlation coefficient was applied.

3. Results

3.1. ^{31}P-MRS parameter

All subjects and patients performed as given an exercise

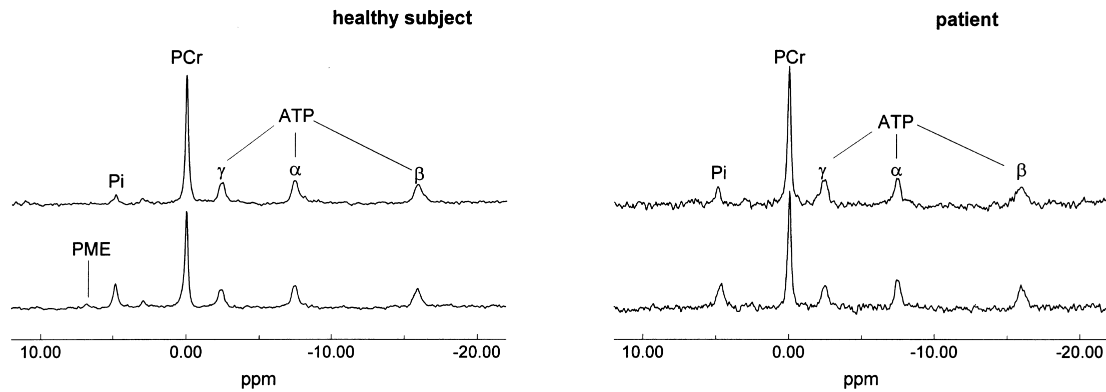

Fig. 1. ^{31}P MR spectra from a healthy (left) and a diseased (right) calf muscle at rest (upper spectrum) and after 3 min isometric foot plantar flexion at 30% maximum voluntary test force under arterial occlusion (lower spectrum). PME, phosphomonoesters; P_i, inorganic phosphate; PCr, phosphocreatine; ATP-γ, -α, -β, adenosine triphosphate.

protocol containing two steps of 3-min isometric plantarflexion at 30% maximum test force with 10 min rest in between. The first contraction was performed under natural perfusion the second under applied arterial occlusion. Maximum test forces of isometric foot plantar flexion performed by healthy subjects were 959 ± 238 (655–1587) N. The patients with BMD performed 1113.5 ± 68.6 (1021–1184) N. The patients with FRDA exhibited lower maximum test forces of 722 ± 365 (326–1334) N. However, this difference was only of borderline significance ($P = 0.07$ compared with healthy subjects and $P = 0.06$ for BMD patients, respectively).

Typical spectra of FRDA patients and healthy subjects are given in Fig. 1. In both groups, patients and control subjects, ATP levels in calf muscles remained constant during all phases of the exercise protocol.

Corresponding with the lower absolute force levels, the depletion of PCr during exercise was lower in FRDA patients than in healthy subjects (see Fig. 2). During ischemic contraction this difference was significant ($P = 0.003$). In BMD patients and in healthy subjects PCr depletion was almost the same during both muscle exercise steps (see Fig. 3). Furthermore, muscle acidification at the end of ischemic contraction was significantly lower in FRDA patients than in controls (−0.05 ± 0.06 in FRDA patients, −0.11 ± 0.05 in healthy controls, $P = 0.0003$; and −0.10 ± 0.06 in BMD patients, $P = 0.035$, see Table 2).

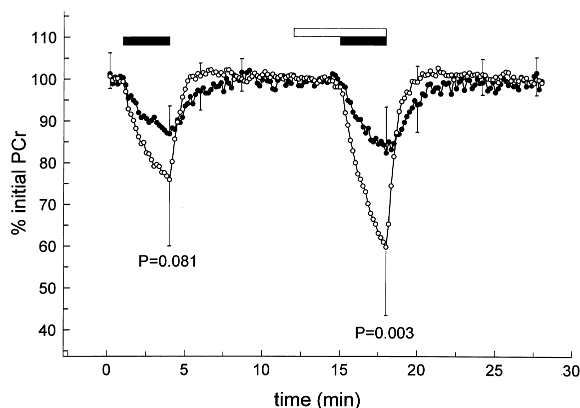

Fig. 2. Consumption of phosphocreatine (PCr) during aerobic and ischemic muscle contraction in FRDA patients. Open circles, healthy subjects (mean ± SD, $n = 15$); closed circles, FRDA patients (mean ± SD, $n = 8$); closed bar, phase with isometric calf muscle contraction (30% maximum test force); open bar, phase with applied arterial occlusion (280–300 mmHg).

Corresponding to the decrease in PCr, the increase in inorganic phosphate was lower in FRDA patients compared with the healthy and the BMD subjects. Only a slight increase in phosphomonoesters was observed during both contraction phases, without significant differences between the three groups.

After muscle contraction oxidative recovery of PCr was severely delayed in patient muscles. After aerobic contraction the values of the time constants (τ) describing the mono-exponential time course of PCr recovery were 79 ± 34 s (38–132 s) in FRDA patients and 26 ± 9 s (16–48 s) in healthy subjects ($P = 0.012$, see Table 2). In BMD patients τ-values were also increased compared with healthy controls (62 ± 5 s, 55–66 s, $P = 0.001$) but they were not significantly different from FRDA patients ($P = 0.271$). After ischemic contraction time constants τ were prolonged in all FRDA patients (119 ± 49 s, 67–194 s) without overlap with the control group (32 ± 11 s, 19–58 s; $P = 0.001$) and significantly different from BMD patients (57 ± 12 s, 42–72 s, $P = 0.008$, see Table 2)

3.2. Correlation of ^{31}P-MRS parameter with clinical and genetic findings

Values of τ after ischemic exercise correlated directly with the repeat length of the smaller allele GAA1 ($r = 0.837$, $P = 0.01$). Furthermore, an inverse correlation was identified with the age of the patients ($r = -0.832$, $P = 0.01$) as well as with disease duration ($r = -0.909$, $P = 0.002$, see Fig. 4). Correlations of τ with further para-

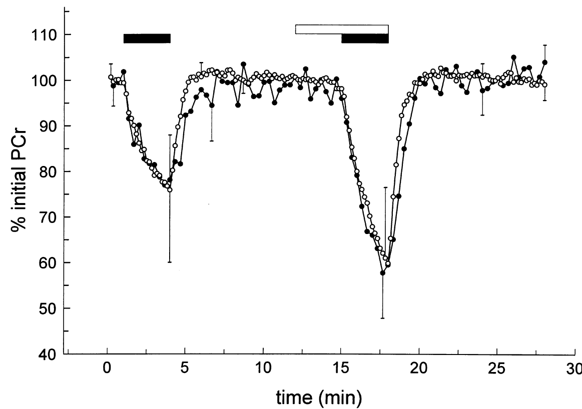

Fig. 3. Consumption of phosphocreatine (PCr) during aerobic and ischemic muscle contraction in BMD patients. Open circles, healthy subjects (mean ± SD, $n = 15$); closed circles, BMD patients (mean ± SD, $n = 4$); closed bar, phase with isometric calf muscle contraction (30% maximum test force); open bar, phase with applied arterial occlusion (280–300 mmHg).

meters (age of disease onset, GAA2, maximum test force, end-exercise [PCr], end-exercise pH) were not significant ($P > 0.05$).

4. Discussion

In this study non-invasive ^{31}P-MRS revealed delayed PCr recoveries after aerobic which were even more pronounced after ischemic exercise in skeletal muscle of FRDA patients compared with healthy controls. Since the time course of PCr recovery after exercise is primarily controlled by mitochondrial oxidative metabolism [7], our data strongly suggest impaired mitochondrial function in FRDA patients similar to mitochondrial defects observed in yeast lacking the *frataxin* homologue [5]. This is in line with the biochemical findings of an abnormal respiratory chain function in endomyocardial biopsies from two FRDA patients [6]. These hints point to similar pathomechanisms in the human disease and the *frataxin*-depleted yeast model.

Although skeletal muscle is not primarily affected in FRDA, we clearly demonstrate mitochondrial dysfunction in skeletal muscle of FRDA patients in vivo. Skeletal muscle involvement in FRDA as a mitochondrial disorder is not surprising since this tissue is rich in mitochondria and depends on efficient oxidative energy metabolism. Additionally, defective respiratory chain function, especially of complexes I, II and III, has been observed before in muscle biopsies of about 30% of FRDA patients [10].

In this study, ^{31}P-MRS depicts mitochondrial dysfunction in all FRDA patients and thus confirms a recently published ^{31}P-MRS study on the calf muscle of FRDA patients [11]. In the study by Lodi et al. [11] PCr recovery in FRDA patients was also significantly delayed after a low-frequency, dynamic plantar-flexion exercise with increasing muscle load. Here we can demonstrate that the impairment of mitochondrial function in FRDA patients is a consequence of both the primary pathology of FRDA and an unspecific adaptation process to the impaired mobility caused by the disease.

Biopsy samples from healthy leg muscles taken in the scope of bedrest or limb-suspension studies have shown that muscle atrophy caused by immobilization results in a reduction of mitochondrial density [12,13]. To minimize these effects of immobilization we included only ambulatory FRDA patients in this study. Furthermore, an ambula-

Table 2
Muscle contraction and energy metabolism[a]

Parameter	FRDA patients		BMD patients		Healthy subjects	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Initial values						
Maximum test force (N)	722 ± 365	326–1334	1113.5 ± 68.6	1021–1184	959 ± 238	655–1587
Initial intracellular pH	7.05 ± 0.04	6.99–7.12	7.06 ± 0.06	6.99–7.12	7.05 ± 0.04	6.98–7.11
Aerobic exercise						
Force integral (N min)	601 ± 251	308–1011	1002 ± 53	943–1062	912 ± 266	567–1516
End-exercise [PCr] (%[PCr]$_I$)	84 ± 6	75–91	78 ± 10	69–89	75 ± 16	38–94
End-exercise pH	7.00 ± 0.05	6.94–7.07	6.97 ± 0.12	6.80–7.06	7.00 ± 0.07	6.86–7.07
τ-value of PCr recovery (s)	79 ± 34[b]	38–132	62 ± 5[b]	55–66	26 ± 9	16–48
Ischemic exercise						
Force time integral (N min)	611 ± 278	302–1003	996 ± 48	959–1063	904 ± 224	599–1353
End-exercise [PCr] (%[PCr]$_I$)	82 ± 11[b,c]	59–93	60 ± 7	53–68	60 ± 17	30–81
End-exercise pH	7.00 ± 0.04[b,c]	6.95–7.09	6.96 ± 0.07	6.87–7.02	6.94 ± 0.06	6.82–7.00
τ-value of PCr recovery (s)	119 ± 49[c,d]	67–194	57 ± 12[b]	42–72	32 ± 11	19–58

[a] Metabolite concentrations in % of [PCr] at initial rest (%[PCr]$_i$).
[b] Significantly different ($P < 0.05$) from healthy subjects.
[c] Significantly different ($P < 0.05$) from BMD patients.
[d] $P < 0.01$ and no overlap from healthy subjects.

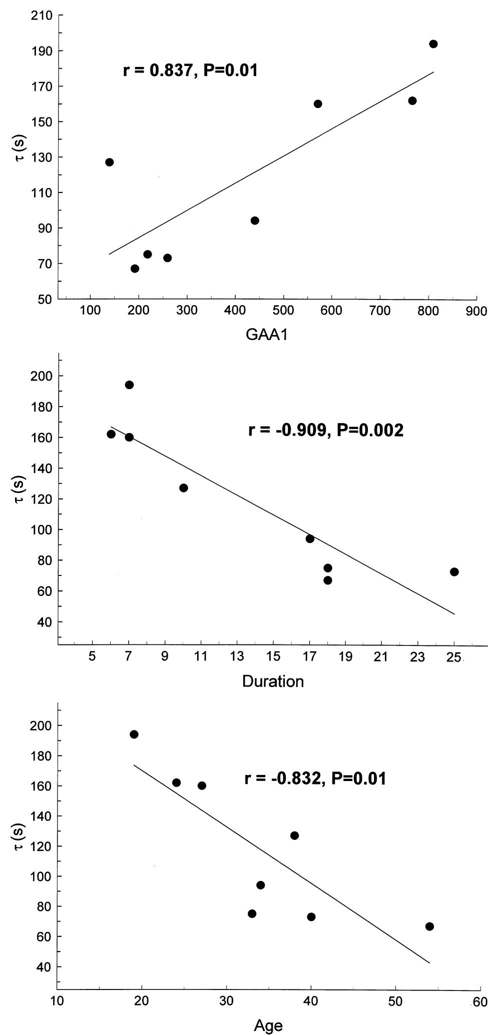

Fig. 4. Correlation between the time constants τ of phosphocreatine recovery after ischemic contraction with the repeat length of the smaller allele GAA1, the disease duration, and the age of the patients.

tory group of BMD patients was included as a disease control group. In contrast to the study by Lodi et al. [11] and probably due to a better time resolution of measurements, we could also demonstrate a significantly slower PCr recovery in BMD patients compared with the healthy controls. After the aerobic test paradigms τ-values of PCr recovery from BMD and FRDA patients were not significantly different. This indicates an impairment of mitochondrial function in both patient groups due to nonspecific immobilization factors, such as muscle atrophy and motor handicap. However, FRDA patients revealed a significantly prolonged PCr recovery after ischemic muscle contraction compared with healthy subjects as well as BMD patients, although reduction of PCr levels at the end of exercise was significantly smaller due to a lower working capacity. There was no overlap between healthy controls and FRDA patients in PCr recovery in the ischemic test paradigm, but some overlap with data from BMD patients. These findings strongly suggest that the impairment of mitochondrial function is a consequence of immobilization, which is also present in the BMD patients as an unspecific symptom, and an additional genetically determined mitochondrial dysfunction.

This conclusion is underlined by correlation analysis. PCr recovery correlated directly with the smaller GAA repeat. Genotype/phenotype analyses demonstrated significant influence of the smaller but not of the larger GAA repeat on age at onset, progression to wheelchair, development of weakness, scoliosis and atrophy in FRDA [14,15]. Moreover, PCr recovery correlates with age of patients and duration of the disease.

In conclusion, all these data indicate PCr recovery as an important feature in FRDA pathogenesis. ^{31}P-MRS provides a direct in vivo approach to reveal mitochondrial respiratory dysfunction in FRDA. Therefore, ^{31}P-MRS represents the first non-invasive tool for properly monitoring therapeutic trials in FRDA patients, which should not only include pharmaceutical treatment supporting mitochondrial function, but also muscle exercise as a countermeasure against immobilization effects.

Acknowledgements

This study was supported by a grant from the medical faculty of the Ruhr University Bochum (FORUM program).

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