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^{31}P magnetic resonance spectroscopy in fibromyalgic muscle

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Abstract

Objective. To measure inorganic phosphate (Pi), phosphocreatine (PCr), ATP and phosphodiesters (PDE) in fibromyalgic muscle tissue by ^{31}P magnetic resonance spectroscopy.

Methods. A 1.5 Tesla scanner with a P 100 surface coil was used to examine 15 patients (mean age 49.9 $\pm$ 14.3 yr) with fibromyalgia, according to the American College of Rheumatology criteria, and 17 healthy controls (mean age 30.2 $\pm$ 5.8 yr).

Results. Compared with the controls, there were increases in the levels of PDE (+22%, $P = 0.032$) and Pi (+19%, $P = 0.019$) in the spectra of fibromyalgia patients, but there was no difference in pH.

Conclusion. The metabolic differences we found may have been related to weakness and fatigue in the fibromyalgia patients, but they do not fully explain the fibromyalgia symptoms.

KEY WORDS: Fibromyalgia, Magnetic resonance spectroscopy, Phosphate, ATP, Phosphocreatine, Phosphodiesters.

The causation and pathophysiology of fibromyalgia are poorly understood. Earlier studies have indicated a correlation between clinical symptoms and metabolic changes in the muscle tissue of fibromyalgia patients [1, 2]. These studies differed in design, technical performance and the anatomical regions that were examined. The aim of our study was to measure levels of inorganic phosphate (Pi), phosphocreatine (PCr), ATP and phosphodiesters (PDE) in fibromyalgia muscle tissue by ^{31}P magnetic resonance spectroscopy.

Methods

Subjects

Fifteen patients with fibromyalgia (one male, 14 females) and 17 healthy controls (three males, 14 females) were investigated. The controls were recruited among co-workers of the patients and their friends, and had levels of daily activity similar to those of the patients. Daily activity was assessed by asking about walking, running, cycling, lap-swimming, callisthenics, rowing, tennis and squash in an interview. The mean age was 49.9 $\pm$ 14.3 yr for the fibromyalgia patients and 30.2 $\pm$ 5.8 yr for the control group. The clinical status of the fibromyalgia patients and controls was determined by a physician trained in rheumatology, using the criteria of the American College of Rheumatology [3].

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Magnetic resonance spectroscopy

^{31}P-magnetic resonance spectroscopy (MRS) measurements were performed with a Philips Gyroscan ACSII system operating at 1.5 Tesla. Measurements were made on the erector muscle, which is one of the tender points recommended by Müller and Lautenschläger [4]. Subjects were investigated only during the resting state. To obtain a selective spectroscopic signal of muscle tissue, an ISIS sequence (image guided *in-vivo* spectroscopy sequence with volume-selective adiabatic high-frequency pulses) was used with a standard transmit–receive surface coil (P 100). The sites of the surface coil and volume selection were controlled by imaging 17 slices in three orthogonal orientations using the body coil. The volume of interest was sited in the erector muscle and adapted to the size of the muscle. The mean value of voxel size was 92.7 $\pm$ 16.0 cm^3 for the controls and 93.7 $\pm$ 15.2 cm^3 for the fibromyalgia patients. Before the ^{31}P-MRS measurements were made, the homogeneity of the magnetic field was adjusted by shimming the water peak to $<$18 Hz (full width at half maximum). Spectroscopic measurements were made with TR (time of repetition) = 2500 ms. The sampling rate was 2000 Hz and the number of sampling points

was 1024. To improve the signal-to-noise ratio, the spectrum was averaged over 768 measurements. Spectral postprocessing was carried out using the scanner software package. Baseline corrected spectra were quantified with an automatic curve-fitting procedure including prior knowledge of the known phosphorus compounds. The reproducibility of the quantification was confirmed by quantifying some artificial line profiles with the middle signal-to-noise ratio of the measurements. These artificial spectra were constructed from an adequate sum of Lorentz profiles and different noise profiles. The standard deviations of the estimated peak areas were $<5\%$ for the PCr peak and 10–20% for the other peaks. Figure 1a gives an example of a spectrum superimposed on the modelled spectrum, and the difference spectrum. The modelled spectrum consisted of a superposition of Gauss–Lorentz-profiles for PME (phosphomonoesters = phosphorylcholine + phosphorylethanolamine), PDE (phosphodiesters = glycerophosphorylcholine + glycerophosphorylethanolamine), Pi, PCr and nucleoside triphosphate (NTP; duplets for α-NTP and γ-NTP and a triplet for β-NTP). Only relative concentrations were estimated. Areas of single peaks were normalized by the total integrated area of the spectrum (the sum of the peak areas of all estimated metabolites of the spectrum).

Statistics

All relative concentrations of the metabolites and the ratios Pi/β-NTP and Pi/PCr were compared between the groups of fibromyalgia patients and controls by analysis of variance using SPSS 7.5.

Results

The mean values of the estimated relative concentrations and the results of the statistical analysis are summarized in Table 1. When age was not considered, the fibromyalgia patients had significantly higher values than the control group for the relative signal intensity for Pi (+ 19%, $P = 0.019$) and PDE (+22%, $P = 0.032$), and showed an increase (+46%, $P = 0.055$) for PME. The α-NTP values of the fibromyalgia patients were significantly smaller than those of the controls (−10%, $P = 0.003$), whereas no significant differences were observed in PCr and pH. A comparison between the spectra of a patient with fibromyalgia and a control subject is shown in Fig. 1b. Age-dependence was assessed by regression analysis of the fibromyalgia group (which had a broader age distribution). The mean age of the control group (30.2 yr) and the values predicted by the regression analysis of the fibromyalgia patients for the age of 30.2 yr are listed in Table 1. The comparison indicates a vanishing difference between the groups for PCr and reduced differences for Pi, PDE and α-NTP.

Discussion

The observed ^{31}P-MRS data support the results of earlier studies that have indicated that clinical symptoms of pain and fatigue are correlated with metabolic changes in the muscle tissue of fibromyalgia patients [2, 5, 6]. Whereas no abnormalities were detected in several earlier studies [7], metabolic differences between fibromyalgia patients and controls were found in the recent studies of Jubrias *et al.* [6] and Park *et al.* [1]. An increased level of phosphodiesters but no change in the Pi/PCr ratio were observed in fibromyalgia patients by Jubrias *et al.*, who investigated skeletal muscle in fibromyalgia patients. Park *et al.* [1] investigated the quadriceps muscles during rest and the performance of an exercise in fibromyalgia patients and healthy controls. During rest, they observed significantly lower concentrations of PCr and NTP for fibromyalgia patients but no change in Pi.

One critical issue in the spectroscopic estimation of metabolic ratios is the fact that changes in two absolute metabolic concentrations in the same direction can occur without an observable change in their ratio. According to Park *et al.* [1], this could be the reason for undetected differences in previous spectroscopic studies of fibromyalgic muscles. Using ratios between single peak areas versus total peak areas, calculated as the sum of all peak areas of the spectrum (i.e. the sum of the areas of the PME, Pi, PDE, PCr, γ-NTP, α-NTP and β-NTP peaks), may help to reduce this shortcoming [8].

Experimental limitations such as partial volume and saturation effects must also be taken into account. Measured signal intensities may depend on differences in the composition of the investigated volume with regard to different types of muscle fibre [9, 16]. Due to the long T_1 times of spectroscopically observable ^{31}P metabolites (ranging from 2 to 6 s), most spectroscopic investigations at 1.5 T have been performed without fully relaxed magnetization ($TR > 3.5\ T_1$) [13–15]. Changes in signal intensity under these conditions may also reflect changes in relaxation times, as demonstrated by Newcomer and Boska [17] for resting and exercising muscle. Consequently, the effects on metabolic ratios of age, gender and athletic activities have to be taken into account [10, 11].

In contrast to Park *et al.* [1], we did not determine absolute concentrations because the use of uniform NTP concentrations as a reference is questionable and because of the risk of measurement errors due to different measurement conditions for muscle tissue and external references such as different pulse angles or different load correction factors [12]. Although the mean values of the PCr and NTP levels were lower for fibromyalgia patients than for controls, the changes observed were not significant. Because 70–80% of the total signal intensity of the whole spectrum is due to the PCr and NTP peaks, a simultaneous decrease in the concentration of the two metabolites would be only partially detectable by decreased relative peak areas of PCr and NTP (normalized by total peak area). On the other hand, the relative concentrations of Pi, PME and PDE would increase without a real increase in the metabolic concentrations, because they represent a greater part of the total intensity. We observed increases in the relative

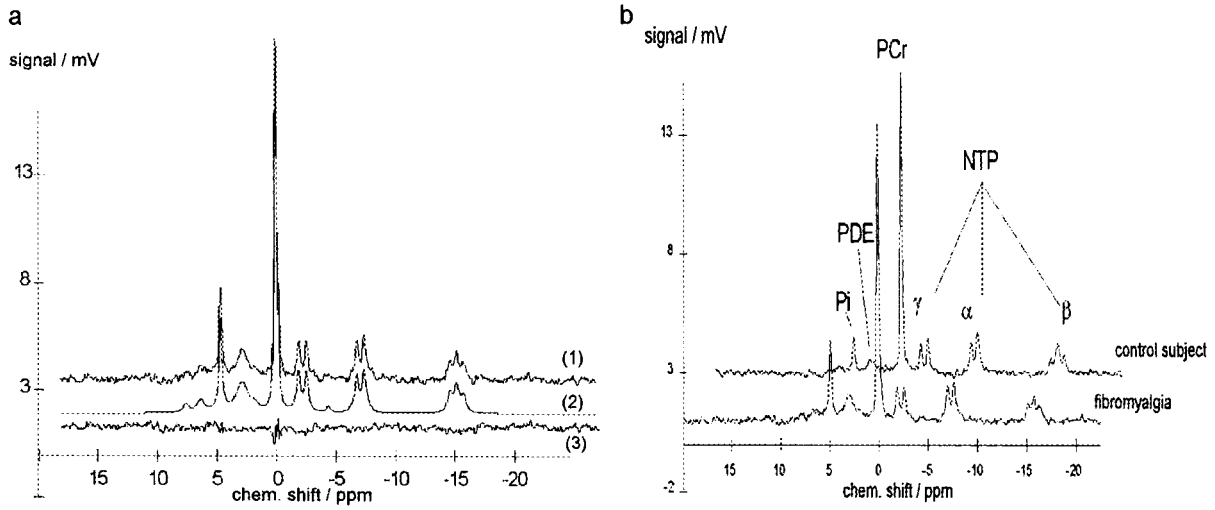

FIG. 1. (a) Original (1), modelled (2) and difference (3) spectra. (b) ^{31}P-MRS spectra of the erector muscle in a control subject and a fibromyalgia patient.

TABLE 1. Mean relative concentrations of Pi, PCr, nucleoside triphosphate β-NTP and PDE in the erector muscle of healthy controls and patients with fibromyalgia, and predicted values for a fibromyalgia group at the age of 30.2 yr

	Fibromyalgia patients	Healthy controls	Difference (fibromyalgia patients minus healthy controls)	P	Fibromyalgia patients (predicted)[b]	r[c]
PME[a]	4.79 ± 2.03	3.28 ± 2.02	+ 1.51 ± 2.86	0.055	3.63 ± 2.27	+ 0.36
Pi[a]	8.68 ± 1.31	7.26 ± 1.32	+ 1.42 ± 1.86	0.019	7.91 ± 1.89	+ 0.08
PCr[a]	37.49 ± 3.88	39.03 ± 3.23	− 1.45 ± 5.16	0.238	39.27 ± 3.97	− 0.50
α-NTP[a]	15.04 ± 1.41	16.77 ± 1.53	− 1.73 ± 2.08	0.003	15.57 ± 1.41	− 0.26
β-NTP[a]	9.76 ± 2.47	10.31 ± 2.03	− 0.55 ± 5.23	0.494	10.01 ± 2.56	− 0.06
γ-NTP[a]	11.21 ± 3.93	12.73 ± 3.45	− 1.52 ± 1.91	0.254	12.04 ± 4.02	− 0.30
PDE[a]	13.03 ± 3.06	10.62 ± 2.98	+ 2.41 ± 4.27	0.032	11.56 ± 2.98	+ 0.27
pH	7.01 ± 0.03	7.02 ± 0.03	− 0.01 ± 0.04	0.594	6.995 ± 0.03	+ 0.43
Pi/PCr	0.23 ± 0.04	0.19 ± 0.04	+ 0.04 ± 0.06	0.008	0.204 ± 0.05	+ 0.05
Pi/β-NTP	0.97 ± 0.43	0.77 ± 0.41	+ 0.20 ± 0.60	0.182	0.583 ± 0.43	+ 0.23

[a]Values are the means ± SEM of the relative concentrations normalised by dividing the single peak area by the total integrated area of the spectrum (estimated as the sum of the peak areas of PME, Pi, PDE, PCr and NTP).
[b]Values predicted by linear regression (± S.E.M.) of the data for fibromyalgia patients for the age of 30.2 yr.
[c]Pearson correlation coefficient for the linear regression analysis of age-dependence in the fibromyalgia group.

signal intensities of these metabolites (PME, Pi and PDE) and only small, insignificant decreases in PCr and β-NTP (Table 1). This either indicates increased metabolic concentrations of PME, Pi and PDE or may be a consequence of decreased absolute concentrations of PCr and NTP. All three NTP resonances showed lower relative intensities for the fibromyalgia group than for the control group; however, only in the case of α-NTP was the difference significant, because of the lower standard deviation of this signal. One reason for this difference may be the triplet structure of the β-peak and distortions of the γ-peak caused by the strong PCr resonance.

The influence of age on metabolic concentrations in the muscle tissue was demonstrated by Schunk *et al.* [10] in a study of 22 young (mean age 27 ± 4 yr) and 10 old (61 ± 5 yr) healthy subjects. They observed higher peak area ratios of Pi/PCr, PME/β-NTP and PDE/β-NTP for the group of old controls. Although the age differences were smaller in our study, the effect of age cannot be neglected. Our regression analysis showed that the observed differences were caused partly by differences in the age distributions. Differences in the spin lattice relaxation time between the two groups cannot be excluded for the phosphorus compounds we investigated; however, changes in measured metabolic ratios or relative concentrations would be affected only if T_1 changed only for a single metabolite and not for the other resonances. Although such changes were observed by Newcomer *et al.* [17] for PCr and Pi (during rest, exercise and recovery), they were not expected in our study, because in the study of Newcomer they were correlated with pH changes and were probably caused by substantial changes in the molecular environment of the metabolites that were investigated. The influence of concentration changes in H^+ and Mg^{2+} on T_1 relaxation time has also been demonstrated in other studies [18, 19]. However, in our study no difference in pH was

observed between the fibromyalgia patients and the controls. The age- and gender-dependence of T_1 times of ^{31}P metabolites of healthy subjects was investigated by Brown *et al.* [14], and showed no significant changes.

In spite of the difference in mean age between the two groups, the results of our study confirm the main findings of the studies of Jubrias *et al.* [6] and Park *et al.* [1], as changes in PDE concentration and PCr/Pi ratio become systemic in fibromyalgia.

We believe that the demonstration of these metabolic changes is helpful in the investigation of the underlying causes of fibromyalgia, although they do not fully explain the symptoms. A decreased concentration of NTP and an increased Pi value in a resting muscle are generally signs of disturbed energy metabolism. These changes are not specifically diagnostic and have been observed in several muscle diseases (inflammatory, mitochondrial and metabolic myopathies) [20–22], or have been caused by lowered perfusion [23]. However, increased Pi values were also observed in association with muscle contraction, with increased capillary perfusion and rising oxygen tension. Increased PME and PDE values are often interpreted as reflecting higher rates of membrane conversion, which indicates disturbed phospholipid metabolism. Although metabolic changes indicate that the muscle in fibromyalgia is stressed, further investigations are necessary to find out the mechanisms responsible for these biochemical abnormalities.

Moreover, the assumption of Park *et al.* [1] that decreased NTP synthesis leads to a vicious circle that becomes systemic and is responsible for the patient's symptoms seems to be rather speculative at the moment.

In summary, metabolic abnormalities are detectable in fibromyalgia using ^{31}P-MRS, and involve certain muscles and possibly the whole musculature. No pH changes were detected in the erector muscle. Significant increases in the relative signal intensities of Pi, PDE and α-NTP and a lower PCr/Pi ratio were observed in the fibromyalgia group. The results support the hypothesis that decreased blood flow may cause focal damage in the muscle due to ischaemia.

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