31P MRS as a Potential Biomarker for Fibromyalgia

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Introduction: Major clinical symptoms in fibromyalgia (FM) are muscle pain, stiffness and fatigue. Studies have shown reduced voluntary strength and exercise capacity, lower endurance and more muscular pain even at low workload. An impaired muscle energy metabolism has therefore been proposed as a result of the disease. An earlier study using magnetic resonance spectroscopy (MRS) showed that at maximal dynamic and static contractions the concentration of inorganic phosphate was lower in FM [1]. A decrease in ATP, ADP and PCr and an increase in AMP and creatine was found in FM biopsies [2]. The purpose of this study was to non-invasively analyze the quantitative content of phosphagens in the resting muscle in FM in comparison to healthy controls using ^{31}P MRS of the quadriceps muscle.

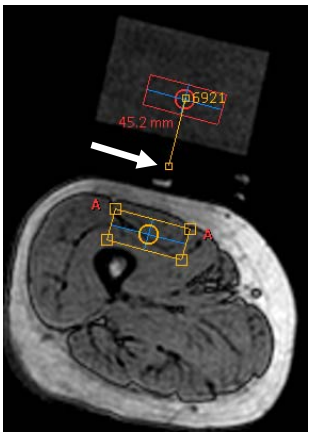

Fig.1 *Experimental setup, the red and yellow rectangle corresponds to the two MRS voxels in the external reference and quadriceps muscle respectively. The Arrow indicates the center of the ^{31}P-coil.*

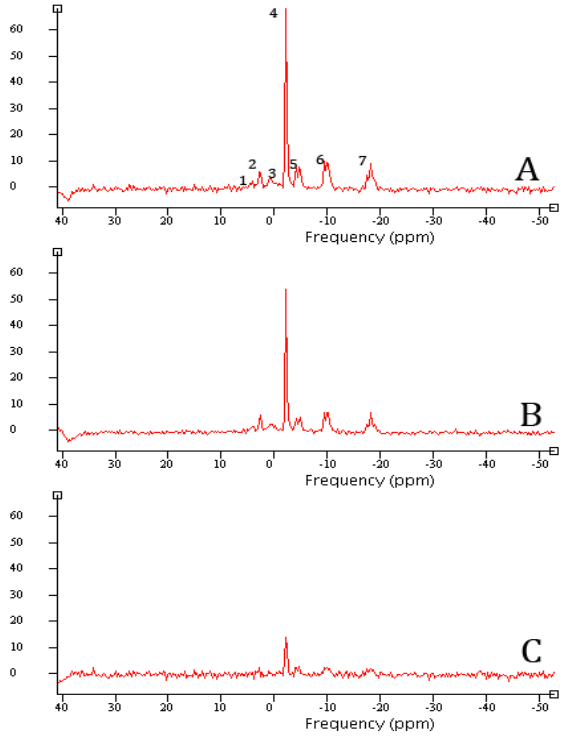

Fig.2 *The spectrum in **Panel A-C** corresponds to the mean of both legs for the control group (n=14), patient group (n=19) and the difference between control and patient groups respectively. The spectra are the mean of all spectrum in the group normalized using the external reference. In **Panel A** the resonances in the spectra corresponds to; 1) Phosphomonoesters, 2) Inorganic phosphate, 3) Phosphodiesters, 4) Phosphocreatine 5-7) γ-,α-,β-nucleotide triphosphate, 6) also includes NAD(H)*

Materials and Methods: 19 female patients (median age: 51, range: 38-61 years) and 14 female controls (median age: 46, range: 39-62 years) were examined prospectively. A 1.5T MR-scanner (Achieva, Phillips Medical Systems, Best, The Netherlands) was used together with a flat 10 cm non-flexible circular ^{31}P T/R surface coil ('P-100'). Attached to the ^{31}P coil was a cylindrical (r=50 mm, h=80 mm) external reference (**Fig. 1**), filled with *dimethyl methylphosphonate* (DMMP) 85 mM. Two MRS voxels (2x5x4 cm^3) were placed at equal distances from the centre of the ^{31}P coil (1) within the quadriceps muscle and (2) the external reference (**Fig. 1**). ^{31}P MRS was simultaneously acquired in both MRS voxels using the same sequence parameters (TR = 4s, 128 averages, TE = 94.6μs, ISIS volume selection). The built-in body coil was used to obtain localizer images. All subjects were placed feet first in a supine position and measurements were performed in both legs for all subjects. jMRUI [3] was used for processing of the ^{31}P MRS using the AMARES algorithm with prior knowledge [4] for relative quantification of the resonances. PCr was used as a chemical shift reference assigned to -2.35 ppm (85% H_3PO_4 = 0.00 ppm). For absolute quantification of PCr and NTP-Mg (mainly ATP), a correction factor was derived based on the assumption that the quadriceps resting ATP concentration is 8.6 mM, as previously reported [5] in healthy muscle. The correction factor was calculated from the control group mean of the relative NTP-Mg concentrations. Similarly PCr was normalized using the DMMP resonance ($[PCr]_{DMMP}$) by relating NTP, normalized using DMMP, to literature value [5].

Results: In **Table 1** the determined metabolite concentrations are shown together with the corresponding estimates normalized using DMMP, based on the mean in both legs per patient, thus giving a quantitative measure of phosphagens. With respect to $[PCr]_{DMMP}$ and $[NTP\text{-}Mg]_{DMMP}$ the patient group has on average 28% and 29% decrease in metabolite concentrations respectively. For [PCr] and [NTP-Mg] the decrease was 12% and 14% respectively. Significant group differences were found in $[PCr]_{DMMP}$ and $[NTP\text{-}Mg]_{DMMP}$ (**Table 1**). **Fig. 2A** shows the control group mean spectrum, normalized with the external reference. The spectral assignments are presented in **Fig. 2A.** The patient group mean spectrum, normalized using the external reference, is shown in **Fig. 2B**. The difference between controls and patients can clearly be appreciated in **Fig. 2C** in which decreased amplitudes of both PCr and NTP are apparent.

Table 1 *Mean and standard deviation for PCr and NTP-Mg concentrations determined using quantitative h ^{31}P MRS, as well estimates normalized using the external reference (DMMP). P-values and confidence interval (CI) for the difference were calculated using Students T-test.*

	Control(*n*=14)	Patient(*n*=19)	95% CI for difference	p-value
$[PCr]_{DMMP}$	30.60 ± 7.34 mM	21.92 ± 6.66 mM	(3.69; 13.68) mM	0.001
[PCr]	30.29 ± 6.73 mM	26.65 ± 6.41 mM	(-1.06; 8.34) mM	0.125
$[NTP\text{-}Mg]_{DMMP}$	8.67 ± 2.67 mM	6.14 ± 2.47 mM	(0.69; 4.36) mM	0.009
[NTP-Mg]	8.60 ± 2.53 mM	7.42 ± 2.31 mM	(-0,56; 2,90) mM	0.176

Discussion and Conclusions: Our results show that there is a significant difference in resting muscle metabolite absolute concentrations for patients suffering of FM, which is in agreement with [6, 2]. The results suggest that ^{31}P MRS can be used as a clinical biomarker for FM. A more elaborate use of an external reference for metabolite normalization can potentially provide more accurate absolute quantifications, in addition to serving as an institutional robust normalization procedure in longitudinal studies, and as a complement to the use of literature values. Further work on validation would be of value in order to assess specificity and sensitivity of the method for clinical use.

References: [1] Lund E *et al* Scand J Rheumatol 2003;32:138-145. [2] Bengtsson A *et al* Arthr Rheum 1986;29:817-21. [3] Naressi A et al MAGMA 2001;12;141-52. [4] Vanhamme L et al J Magn Reson 1997;129:35-43. [5] Kemp G.J. et al NMR Biomed 2007;20:555-65. [6] Sprott H et al Rheumatology 2000;39:1121-2.