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Adolfo Sanchez

Donald Wenner

Leslie Morrison

Paul Mullins

Charles Gasparovic

*See next page for additional authors*

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**Authors**

Adolfo Sanchez, Donald Wenner, Leslie Morrison, Paul Mullins, Charles Gasparovic, Clifford Qualls, and  
Linda Friesen

## **P-31 Magnetic Resonance Spectroscopy in OPMD and Myotonic Dystrophy**

**Medical Students: Donald Wenner, Adolfo Sanchez**

**PI: Leslie Morrison MD.**

**Co-Pi: Paul Mullins Ph.d., Charles Gasparovic Ph.d., Clifford Qualls Ph.d., Linda Friesen**

### **Introduction:**

Oculopharyngeal muscular dystrophy (OPMD) is a disorder characterized by the onset of progressive ocular ptosis, dysphagia, and proximal muscle weakness during middle age. The cause of this disorder is an autosomal dominant gene mutation that consists of a stable trinucleotide repeat in PABPN1 on chromosome 14. The trinucleotide repeat ranges from (GCG)<sub>8-13</sub> with some mutations also containing GCA.<sup>1</sup> Thus far only (GCG)<sub>9</sub> has been seen in Hispanic New Mexican families.<sup>2</sup> Although this disease does not appear to shorten lifespan, it clearly diminishes quality of life. It is potentially amenable to treatment, as demonstrated in a recent mouse model treated with doxycycline in which typical intranuclear inclusions were decreased and muscle strength and function increased.<sup>3</sup> As such, it has become important to devise noninvasive measures of muscle function in order to evaluate potential treatment effects. A single prior P-31 Magnetic Resonance Spectroscopy study of forearm muscle has been conducted in patients with OPMD.<sup>4</sup>

Myotonic muscular dystrophy type I (DM1), an autosomal dominant disorder with uneven penetrance, is the most common muscular dystrophy of adults with an estimated lifetime incidence of approximately 1 in 8000.<sup>5</sup> This disease is due to an unstable trinucleotide (CTG) repeat located in the 3' untranslated region of the DMPK (dystrophica myotonica protein kinase) gene on chromosome 19q13.3.<sup>6,7,8</sup> Normal individuals have DMPK alleles with between 5 and 35 (CTG)<sub>n</sub> repeats that are stably transmitted, while diseased individuals have alleles containing 50 to several thousand (CTG)<sub>n</sub> repeats that are no longer stable.<sup>6,9,10</sup> The disease shows a tendency to have an earlier onset in subsequent generations, a phenomenon known as anticipation, which is accompanied by expansions in the size of the CTG<sub>n</sub> repeat region. An inverse correlation between CTG<sub>n</sub> repeat size and age of onset has been noted, as well as a fairly broad correlation between repeat size and the severity of the disease.<sup>9,11</sup> These correlations are quite variable and thus, specific ranges of repeat size cannot be used to predict age of onset or disease severity in individual patients. The majority of persons with DM1 present in adulthood with distal muscle weakness and wasting accompanied by myotonia, a sustained muscle contraction, caused by abnormal activity of the sarcolemma.<sup>12,13,14</sup> DM1 has multisystemic effects that include endocrinopathy, cardiac conduction abnormalities, neurocognitive changes, and the development of cataracts, in addition to the aforementioned effects on skeletal muscle. A treatment trial for weakness in DM1 looked at the efficacy of creatine monohydrate supplementation in DM1 patients, with the finding that there was no benefit.<sup>15</sup> Researchers in this trial used <sup>31</sup>P MRS imaging of the forearm muscles to help measure the response to the treatment. This highlights the

usefulness of the noninvasive technique of  $^{31}\text{P}$  MRS imaging in studying muscular dystrophies and the response of muscle to treatments.

Magnetic resonance imaging can be used to evaluate muscle size, signal characteristics and to distinguish the pattern and degree of atrophy.  $^{31}\text{P}$  MRS has been used in the investigation of muscle energy metabolism in health and disease for over 20 years.<sup>16</sup> By measuring unbound phosphorus metabolites with this technique, muscle energy metabolism can be investigated in a noninvasive and painless fashion during rest, exercise, and recovery from exercise for serial monitoring of oxidative and glycolytic metabolism of muscle. It is possible to measure unbound phosphorus metabolites in living human muscle with concentrations of at least 1 mM. Phosphorus spectra from muscle contain five major peaks related to energy metabolism: three from adenosine triphosphate (ATP), one from phosphocreatine (PCr) and one from inorganic phosphates (Pi). Two additional peaks arise from phosphomonoesters (PME) and (PDE).<sup>17</sup>

The aim of this study was to further characterize the bioenergetics of muscle in patients with OPMD and DM1. To date there has not been a good noninvasive way to follow the course of muscle in OPMD and DM1 other than with clinical manifestations such as muscle weakness.  $^{31}\text{P}$  MRS is a promising technology to evaluate disease course over time or to determine response to treatment. The response of muscle to exercise in these and other muscular dystrophies also remain unclear, with some types of exercise showing benefit and others detriment depending on the specific disease.<sup>18</sup> More knowledge about the bioenergetics of muscle in OPMD and DM1 could help clinicians determine whether exercise is beneficial or detrimental. Thus far, there have been no studies reporting  $^{31}\text{P}$  MRS in the gastrocnemius muscle of OPMD, and only two in DM1.

## **Materials and Methods:**

### ***Subjects:***

A total of 34 subjects (12 OPMD, 11 DM1, 11 controls) were recruited to participate in this study. Preexisting clinical databases of patients with previously diagnosed OPMD and DM1 were used to identify potential subjects. The recruited subjects had no contraindications to magnetic resonance testing. An effort was made to age and sex-match the participants in each group; however with the limited numbers of patients available this was not entirely possible. See table 1 for age and gender distribution. This study was approved by the University of New Mexico Human Research Review Committee, and informed verbal and written consent was obtained from each participant.

Scans were performed and data obtained from all patients in the OPMD and control groups. Data from three patients in the DM1 group was not usable. One male patient with DM1 was unable to complete the study due to the presence of a leg brace that interfered with proper functioning of the exercise apparatus. The data for the second DM1 male was unusable, due to poor quality spectra with peaks indistinguishable from noise. This participant was noted to have extensive muscle wasting, and was unable to return for a repeat scan due to his declining health status. The data for the third DM1 female patient was not obtained according to protocol with incorrect resting and exercise duration. This participant did not respond to messages requesting that she return for a repeat scan.

### **<sup>31</sup>P MRS methodology:**

Experiments were performed on the 4 Tesla MRI scanner at the Mind Imaging Center (Bruker Biospin, Billerica MA). Subjects lay supine in the bore of the magnet with a dual tuned proton/phosphorus (<sup>1</sup>H/<sup>31</sup>P) transmit/receive coil secured around the lower right leg at the level of the gastrocnemius and the foot was secured into the exercise apparatus. The location of the gastrocnemius within the magnet was confirmed by T<sub>1</sub> weighted <sup>1</sup>H localizing images obtained in the axial plane. An anatomical scan to allow the placement of the chemical shift imaging slab was then acquired using a 3D T<sub>1</sub> weighted gradient refocused echo sequence (fov = 200 x 200 mm, matrix = 256 x 256, 96 slices, with a 1 mm slice thickness giving a final resolution of 0.8 mm x 0.8 mm x 1.0 mm.). Shimming on the proton signal from tissue water optimizes magnetic field homogeneity. Chemical shift imaging (CSI) <sup>31</sup>P MRS data were then acquired at 60 MHz from a 40mm thick axial slice, which includes the gastrocnemius muscle. The sensitive volume of the coil limits the volume of the tissue sampled in the anterior posterior direction in the leg. A custom developed exercise apparatus that allows for <sup>31</sup>P MRS data acquisition during exercise in the magnetic resonance image (MRI) scanner was used. This apparatus has been used previously in a study on sarcopenia by Waters et. al.<sup>19</sup> The exercise apparatus is a rigid frame with a wheel that turns around its axis by pushing down on a pedal with the foot. A static line connects the outer radius on one side of the wheel, over a pulley to a hook suspending calibrated weights from a frame. The weight to be lifted was chosen for each subject based on subject assessment of effort and ability to finish the six minutes of exercise. Contraction of the gastrocnemius allows for a 9 cm vertical displacement of the weights.

CSI data from an 8x8 matrix (fov = 200 X 200) are sampled using a spectral width of 3000 Hz, and a recycle time (TR) of 1 second. This will produce one CSI data set in approximately one minute (64 sec). Subjects were asked to rest for the first four CSI acquisitions, then to contract the calf to raise the weights at the rate of 60 repetitions per minute, for six CSI acquisitions, then the subjects were told to rest again, allowing muscle recovery for six CSI acquisitions. The contraction rate of 60 repetitions per minute was standardized and maintained by having the subject control the contraction frequency using the audible gradient noise of the scanner acquisition set at a 1 second recycle time.

### ***Quantification of mitochondrial function:***

Data was analyzed using jMRUI software (jMRUI v. 2.2, European Community) in the time domain. The <sup>1</sup>H localizing image that was taken for alignment of the leg was used to pick voxels that were completely within the gastrocnemius muscle. The data for these voxels was then viewed by two blinded evaluators (LM and LF) and the voxel with the best signal to noise ratio was used for the analysis. For visualization, each free induction decay (FID) was processed with a 5 Hz exponential line broadening before zero filling and Fourier transformation. All spectra were manually phased using zero and first-order phase corrections. Resonance peak areas for PCr, Pi and ATP were measured by line fitting of the spectrum. Signal-to-noise ratios were sufficient to allow PCr, and Pi to be quantified with a temporal resolution of 1 minute during exercise and recovery. Baseline (rest) cytosolic [PCr] and [Pi] were calculated from the signal intensity ratios PCr/ $\beta$ ATP

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and  $P_i/\beta$ ATP respectively, taking ATP at rest to be 8.2 mmol/L cell water as follows:  
 $[PCr] = (PCr/\beta$ ATP) 8.2 mmol/L.<sup>20,21</sup> Metabolite peak areas were normalized to 100% by using the average metabolite value obtained during the four-minute rest (pre-exercise) as a reference. Concentrations are expressed as the percent change from baseline levels defined as 100%. Thus, PCr and  $P_i$ , indicate peak areas obtained from spectra and [PCr] and [P<sub>i</sub>] are calculated concentrations. Cytosolic [ADP] is calculated as follows:  $[ADP] = ([TCr]/[PCr]-1)[ATP]/(K/[H^+])$  where [TCr] = total creatine = 42.5 mmol/L cell water, and K is the equilibrium constant of creatine phosphokinase (1.66 x 10<sup>9</sup>L/mol).<sup>20,21</sup> Relative changes of PCr,  $P_i$ , and [ADP] are expressed graphically as percentages of the resting average.

#### Statistical Analysis:

Data from patients was subdivided into those with DM1, those with OPMD, and control subjects. The overall analysis of the time course of each metabolite, (PCr,  $P_i$ , and ADP) was done by repeated measures ANOVA with time as the repeated factor and the three patient groups (DM1, OPMD, control) as the grouping factor. Post hoc testing was done by unpaired t-tests at each time point. The variables are reported as mean + SE. A value of P<0.05 was considered statistically significant.

## Results:

### Demographics (Table 1)

Table 1

Group	Age (years)	Gender (% female)
OPMD (n=12)	53.2 + 8.9	67% (8)
DM1 (n=11)	43.9 + 9.4	73% (8)
Control (n=11)	44.6 + 10.7	64% (7)

### <sup>31</sup>P MRS baseline concentrations (Table 2)

During the baseline resting period the values for [PCr] were significantly decreased in the DM1 group versus controls, and the OPMD group also showed this trend however it was not statistically significant. The baseline resting [ADP] level was significantly higher in the DM1 group than in controls.

Table 2

	[PCr] (mM)	SE	P value
OPMD	0.472	0.0112	0.0792
DM1	0.4579	0.01	<b>0.0065</b>
Control	0.4958	0.0063	
	Pi (Mm)	SE	P value
OPMD	0.0548	0.0032	0.5057
DM1	0.0601	0.003	0.0508
Control	0.0521	0.0023	
	[ADP] (μM)	SE	P value

OPMD	5.7018	0.4279	0.3239
DM1	6.6995	0.4422	<b>0.0116</b>
Control	5.1897	0.2702	

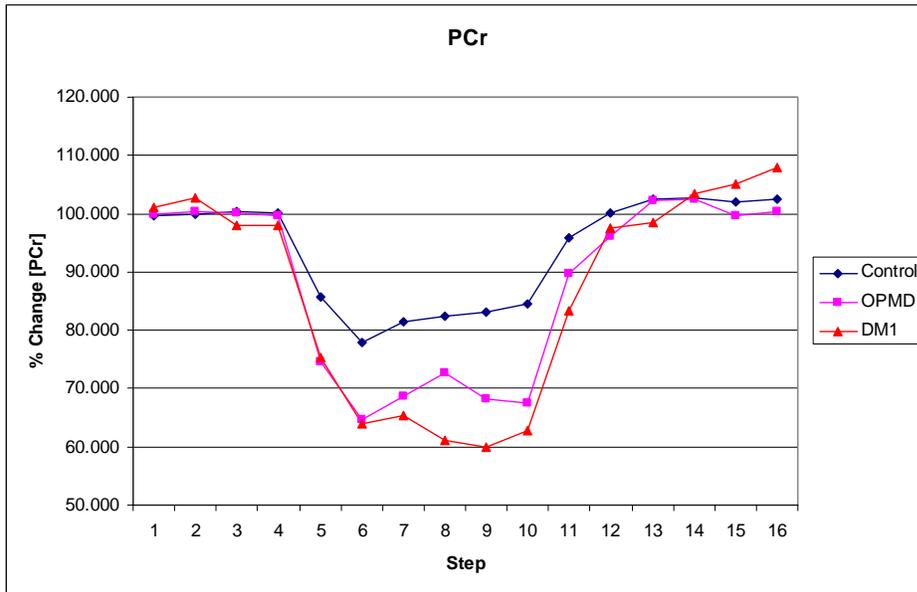
Data reported as mean and standard error (SE). P values are for diseased group compared with controls.

<sup>31</sup>P MRS relative metabolite change normalized to resting baseline during rest, exercise and recovery (Figures 1, 2, and 3).

The minimum [PCr] was significantly lower in OPMD patients than in controls (Fig. 1) after the first minute of exercise (step 5) and at the end of exercise (steps 9 & 10). The differences seen for the DM1 patients versus controls were not statistically significant.

**Comment [H1]:** Do you have OPMD and DM1 inverted

Figure 1

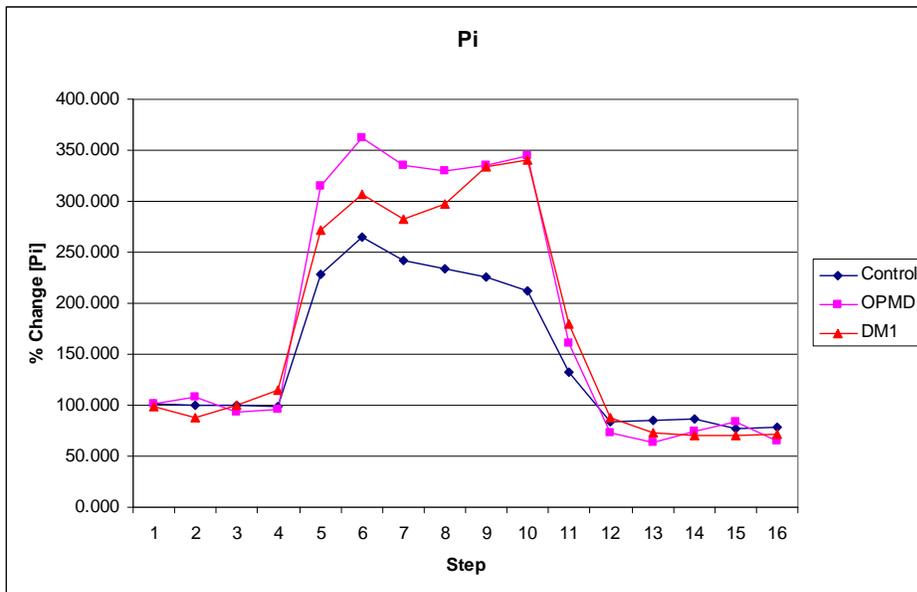


Step refers to each 64 second period of data acquisition. Steps 1-4 are during the rest period, Steps 5-10 are during exercise, and steps 11-16 are during recovery.

The maximum [Pi] was significantly higher in OPMD patients than in controls (Fig. 2) after the first minute of exercise (step 5) and at the end of exercise (steps 9 & 10). The differences seen for the DM1 patients versus controls were not statistically significant.

**Comment [H2]:** Check the data to see if OPMD and DM1 are inverted!

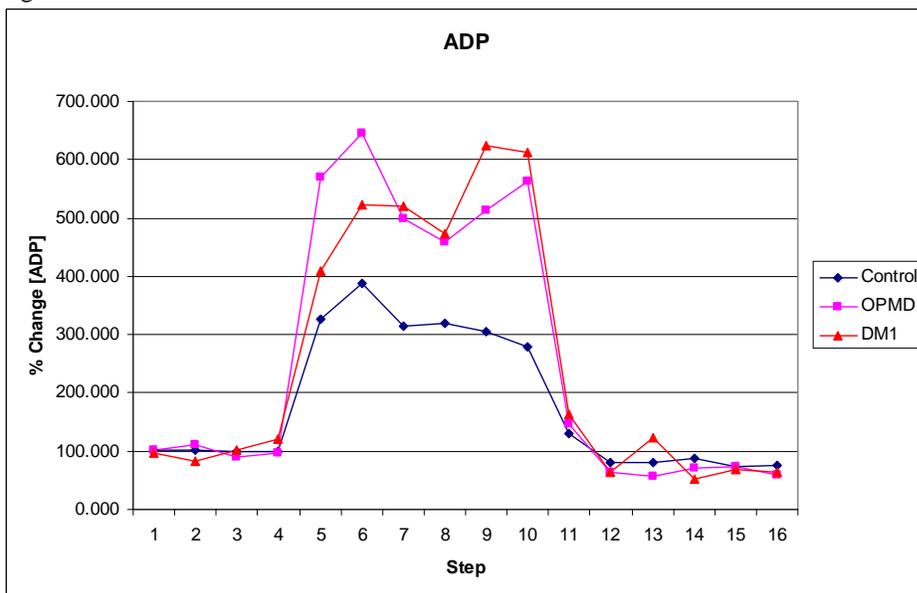
Figure 2



The maximum [ADP] was significantly higher in OPMD patients than in controls (Fig. 3) throughout the exercise period (steps 5,6,7, 9 & 10). The differences seen for the DM1 patients versus controls were not statistically significant.

Comment [H3]: Check these!

Figure 3



## Discussion

The goal of this study was to characterize the bioenergetics of muscle by P-31 MRS at rest, with exercise and in recovery in patients with OPMD and DM1. This is especially important for the patients with OPMD because of the scarcity of studies involving this disease. The only P-31 MRS study in patients with OPMD that was uncovered during our literature search was a small study by Zochodne et al that involved 5 siblings with OPMD.<sup>4</sup> The investigators in this study looked at the forearm flexor muscles during rest, exercise, and recovery periods with the findings that the OPMD patients had reduced PCr/(PCr +Pi) ratios and increased pH in resting muscle compared to normal controls. During exercise they observed an early and prominent fall in PCr/(PCr +Pi) as well as an excessive intracellular muscle acidosis that was slow to recover after exercise had ceased. All of these findings reached statistical significance and are of particular interest since OPMD generally affects proximal limb muscles, but these abnormalities were noted in the more distal muscles of the forearm.

Our study looked at the gastrocnemius muscle which is also a distal muscle that is not severely affected in OPMD patients. We found larger decreases in [PCr] in the OPMD group compared to controls early during exercise and at the end of the exercise period, which is similar to the results obtained in the study by Zochodne et al . A trend toward decreased [PCr] during rest was noted in our study, however this did not reach statistical significance. The [ADP] was found to increase to higher levels during exercise compared with controls. These findings indicate that even though the gastrocnemius muscle is not clinically affected in OPMD patients, there is 31-P MRS evidence of altered function. This lends further evidence to OPMD being a widespread disorder of striated muscle, even when muscle strength is clinically judged as normal.

One possible mechanism for the results that we obtained is that within the muscle there is a reduced number of functioning muscle fibers. The results that we obtained of an early and excessive fall in [PCr] and an elevated [ADP] during exercise could indicate that there is an increase in ATP hydrolysis used for cross-bridge movement in the depleted pool of muscle fibers. This leads to increased ADP, which then undergoes phosphorylation by PCr back into ATP in a reaction catalyzed by creatine kinase. The excessive fall in [PCr] compared to controls is attributed to a greater demand placed on each remaining functional muscle fiber. Another possible mechanism is that there is a normal amount of muscle fibers but they are not functioning normally. At moderate levels of exercise, such as in our study, most of the energy used for muscle contraction is generated by oxidative phosphorylation with glycogen being the major fuel for the first several minutes after exercise begins. The dystrophic process could affect one of the steps in oxidative phosphorylation thus slowing this process. This would lead to increased PCr depletion and elevated end-exercise [ADP].<sup>21</sup>

Our results obtained from the patients with DM1 showed similar trends to the results from the OPMD group; however most of the findings in the DM1 group did not reach statistical significance. We had a small number of patients in each group to begin with and unfortunately data from three of the DM1 participants was unusable for the reasons described previously under the heading Subjects. The smaller number of participants decreased the statistical power of our study to distinguish differences between the DM1 group and the control group. A decreased resting [PCr] and an elevated resting [ADP] compared to controls were found to be significant in our study.

Comment [H4]: Are these inverted?

These results can be compared with those by Barany et al, and Barnes et al, who have previously performed <sup>31</sup>P MRS studies in patients with DM1 on the gastrocnemius muscle.<sup>14,23</sup> Barany et al studied 4 patients with DM1 as part of a larger study on leg neuromuscular diseases, with the finding that DM1 patients had elevated ATP/PCr ratios compared to controls. Barnes et al studied 14 patients with myotonic dystrophy, however only 7 patients were able to exercise for 5 minutes. In these patients an elevated resting intracellular pH was noted, as well as elevated [ADP] levels, and a reduced phosphorylation potential. These findings were even more pronounced in the patients that could not complete the exercise regimen. During exercise in the DM1 patients phosphocreatine was depleted more rapidly, ADP concentrations were higher, and calculated ATP turnover was elevated compared to controls. Our results during exercise, though not statistically significant, show trends towards elevated [ADP] and decreased phosphorylation potential. This would be consistent with the study by Barnes et al with these findings thought to be due to both reduced mitochondrial and glycogenolytic function.<sup>14</sup>

In conclusion we have been able to demonstrate bioenergetic abnormalities in the gastrocnemius muscle during exercise in patients with OPMD. Unfortunately the differences from controls in patients with myotonic dystrophy did not reach statistical significance, likely due to unusable data from several participants and thus decreased statistical power. The findings in the OPMD patients provide promise that <sup>31</sup>P MRS could be used to noninvasively monitor bioenergetic indices that could be followed serially during therapeutic trials. The hope is that improvement in these bioenergetic indices for both OPMD and DM-1 patients might precede or accompany an improvement in clinical measures.

Comment [H5]: BE SURE OF WHICH IS WHICH

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