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Exploring ^{31}P-MRS changes in skeletal muscle of riboflavin-responsive lipid storage myopathy patients: Clinical value and implications

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Abstract

Objective: To investigate the characteristics of skeletal muscle changes observed through ^{31}phosphorus-magnetic resonance spectroscopy (^{31}P-MRS) in patients with riboflavin-responsive lipid storage myopathy (RR-LSM), and to explore the clinical value of these changes in assisting the diagnosis and evaluating the efficacy of LSM treatment. *Methods:* A total of 12 RR-LSM patients underwent ^{31}P-MRS scans pre- and post-treatment, alongside 11 healthy controls. Spectral data were analyzed to derive key metabolite levels, including inorganic phosphate (Pi), phosphocreatine (PCr), and adenosine triphosphate (ATP). Ratios such as Pi/ATP, PCr/ATP, and Pi/PCr were calculated, alongside values for intracellular pH (pH_{int}), adenosine diphosphate (ADP), and phosphorylation potential (PP). Comparative analyses were conducted among three groups: RR - LSM patients before treatment, RR - LSM patients after treatment, and the control group. *Results:* Pre-treatment, RR-LSM patients exhibited significantly lower levels of PCr, PCr/ATP, and PP compared to controls ($P < 0.05$). Conversely, elevated levels of Pi/PCr and ADP were observed ($P < 0.05$). No significant differences were noted in Pi, Pi/ATP, and intracellular pH (pH_{int}) between groups ($P > 0.05$). Post-treatment, significant increases in PCr, PCr/ATP, and PP were observed ($P < 0.05$), while ADP levels decreased markedly ($P < 0.05$). However, Pi, Pi/ATP, Pi/PCr, and pH_{int} remained unchanged after treatment ($P > 0.05$). Post-treatment ADP and 1/PP remained elevated compared to controls ($P<0.05$).
Conclusion: Our study identifies distinct metabolic alterations in RR-LSM patients, particularly in PCr, PCr/ATP, PP, Pi/PCr, and ADP levels, which serve as valuable biomarkers for diagnosis in RR-LSM. The observed improvements in PCr and related ratios post-treatment highlight the utility of ^{31}P-MRS in tracking metabolic recovery and evaluating the efficacy of riboflavin therapy. These findings underscore the potential of ^{31}P-MRS as a non-invasive tool for clinical management of RR-LSM.

Keywords: ^{31}P-MRS, riboflavin-responsive lipid storage myopathy, metabolic biomarkers

INTRODUCTION

Lipid storage myopathies (LSM) represent a spectrum of metabolic disorders characterized by defects in lipid metabolism, leading to pathological accumulation of lipids within muscle fibers.[1] Among these, riboflavin-responsive lipid storage myopathy (RR-LSM) is a notable subtype distinguished by its remarkable response to riboflavin (vitamin B_2) therapy.[2] This condition manifests across a wide age range, from infancy to adulthood, with clinical features including episodic muscle weakness, exercise intolerance triggered by specific factors, and, occasionally, multisystem involvement beyond the musculature.[2,3] Histologically, RR-LSM is characterized by the excessive accumulation of lipid droplets within muscle fibers, reflecting underlying defects in mitochondrial fatty acid oxidation.

Therapeutic intervention with riboflavin has demonstrated significant efficacy in alleviating symptoms associated with RR-LSM.[4] Genetic studies have shown that over 90% of RR-LSM patients carry pathogenic variants in the gene encoding *ETFDH* gene, while a minority exhibit biallelic variants in the *COASY* gene.[2]

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Additionally, mutations in genes encoding riboflavin transporters (*SLC52A1*, *SLC52A2*, *SLC52A3*), mitochondrial folate transporter (*SLC25A32*), and FAD synthase (*FLAD1*) have been implicated in RR-LSM or related riboflavin-responsive disorders.[5-7] These genes are critical for riboflavin uptake, mitochondrial folate metabolism, and flavin cofactor synthesis, respectively. Despite advancements in understanding the genetic basis of RR-LSM, diagnostic and therapeutic monitoring remain challenging due to the invasive nature of conventional assessment methods such as muscle biopsy. While muscle biopsy remains the gold standard for diagnosis, it is not feasible for repeated evaluation or long-term follow-up. Therefore, there is an urgent need for non-invasive diagnostic tools capable of effectively assessing metabolic dysfunction in RR-LSM.

^{31}P-MRS offers a promising non-invasive solution to address this clinical need. By quantifying phosphorus-containing metabolites such as ATP, phosphomonoester (PME), Pi, and PCr, and indirectly calculating parameters such as Pi concentration, PCr concentration, ADP concentration, pH_{int}, PP, Pi/ATP ratio, PCr/ATP ratio, and Pi/PCr ratio, ^{31}P-MRS provides valuable insights into the dynamic metabolic processes of skeletal muscle.[8-10] Alterations in these metabolites can reflect underlying mitochondrial dysfunction and lipid metabolic abnormalities.[10,11]

In the context of RR-LSM, defects in fatty acid oxidation may disrupt ATP production and phosphocreatine resynthesis, leading to metabolic perturbations detectable via ^{31}P-MRS. This study aims to investigate skeletal muscle energy metabolism in a cohort of 12 patients with RR-LSM using ^{31}P-MRS, both before and after riboflavin treatment. By exploring the metabolic signatures and therapeutic responses, this research seeks to evaluate the potential of ^{31}P-MRS as a non-invasive diagnostic and therapeutic monitoring tool for RR-LSM.

METHODS

This study enrolled 15 patients diagnosed with LSM at the Second Affiliated Hospital of Nanchang University over a three-year period. All participants underwent ^{31}P-MRS before treatment. Of these, 12 patients showed unequivocal clinical responses to riboflavin therapy and were included in the final study cohort, with repeat 31P-MRS performed post-treatment.

Inclusion criteria were: Significant lipid droplet accumulation within skeletal muscle fibers, confirmed by muscle biopsy (Oil Red O staining); Absence of secondary causes of lipid deposition (e.g., mitochondrial myopathies, glycogen storage disorders, or steroid myopathy); Documented clinical improvement following ≥20 days of Vitamin B2 supplementation (200–300 mg/day).

Exclusion criteria were: Elevated serum lactate levels (>2.5 mmol/L at rest or post-exercise); Multisystem involvement (e.g., encephalopathy, cardiomyopathy) indicative of mitochondrial disease; All participants provided informed consent.

The control group comprised 11 healthy individuals, demographically matched to the RR-LSM patient cohort, recruited from hospital staff, including medical personnel and other employees.

^{31}P-MRS scanning

Scanning was conducted using a GE 1.5T Signa Twin Speed magnetic resonance imaging system. An initial scan was performed to optimize magnetic field homogeneity. Participants lay supine with their feet forward, focusing on the upper thigh. A body coil was employed for radiofrequency transmission, while a dedicated ^{31}P surface coil served as the receiver. A standard transverse fast spin-echo (FSE) T2-weighted pre-scan of the mid-femur was executed with the following parameters: TR/TE = 53.9/1.6 ms, ETL 19, slice thickness and gap of 20 mm each, FOV = 20 cm, 2 excitations, and matrix size 256 × 128. Subsequently, the ^{31}P-MRS scan began, maintaining the same position. The volume of interest (VOI) was centered on the quadriceps muscle, avoiding bone tissue. The Spin Echo MRS sequence was used with parameters including: TR = 2000 ms, flip angle 60°, slice thickness and gap of 20 mm each, FOV = 20 cm, 2 excitations, matrix size 1 × 1, spectral width = 2500 Hz, and data point resolution of 1024. A spectral prescan was conducted to ensure satisfactory free induction decay signals, after which MRS signal acquisition was initiated, totaling 4 minutes and 32 seconds.

^{31}P-MRS data processing

Data processing was performed using Sage software on an ADW4.0 workstation. The chemical shifts of different metabolites in the spectrum were analyzed to identify the resonance peaks for Pi, PCr, and the γ-, α-, and β-forms of ATP. The following metabolic indices were calculated: 1) The area under the peaks for Pi, PCr, and ATP was determined, and the ratios of Pi/ATP, PCr/ATP, and Pi/PCr were recorded. The

intracellular ATP concentration was assumed to be 8.2 mmol/L to calculate the concentrations of Pi and PCr. 2) pH_{int} was calculated using the formula: $pH_{int} = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$, where δ represents the chemical shift (ppm) between the Pi and PCr peaks. 3) The concentration of adenosine diphosphate (ADP) was calculated using the formula: [ADP] = ([TCr] - [PCr]) [ATP] / (K_{eq} [PCr][H^+]), where $K_{eq} = 1.66 \times 10^9$ /M (38°C, pH = 7) and [TCr] = 42.5 mmol/L. 4) Phosphorylation potential (PP) or 1/PP ($\times 10^7$) was calculated using the formula: 1/PP = [ADP] [Pi]/[ATP].

Statistical methods

Statistical analysis was performed using SPSS version 17.0. All continuous data are presented as mean ± standard deviation (±s). Normality was assessed using the Kolmogorov-Smirnov (K-S) test within both the RR-LSM group and the control group. For normally distributed data, independent samples t-tests were used to compare the RR-LSM group pre-treatment with the control group, and paired t-tests were applied to compare pre- and post-treatment results within the RR-LSM group. For non-normally distributed data, the Mann-Whitney U test was used for comparisons between the RR-LSM group and the control group, while the Wilcoxon signed-rank test was used for pre- and post-treatment comparisons within the RR-LSM group. A p-value of <0.05 was considered statistically significant.

RESULTS

Patient characteristics

The study group consisted of 8 males and 4 females, with an age range of 19 to 49 years (mean age: 32.0 ± 8.4 years) and a disease duration range of 1 to 108 months (mean: 32.5 ± 39.0 months). Detailed clinical data for each case are presented in Table 1.

Comparison of quadriceps ^{31}P-MRS parameters between control and RR-LSM groups before treatment

At baseline, patients with RR-LSM demonstrated significantly reduced levels of PCr, the PCr/ATP ratio, and PP compared to the healthy control group ($P < 0.05$).

Conversely, the Pi/PCr ratio and ADP levels were markedly elevated in the RR-LSM group relative to the control group ($P < 0.05$). No statistically significant differences were noted in the Pi, Pi/ATP ratio, or pH_{int} between the two groups ($P > 0.05$) (Table 2, Figures 1 and 2a).

Table 1: Patient characteristics in the RR-LSM group

ID	S	A	Sym	Pre-Tx MStr	EMG	Post-Tx MStr	D	Dur	VitB2
1	M	30	Myasthenia	Prx 4- Dt 5-	MCh	Prx 5- Dt 5	49	7	250
2	M	19	Myasthenia	Prx 4 Dt 5	Nrm	Prx 5- Dt 5	51	7.3	250
3	F	35	Myas, ExI	Prx 4 Dt 5	MCh	Prx 5 Dt 5	57	8.1	250
4	M	27	Myas, ExI	Prx 5- Dt 5	Nrm	Prx 5 Dt 5	65	9.3	250
5	M	31	Myas, ExI	Prx 4- Dt 5	NCh	Prx 5- Dt 5	54	7.7	300
6	F	30	Myasthenia	Prx 3 Dt 5-	SMCh	Prx 5- Dt 5	63	9.0	250
7	F	23	Myas, ExI	Prx 4- Dt 5-	Nrm	Prx 5- Dt 5	20	2.9	250
8	M	31	Myas, Myg	Prx 2 Dt 4	MCh	Prx 4- Dt 5-	64	9.1	250
9	F	49	Myasthenia	Prx 4+ Dt 5-	Nrm	Prx 5 Dt 5	49	7.0	300
10	M	44	Myas, ExI	Prx 4 Dt 5	SMCh	Prx 5 Dt 5	46	6.6	300
11	M	37	Myas, ExI	Prx 4 Dt 5	Nrm	Prx 5 Dt 5	39	5.6	250
12	M	28	Myas, ExI	Prx 5- Dt 5	Nrm	Prx 5 Dt 5	34	4.9	200

Note: ID: Patient Identifier; S: Sex (M: Male, F: Female); A: Age (years); Sym: Symptoms (Myas: Myasthenia, ExI: Exercise Intolerance, Myg: Myalgia); Pre-Tx MStr: Limb Muscle Strength Before Treatment (Prx: Proximal, Dt: Distal); EMG: Electromyography Results (MCh: Myopathic Changes, Nrm: Normal, NCh: Neuropathic Changes, SMCh: Suspected Myopathic Changes); Post-Tx MStr: Limb Muscle Strength After Treatment (Prx: Proximal, Dt: Distal); D: Days Between Two 31P-MRS Scans; Dur: Treatment Duration (weeks); VitB2: Vitamin B2 daily dosage (mg/d).

Table 2: Comparison of ^{31}P-MRS parameters between control and RR-LSM groups before treatment

Parameter	Control Group (Mean ± SD)	LSM Group (Mean ± SD)	P-value
Pi (mmol/L)	3.23 ± 0.62	3.78 ± 1.69	0.400
PCr (mmol/L)	24.84 ± 3.08	18.99 ± 3.25*	0.002
Pi/ATP	0.39 ± 0.08	0.46 ± 0.21	0.400
PCr/ATP	3.03 ± 0.38	2.32 ± 0.40*	0.002
Pi/PCr	0.13 ± 0.03	0.20 ± 0.10*	0.027
pH_{int}	7.07 ± 0.03	7.12 ± 0.11	0.482
ADP (μmol/L)	42.68 ± 13.97	84.24 ± 24.27*	0.002
1/PP ($\times 10^7$)	16.72 ± 6.56	36.39 ± 18.08*	0.012

*Note: * indicates a statistically significant difference (P < 0.05) when comparing the control and pre - treatment data.*

Comparison of quadriceps ^{31}P-MRS parameters between RR-LSM patients before and after treatment

Following treatment, significant improvements were observed in the PCr levels, PCr/ATP ratio, and PP in RR-LSM patients when compared to their pre-treatment values (P < 0.05). Additionally, ADP levels decreased significantly post-treatment (P < 0.05). However, no significant changes were detected in the Pi, Pi/ATP ratio, Pi/PCr ratio, or pH_{int} between pre- and post-treatment assessments in the RR-LSM group (P > 0.05) (Table 3, Figure 2b).

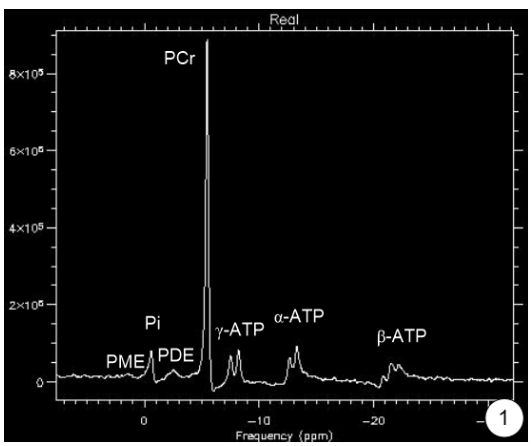

Figure 1: ^{31}P-MRS Spectrum of Normal Human Quadriceps Muscle
The spectrum illustrates the resonance peaks of seven phosphorus metabolites, ordered from left to right based on chemical shift: PME, Pi, PDE, PCr, and the γ-, α-, and β-forms of ATP. Notably, the peaks for PCr and ATP are more prominent, while those for PME and PDE are relatively less intense.

Comparison of quadriceps 31P-MRS parameters between RR-LSM patients after treatment and the control group

Post-treatment, RR-LSM patients exhibited persistent elevations in ADP and 1/PP compared to controls (P < 0.05), despite normalization of Pi-related ratios and pH_{int}. PCr and PCr/ATP showed improvement but did not fully recover to control levels (P = 0.087 and 0.102, respectively) (Table 4).

DISCUSSION

This study reveals that patients with RR-LSM exhibit substantial modifications in ^{31}P-MRS indicators, specifically marked declines in PCr, the PCr/ATP ratio, and PP, together with significant elevations in the Pi/PCr ratio and ADP levels.

PCr serves as a critical energy buffer; during periods of heightened ATP consumption, it supports rapid ATP replenishment via the creatine kinase reaction: PCr + ADP ⇌ ATP + Cr.[12] This process operates at a substantially faster rate compared to ATP generation through oxidative phosphorylation.[8,13] The observed reduction in PCr concentration in LSM patients might be due to impaired lipid utilization or aberrant oxidative metabolism, leading to mitochondrial dysfunction and accelerated PCr depletion. The notably decreased PP value implies compromised mitochondrial respiratory efficiency in these patients. Under circumstances of increased ATP use or inadequate production, intracellular ADP concentration increases to spur mitochondrial ATP synthesis.[14] Conversely, insufficient ADP levels would retard mitochondrial ATP production. The substantial rise in ADP levels observed in LSM patients could stem from lipid metabolism irregularities, causing relative ATP deficiency

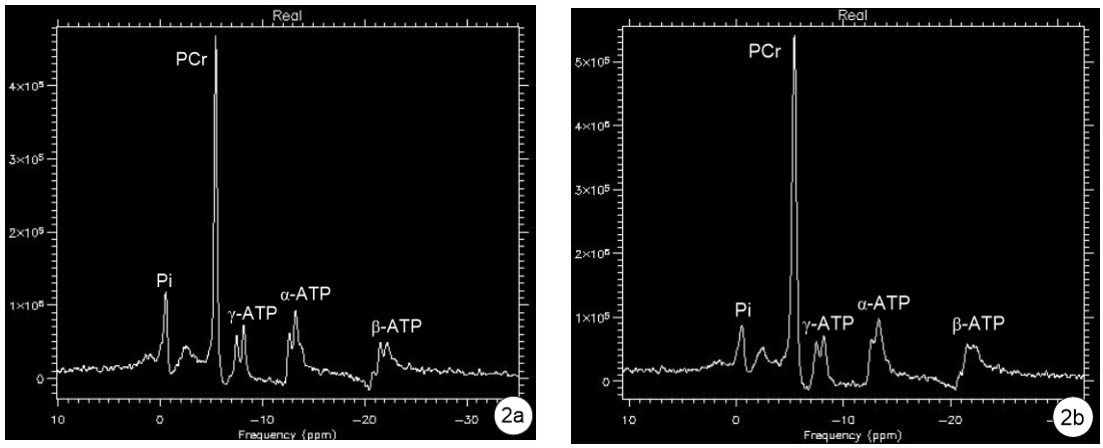

Figure 2: ^{31}P-MRS Spectrum of Quadriceps Muscle in LSM Patient
(a) Pre-treatment ^{31}P-MRS Spectrum: This spectrum shows a low PCr peak and a high Pi peak.
(b) Post-treatment ^{31}P-MRS Spectrum: After treatment, there is a significant increase in the PCr peak and a substantial decrease in the Pi peak.

within mitochondria, disrupting ATP homeostasis, and necessitating a compensatory elevation in ADP concentration to boost ATP synthesis rate.[15] These observations collectively underscore that ^{31}P-MRS is a valuable non-invasive method for identifying energy metabolism anomalies in RR-LSM patients. It is important to note that prior studies have shown that skeletal muscle ^{31}P-MRS changes in mitochondrial diseases and glycogen storage disorders primarily involve decreases in PCr and PP, accompanied by increases in Pi, the Pi/PCr ratio, and ADP.[16-18] This suggests that while ^{31}P-MRS exhibits high sensitivity in detecting skeletal muscle energy metabolism abnormalities, its diagnostic specificity may be constrained.

In recent times, MRS has emerged as an invaluable tool for assessing the efficacy of experimental therapeutic interventions.[19] Unlike invasive muscle biopsy procedures, MRS allows for repeated evaluations of treatment responses and longitudinal tracking of disease progression. In the pioneering study by Bendahan *et al*., two patients with mitochondrial myopathy displayed significant clinical enhancement after 10 months of coenzyme Q_{10} therapy, evidenced by an increased PCr/Pi ratio post-treatment, indicating improved mitochondrial function.[20] Additional studies have documented the use of ^{31}P-MRS in monitoring therapeutic responses to riboflavin, niacin, and cortisol treatments in mitochondrial disorders.[21,22] In our present investigation, LSM patients demonstrated significant alleviation in clinical symptoms after $VitB_2$ therapy, accompanied by notable increases in PCr, the PCr/ATP ratio, and PP, along with a significant reduction in ADP levels compared to pre-treatment values. These findings suggest an improvement in lipid metabolism irregularities and enhancement

Table 3: Comparison of ^{31}P-MRS parameters in LSM patients before and after treatment

Parameter	Pre-treatment (Mean ± SD)	Post-treatment (Mean ± SD)	P-value
Pi (mmol/L)	3.78 ± 1.69	3.52 ± 1.34	0.764
PCr (mmol/L)	18.99 ± 3.25	22.60 ± 3.79*	0.012
Pi/ATP	0.46 ± 0.21	0.43 ± 0.16	0.764
PCr/ATP	2.32 ± 0.40	2.76 ± 0.46*	0.012
Pi/PCr	0.20 ± 0.10	0.16 ± 0.06	0.266
pH_{int}	7.12 ± 0.11	7.09 ± 0.04	0.467
ADP (μmol/L)	84.24 ± 24.27	55.44 ± 16.50*	0.034
1/PP ($\times 10^7$)	36.39 ± 18.08	22.86 ± 10.11*	0.018

*Note: * indicates a statistically significant difference ($P < 0.05$) when comparing the pre - treatment and post - treatment data.*

Table 4: Comparison of 31P-MRS parameters between control and RR-LSM groups after treatment

Parameter	Control Group (Mean ± SD)	Post-treatment (Mean ± SD)	P-value
Pi (mmol/L)	3.23 ± 0.62	3.52 ± 1.34	0.512
PCr (mmol/L)	24.84 ± 3.08	22.60 ± 3.79	0.087
Pi/ATP	0.39 ± 0.08	0.43 ± 0.16	0.480
PCr/ATP	3.03 ± 0.38	2.76 ± 0.46	0.102
Pi/PCr	0.13 ± 0.03	0.16 ± 0.06	0.214
pH_{int}	7.07 ± 0.03	7.09 ± 0.04	0.302
ADP (μmol/L)	42.68 ± 13.97	55.44 ± 16.50*	0.032
1/PP ($\times 10^7$)	16.72 ± 6.56	22.86 ± 10.11*	0.026

*Note: * indicates a statistically significant difference (P < 0.05) when comparing the control and post - treatment data.*

of mitochondrial respiratory function consequent to therapeutic intervention.

The sustained elevation of ADP and 1/PP post-treatment suggests ongoing mitochondrial inefficiency, even in clinically asymptomatic patients. This aligns with prior reports of delayed metabolic recovery in mitochondrial disorders, where biochemical normalization may lag behind symptomatic improvement.[20] 31P-MRS thus provides critical insights into subclinical pathology, underscoring its utility for long-term monitoring and tailored therapeutic strategies.

This study has certain limitations. Although we employed rigorous clinical and histopathological criteria to distinguish RR-LSM from mitochondrial disorders, the absence of genetic confirmation (e.g., *ETFDH* or *COASY* sequencing) may introduce diagnostic uncertainty. Future studies integrating next-generation sequencing with functional assays would further enhance diagnostic precision.

In conclusion, this study employs ^{31}P-MRS to unveil significant energy metabolism alterations in RR-LSM patients, such as reduced PCr, PCr/ATP ratio, and PP, and increased Pi/PCr ratio and ADP levels, indicating mitochondrial dysfunction and impaired ATP homeostasis. Additionally, ^{31}P-MRS shows potential in evaluating therapeutic interventions, as VitB2 therapy led to clinical improvements, marked by increased PCr, PCr/ATP ratio, and PP, and reduced ADP levels, indicating enhanced mitochondrial function and normalized lipid metabolism. Overall, ^{31}P-MRS is a valuable tool for diagnosing and monitoring energy metabolism disorders in RR-LSM, offering insights into both diagnostic precision and therapeutic optimization.

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