Investigating Muscle Function After Stroke Rehabilitation with 31P-MRS: A Preliminary Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: New evidence reveals significant metabolic changes in skeletal muscle after stroke. However, it is unknown if 31P magnetic resonance spectroscopy (31P-MRS) can evaluate these metabolic changes. Our objective here was to investigate: (a) if muscle energy metabolism changes in the affected side; (b) if muscle energy metabolism changes after rehabilitation; and (c) if energy metabolism measured by 31P-MRS can reflect changes in the Modified Modified Ashworth Scale (MMAS) and Fugl-Meyer assessment-lower extremity (FMA-LE) scores after rehabilitation.

Material/Methods: We enrolled 13 patients with stroke symptoms and hemiplegia. Lower-limb motor status on the affected side was evaluated by FMA-LE and MMAS. The 31P-MRS measures included phosphocreatine (PCr), inorganic phosphate (Pi), PCr/Pi, and pH. We statistically compared these measures in the affected and unaffected lower leg muscles before rehabilitation and after rehabilitation on the affected side. Spearman correlational analyses was performed to determine correlations between change in energy metabolism and change in FMA-LE score and MMAS score after rehabilitation.

Results: PCr and PCr/Pi were significantly lower in the affected muscle compared to the unaffected muscle; however, there were no significant differences in Pi or pH. After rehabilitation, PCr, Pi, PCr/Pi, and pH did not significantly change. However, FMA-LE and MMAS score improved significantly after rehabilitation. Changes in energy metabolism measured by 31P-MRS had no correlation with FMA-LE change after rehabilitation. However, changes in PCr and PCr/Pi were correlated with change in MMAS score after rehabilitation.

Conclusions: 31P-MRS can evaluate changes in muscle energy metabolism in patients with stroke. PCr measured by 31P-MRS can reflect changes in MMAS after rehabilitation.

MeSH Keywords: Magnetic Resonance Imaging • Mitochondria, Muscle • Physical and Rehabilitation Medicine • Stroke

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Background

In 2010, stroke remained the leading cause of death and the second leading cause of life-years lost in China. There are 1.5 to 2 million new strokes every year in China [1,2]. Studies in China showed that the burden of stroke has gradually increased over the past 30 years, and compared to other similar surveys, China has the highest incidence and mortality rate of stroke in the world [3]. Despite advances in stroke management, many stroke patients exhibit significant residual impairments. Of these, the most common are motor dysfunctions contralateral to the side of the stroke lesion [4]. Rehabilitation treatment seeks to facilitate recovery of impaired muscle movement in patients with stroke [5]. As an essential part of the post-stroke care continuum, stroke rehabilitation can minimize disability and improve quality of life [6]. New evidence reveals significant structural and metabolic changes in skeletal muscle after stroke, and rehabilitation improves muscle function. However, it is unknown if 31P-MRS can evaluate the metabolic changes in patients with stroke. Our objective was to investigate: (a) if hemiparetic-side muscle energy metabolism, as measured by 31P-MRS, differs from that of the unaffected side; (b) if hemiparetic-side muscle energy metabolism changes after physical rehabilitation; and (c) if the energy metabolism measured by 31P-MRS can reflect changes in Modified Modified Ashworth Scale (MMAS) and Fugl-Meyer assessment-lower extremity (FMA-LE) after rehabilitation.

Material and Methods

Subjects

We enrolled 13 patients with first-time stroke, between June 2016 and May 2017, from the Stroke Rehabilitation Department at Beijing Hospital. The Institutional Review Board at Beijing Hospital approved this study and all patients provided written consent prior to participation. Patient inclusion criteria were: (1) between 40 and 60 years of age; (2) no known history of neurologic, psychiatric, or developmental disabilities; (3) lower-extremity motor impairment resulting from ischemic stroke and causing spastic hemiparesis; (4) completion of 5~6 weeks of physical rehabilitation after stroke; and (5) chronic stroke symptoms of 2~4 months duration. We established age limitations to control for potential age-related mitochondrial dysfunction [7]. Patients with chronic stroke were selected to ensure stability of patient condition and to avoid any potential confounders associated with early neurological recovery effects on skeletal muscle structure and function. Exclusion criteria were: (1) MRI contraindications; (2) other muscle metabolic diseases such as diabetes or mitochondrial myopathy; (3) patients in critical condition; (4) prior history of other organic

brain injury; (5) severe cognitive dysfunction affecting ability to understand; and (6) hemorrhagic stroke.

Patient characteristics

Thirteen patients (all males, age: 49.46±4.46 years) were eligible for the study. Stroke-related diagnoses included frontal lobe infarct (n=1), occipital lobe infarct (n=3), brainstem infarct (n=4), and basal ganglia infarct (n=5). All patients were medically stable during the examination period and all MR examinations were performed successfully in all patients, without any complications. All images were suitable for analyses. Overall MRI time was 20~30 min, and it took approximately 10 min to obtain all the measurements.

Lower-limb function status measure

One occupational therapist evaluated lower-limb function and spasticity for all subjects with stroke using the FMA-LE scores and the MMAS scores. The same occupational therapist performed all clinical evaluations to avoid inter-rater variation. We acquired 31P-MRS data, FMA-LE scores (mobility and locomotion status), and MMAS at 2 time points: before and upon completion of 5~6 weeks rehabilitation.

Fugl-Meyer assessment (FMA) is a highly reliable and valid tool for assessing lower-limb motor recovery after stroke, and is widely used in rehabilitation settings [8,9]. FMA-LE total score ranges from 0 (no motor function) to 34 (good motor recovery). The FMA-LE evaluates movement, coordination, and reflex action of the hip, knee, and ankle. Each domain consists of multiple items, each scored on a 3-point ordinal scale (0=cannot perform, 1=performs partially, 2=performs fully).

The MMAS is the most commonly used clinical measure of spasticity and is easy to use largely because it requires no equipment. Spasticity of ankle plantar flexors was evaluated using the Modified Modified Ashworth Scale (MMAS). The MMAS describes the severity of spasticity as follows [10]: 0=no increase in muscle tone; 1=Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion when the affected part(s) is moved in flexion or extension; 2=Marked increase in muscle tone, manifested by a catch in the middle range and resistance throughout the remainder of the range of motion, but affected part (s) easily moved; 3=Considerable increase in muscle tone, passive movement difficult; and 4=Affected part(s) rigid in flexion or extension.

Rehabilitative treatment

All patients received 5~6 weeks of physical rehabilitation therapy protocol. We used various devices such as paddle cycle,
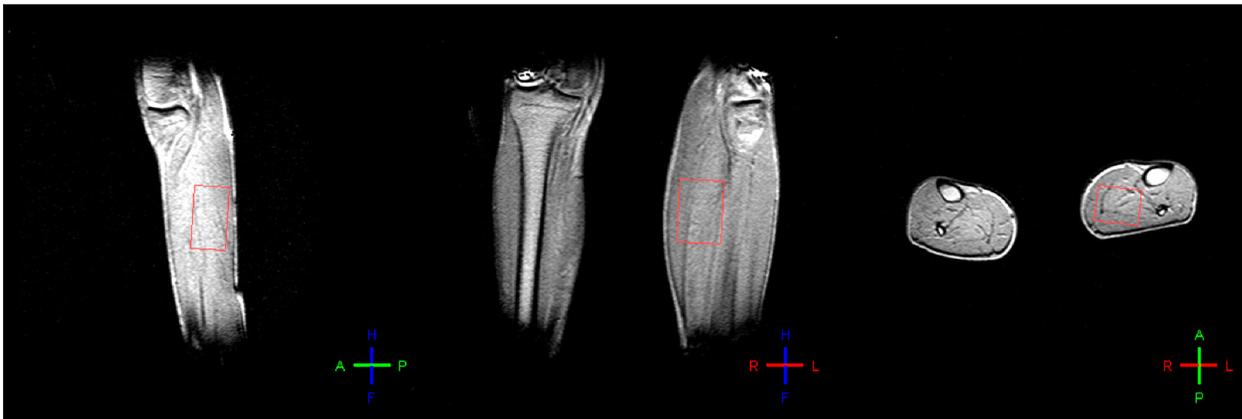
Figure 1. The volumes of interest (VOI) were selected in axial, coronal, and sagittal orientation and positioned in the human soleus/gastrocnemius complex.

rocker board, foot roller, and neuromuscular electrical stimulation to assist lower-extremity motor control. Patients received occupational or speech therapy as needed.

31P-MRS

31P-MRS measures were obtained using a 3.0-T MRI system (Achieva, Philips Medical Systems, Best, the Netherlands). Spectroscopic measurements used a A31P transmit-receive surface coil, with a diameter of 14 cm, provided by the manufacturer. Subjects fasted and abstained from caffeinated beverages for at least 6 h and from exercise for at least 2 h prior to testing. We fastened the coil to the underbelly of the calf muscle, at the point of maximum circumference. 31P-MRS parameters were repetition time (TR)=4500 ms, echo time (TE)=0.1 ms, 2500 Hz spectral bandwidth, and 8 phase cycles to encode ISIS voxels. We selected volumes of interest (VOI) from axial, coronal, and sagittal orientations within the human soleus/gastrocnemius complex. VOIs were as large as possible and were individually determined for each muscle in each subject (Figure 1). Phosphomonoester (PME), phosphodiester (PDE), phosphocreatine (PCr), adenosine-triphosphate (α-, β-, and γ-ATP), and inorganic phosphate (Pi) peaks were determined from the spectral curves of 31P-containing molecules (Figure 2). After manual determination of eligible peaks by the operator, areas under the peak (amplitudes in the time domain) were automatically calculated by the software. The area under the peak of compound represents the relative content of material. Absolute concentrations of PCr and Pi were calculated using γ-ATP as an internal standard by assuming an γ-ATP concentration of 8.2 mmol/l [11]. PCr/Pi were calculated from the relative area of appropriate peaks from each spectrum after processing. Intramuscular pH was calculated based on the chemical shift (dis) of Pi, relative to PCr, in parts per million: pH=6.75 + log (dis-3.27)/(5.69-dis) [12].

Statistical analysis

The Statistical Package for the Social Sciences version 19.0 (SPSS Inc., Chicago, USA) was used for all statistical analyses. We tested continuous variable for normal distribution of the data using the Kolmogorov-Smirnov test. PCr, Pi, PCr/Pi, and pH values were statistically compared between the affected and unaffected sides before rehabilitation, and between before and after rehabilitation on affected side, using paired t tests. FMA-LE scores were statistically compared before and after rehabilitation on the affected side using paired t tests. Changes in MMAS scores between before and after rehabilitation on affected sides were determined using the Mann-Whitney U test. We used Spearman correlational analyses to investigate correlations between changes in energy metabolism (δPCr, δPi, δPCr/Pi, and δpH) and changes in FMA-LE and MMAS (δFMA-LE and δMMAS) after rehabilitation. Continuous variable data are presented as means ±SD. All reported p-values are two-sided and considered to indicate statistical significance when p<0.05.

Results

Comparison between affected and unaffected sides before rehabilitation

The mean values of energy metabolism of muscles on the affected and unaffected sides before rehabilitation are summarized in Table 1. PCr and PCr/Pi content were lower in affected than in unaffected muscles (29.05±3.50 *vs.* 30.22±3.60, 5.71±0.60 *vs.* 6.11±0.82, p=0.046 and 0.008, respectively); however, there were no significant differences in Pi or pH (5.11±0.55 *vs.* 4.98±0.47, 7.01±0.02 *vs.* 7.02±0.03, p=0.098 and 0.335, respectively) (Figures 2, 3).
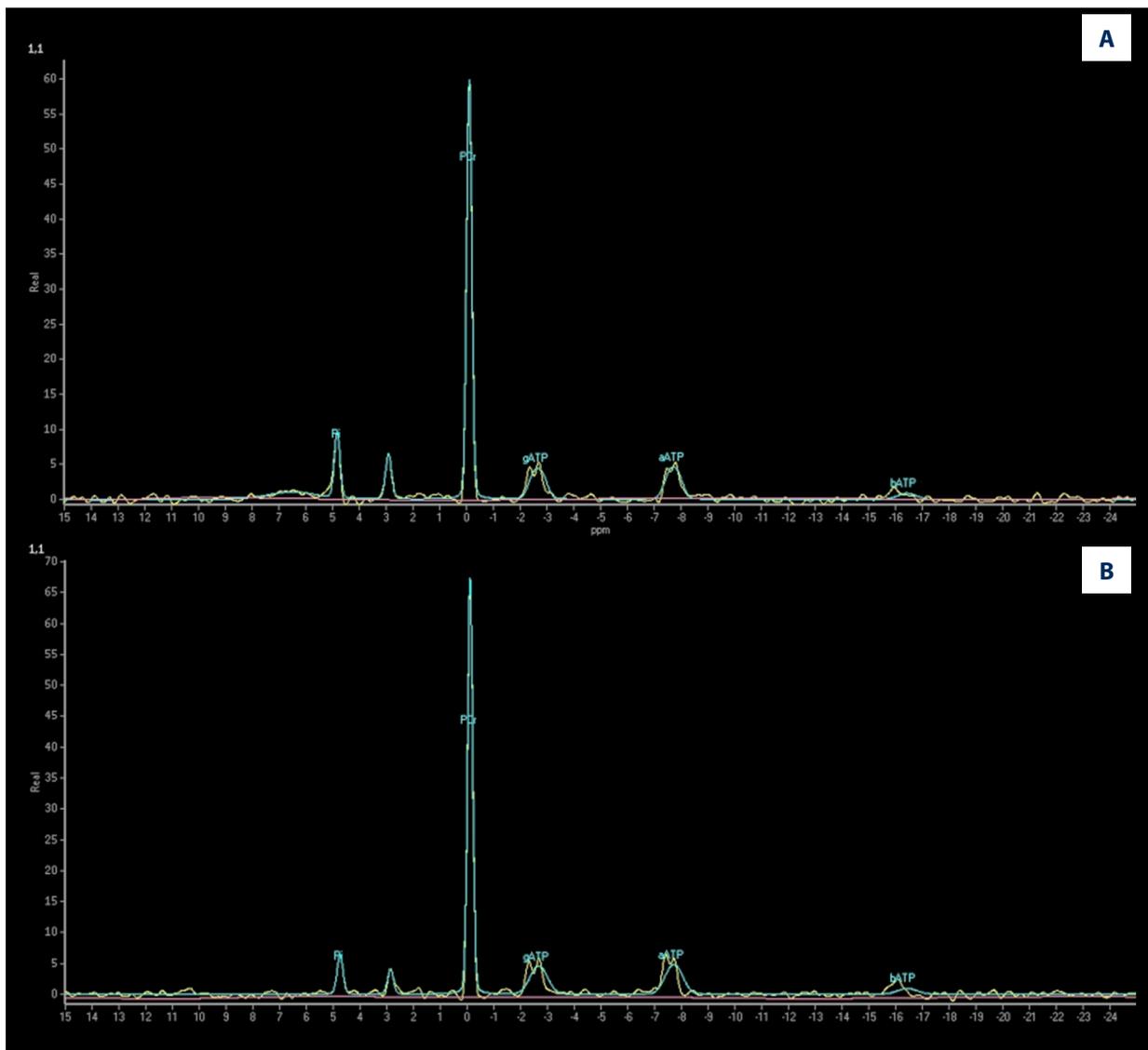
Figure 2. Representative spectra recorded in a patient with stroke: energy metabolism of muscles in affected side (**A**) and unaffected side (**B**). PCr and PCr/Pi ratio are lower in the affected side.

Table 1. Metabolic variables of affected and unaffected side.

Variable	Affected side	Unaffected side	T	P-value
PCr (mmol/L)	29.05±3.50	30.22±3.60	−2.23	0.046
Pi (mmol/L)	5.11±0.55	4.98±0.47	1.80	0.098
PCr/Pi	5.71±0.60	6.11±0.82	−3.20	0.008
pH	7.01±0.02	7.02±0.03	−1.004	0.335

Data are shown as mean ±SD (range). PCr – phosphocreatine; Pi – inorganic phosphate.

Comparison between before and after 5~6 weeks of rehabilitation

The mean energy metabolism values of the affected side before and after rehabilitation are summarized in Table 2. After rehabilitation, PCr, Pi, PCr/Pi, and pH exhibited no significant changes (29.05±3.50 *vs.* 29.47±2.02, 5.11±0.55 *vs.* 5.09±0.50, 5.71±0.60 *vs.* 5.83±0.60, 7.01±0.02 *vs.* 7.02±0.04, p=0.528,
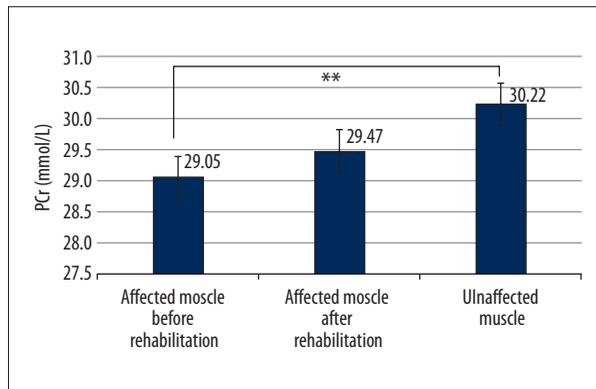
Figure 3. The content of PCr measured by 31P-MRS in affected muscle is significantly lower than in the unaffected muscle. The content of PCr in the affected muscle increased after rehabilitation; however, there was no significant difference.

0.844, 0.324, and 0.317, respectively) (Figure 3). However, FMA-LE scores changed significantly after rehabilitation, from 16.92±3.33 to 20.38±3.18 (p=0.001). MMAS decreased significantly after rehabilitation (p=0.01). The MMAS of the affected side before and after rehabilitation are summarized in Table 3.

The correlation of changes in metabolism with changes of FMA-LE and MMAS before and after rehabilitation

Although FMA-LE scores increased significantly after rehabilitation, the Spearman correlation analysis indicated that changes in PCr, Pi, PCr/Pi, and pH measured by 31P-MRS had no correlation with FMA-LE change after rehabilitation (r=−0.02,

−0.39, 0.32, 0.30; p=0.95, 0.19, 0.29, and 0.31, respectively) (Figure 4A, Table 4).

The Spearman correlation analysis indicated changes in PCr and PCr/Pi measured by 31P-MRS had significant correlation with change in MMAS after rehabilitation (r=−0.61, p=0.03; r=−0.59, p=0.03, respectively); however, changes in Pi and pH had no significant correlation with changes in MMAS (r=−0.06, p=0.85; r=0.33, p=0.28, respectively) (Figure 4B, Table 5).

Discussion

New evidence reveals significant structural and metabolic changes in skeletal muscle after stroke, and rehabilitation improves muscle function. Biomarkers that track muscle recovery in patients with stroke who are receiving rehabilitation would add information helpful to clinical practice. 31P-MRS is widely used for non-invasive measurement of muscle metabolism and can quantifiably estimate mitochondrial capacity with high precision and accuracy [13,14]. However, it is unknown if 31P-MRS can evaluate the change in muscle function in patients with stroke. To the best of our knowledge, this is the first study to examine muscle energy metabolism in patients with stroke by use of 31P-MRS, and is also the first to analyze the correlation of changes in energy metabolism with FMA-LE and MMAS after rehabilitation.

Stroke subjects offer a unique opportunity for studying the biological changes in hemiparetic muscle because the subjects' nonparetic muscle serves as their own internal controls. Kim

Table 2. Metabolic variables and FMA-LE Score before and after rehabilitation on affected side.

Variable	Before rehabilitation	After rehabilitation	T	P-value
PCr (mmol/L)	29.05±3.50	29.47±2.02	−0.65	0.528
Pi (mmol/L)	5.11±0.55	5.09±0.50	0.20	0.844
PCr/Pi	5.71±0.60	5.83±0.60	−1.03	0.324
pH	7.01±0.02	7.02±0.04	−1.04	0.317
FMA-LE Score	16.92±3.33	20.38±3.18	−9.86	0.001

Data are shown as mean ±SD (range). PCr – phosphocreatine; Pi – inorganic phosphate; FMA-LE – Fugl-Meyer assessment lower extremity.

Table 3. MMAS scores before and after rehabilitation on affected side.

MMAS scores	0	1	2	3	4
Before rehabilitation	0	2	7	3	1
After rehabilitation	2	6	4	1	0

MMAS scores before and after rehabilitation on affected side, P=0.01.
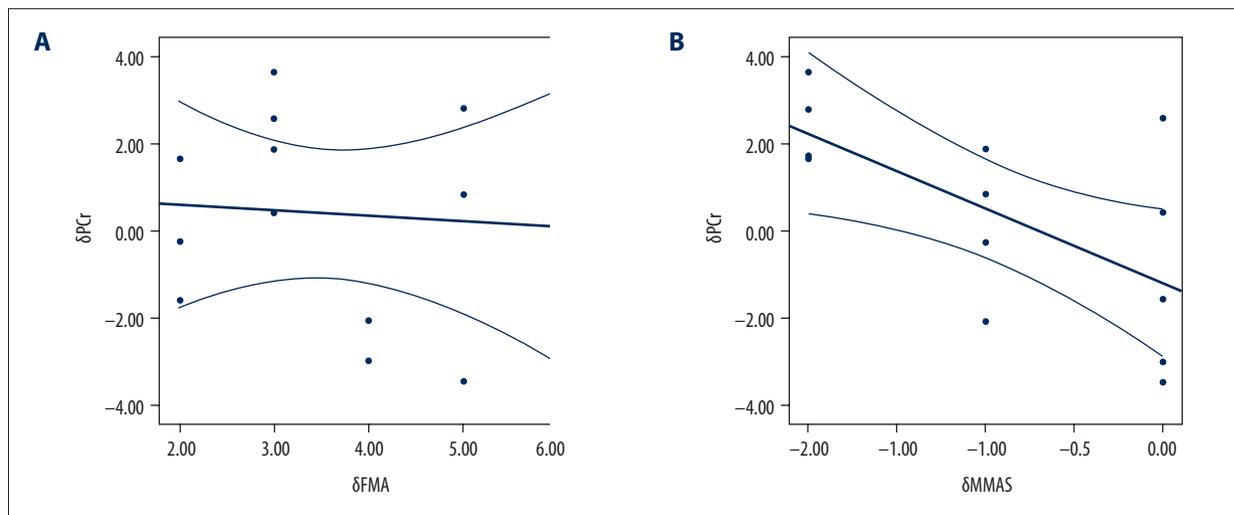
Figure 4. Scatter plot of correlation showing the change of PCr with FMA-LE (**A**) (r=−0.02, P=0.95) and MMAS scores (**B**) (r=−0.61, P=0.03) before and after rehabilitation.

Table 4. Spearsman correlation coefficients (r) for the correlation between change of energy metabolism (δPCr, δPi, δPCr/Pi, δpH) and change of FMA-LE (δFMA-LE) before and after rehabilitation.

Variable	Correlation coefficient(r)	P-value
δPCr (mmol/L)	−0.02	0.95
δPi (mmol/L)	−0.39	0.19
δPCr/Pi	0.32	0.29
δpH	0.30	0.31

δPCr, δPi, δPCr/Pi, δpH – change of energy metabolism before and after rehabilitation. δFMA-LE – change of Fugl-Meyer assessment lower extremity before and after rehabilitation.

Table 5. Spearsman correlation coefficients (r) for the correlation between change of energy metabolism(δPCr, δPi, δPCr/Pi, δpH) and change of MMAS (δMMAS) before and after rehabilitation.

Variable	Correlation coefficient(r)	P-value
δPCr (mmol/L)	-0.61	0.03
δPi (mmol/L)	-0.06	0.85
δPCr/Pi	-0.59	0.03
δpH	0.33	0.28

δPCr, δPi, δPCr/Pi, δpH – change of energy metabolism before and after rehabilitation. δMMAS – change of Modified Modified Asworth Scale for ankle plantar flexors before and after rehabilitation.

et al. evaluated diaphragm thicknesses during respiration by ultrasonography and confirmed that the diaphragm thickness of the affected side was decreased, and the inspiratory muscle functions were also significantly reduced compared to the unaffected side [15]. Compared with the unaffected side, our results indicated lower PCr and PCr/Pi content in leg muscles on the affected side. PCr is a phosphorylated creatine molecule that is a rapidly deployed reserve of high-energy phosphates in skeletal muscle. PCr plays an important role in skeletal muscle energy metabolism, and decreased intracellular PCr concentration can occur following muscle injury. Although little is known about skeletal muscle phenotypic abnormalities after stroke, the decrease in PCr suggests changes in muscle physiology and composition. Hemiparetic-side skeletal muscle changes after stroke can include gross muscular atrophy, increased intramuscular fat, and a shift toward fast myosin heavy chain phenotypes, with reduced muscle oxidative

capacities [16]. Abnormal expression of several major gene categories or clusters may present in hemiplegic leg muscles, including genes that regulate muscle metabolism, contractile proteins, mitogenesis and growth factors, inflammation, metabolism, and signal transduction pathways [16]. Changes in these mechanisms are complex, and have been the focus of numerous studies. Ryan et al. suggested that the myostatin cascade signaling pathway is involved in muscle physiology changes post-stroke [117]. Myostatin is a key negative regulator of muscle fiber size, and there is significantly more myostatin in paretic limbs compared to nonparetic limbs [17,18]. In addition, post-stroke muscle composition changes, such as decreases in type I muscle fibers, may also account for decreased PCr on the affected side. Type I muscle fibers have a mitochondrial density that is 2- to 3-fold higher compared to type II fibers [19]. After stroke, affected muscles demonstrate

decreases in fiber size, type I fiber density, and capillary density in hemiparetic limbs of stroke patients [20]. Mitochondrial dysfunction is more prominent in muscles with lower type I muscle fiber content [7]. PCr/Pi ratio decreased due to the decrease of PCr in affected muscle.

In our study we found no change in the Pi or pH between the affected and unaffected sides. Pi levels change when depleted for ATP regeneration [21]. Unlike in patients with hyperactivity [22], metabolic rates do not change in patients with stroke, so no Pi changes were observed in our study. Lactic acid accumulates in muscles after movement or activity [23], changing the muscle pH. We did not have patients perform dynamic exercise; therefore, pH did not change during rest. PDE is associated with cell membrane decomposition, while PME reflects phospholipid metabolites, which are related to cell membrane stability. PDE and PME content is low in normal skeletal muscle, and PDE and PME are poorly correlated with motor function. Therefore, we did not compare PDE and PME content.

FMA-LE scores increased significantly after rehabilitation, consistent with earlier findings [24], indicating that rehabilitation treatment effectively causes motor improvement. However, PCr and PCr/Pi ratio did not change after rehabilitation as we expected. There are several potential reasons for this. First, our study follow-up period was short and recovery may require more time. Second, the fact that muscle motor function on the affected side had not fully recovered suggests that rehabilitation treatment for these patients is a long process. Third, the difference in PCr and PCr/Pi ratio is small after rehabilitation, and P31-MRS at 3.0T MR was not sensitive enough to detect this difference.

Previous studies suggested that sex, age, side, and type of stroke do not predict stroke outcome [25–27]. To control for other factors that affect motor outcome, we limited duration of stroke to the chronic (2~4 months post-onset) phase of stroke recovery. Since subjects' nonparetic leg muscle served as their own internal control, we inferred that change in PCr reflected abnormal muscle metabolism on the affected side. However, the Spearman correlation analysis indicated that changes in energy metabolism measured by 31P-MRS had no correlation with FMA-LE change before and after rehabilitation. We infer that the outcome of patients with stroke after rehabilitation treatments are complicated, and mainly involve brain tissue lesions [28,29], including the lesion location and size; nevertheless, changes in energy metabolism mainly reflected the metabolism of muscle. Therefore, the change in energy metabolism before and after rehabilitation cannot reflect FMA-LE change after rehabilitation.

Skeletal muscle following stroke may be affected by the altered central neural activation and spasticity. The spasticity has many influences on the muscle, causing both reduced motor unit recruitment and excessive cocontraction, with an overactive stretch reflex [20]. Passive joint stiffness in subjects with chronic stroke, including increased resistance to passive movement, have a multitude of functional implications, including alterations in gait [30]. Fortunately, rehabilitation therapies to improve passive joint stiffness can improve functional outcomes. The pathophysiology of spasticity can occur as a result of abnormalities on different levels, including muscular and spinal properties, as well as supraspinal mechanisms [31]. Patients in our study had a significant recovery after the rehabilitation treatment based on the MMAS scores, which was consistent with previous research results [32,33]. In addition, the Spearman correlation analysis indicated that changes in PCr and PCr/Pi-measured 31P-MRS were negatively correlated with MMAS change before and after rehabilitation. We infer that spasticity can cause muscle weakness, and with the MASS recovery, the degree of weakness was reduced, causing more reserve of energy sources such as PCr [32].

We acknowledge several limitations of our study. First, the structure and metabolism in skeletal muscle of the unaffected side can also change after stroke, so we did not use normal subjects as normal controls. Although little evidence shows sex differences in energy metabolism in skeletal muscle, the size of the sample was small and there were no women in our study. Second, the location and features of lesions in the brain affected by stroke and time of onset may be associated with lower-limb movements. Third, because patients with stroke experience difficulty controlling lower-limb movements, we did not use 31P-MRS to complete the dynamic exercise study. Future studies that include larger participant samples are needed to comprehensively evaluate rehabilitation effects in this field.

Conclusions

We have shown not only that PCr and PCr/Pi measured by 31P-MRS decreased on the affected side, but also that PCr reflected changes in MMAS. This finding supports the pathophysiological changes of muscles after stroke. Rehabilitation treatment facilitates recovery of impaired muscle movement in patients with stroke; however, the outcome differs among patients. Thus, 31P-MRS, as a non-invasive measurement of muscle metabolism, is especially pertinent for patients in chronic stage ready for rehabilitation treatment, which can provide an adequate diagnostic and follow-up protocol in patients with stroke.

Conflicts of interest

There are no conflicts of interest.

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