ORIGINAL ARTICLE

Quantification of Muscle Metabolites Using Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) for Incomplete Spinal Cord Injury Patients: Preliminary Study

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ABSTRACT

Introduction: This preliminary study aimed to non-invasively evaluate choline (CHO), creatine (Cr) and intramyocellular lipid (IMCL) metabolites in skeletal muscles at pre- and post-functional electrical stimulation (FES) exercise among incomplete spinal cord injury (SCI) of American Spinal Injury Association Impairment Scale (ASIA-AIS) D patients using proton magnetic resonance spectroscopy or ^{1}H-MRS. **Methods:** These metabolites were measured from the vastus lateralis and semitendinosus muscles of three incomplete SCI ASIA-AIS D patients who completed the FES exercise and later underwent 3 Tesla (T) MRI (repetition time/echo time; TR/TE of 3500ms/100ms, field-of-view; FOV of 20cm, slice thickness of 6mm) and ^{1}H-MRS (TR/TE of 2000ms/31ms, voxel size of 20mm x 20mm x 35mm). **Results:** Out of those selected metabolites, only CHO value of vastus lateralis showed a statistically significant difference between pre- and post FES exercise ^{1}H-MRS scanning ($p = 0.01$). **Conclusion:** Therefore, this preliminary finding has postulated that the quantification of muscle metabolites using ^{1}H-MRS imaging could be used as a potential indicator in evaluating the muscle strength for incomplete SCI ASIA-AIS D patients after the completion of FES cycling exercise.

Keywords: ^{1}H-MRS, Muscle metabolites, CHO, Cr, IMCL, FES

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INTRODUCTION

Magnetic resonance spectroscopy (MRS) is a non-harmful companion imaging for conventional magnetic resonance imaging (MRI). Conventional MRI focuses on the water proton spatial distribution, while MRS determines the chemical content of different elements such as hydrogen element (^{1}H), carbon element (^{12}C), and phosphorus element (^{31}P). Furthermore, MRS is valuable in evaluating muscle metabolites because metabolite nuclei have their chemical properties and environment, which determine the frequency in the MR spectrum that showing peaks for all types of metabolites. Therefore, MRS can access a variety of biological composites for many tissue types (1).

MRS in human skeletal muscle is different from the other applications like brain MRS, which is a major region for spectroscopy procedure. Indeed, MRS of human skeletal muscle is more metabolically organised, and precise localisation is not a matter of concern due to geometrical differences of any lesion. However, an accurate volume measurement is required for some applications to exclude abundant signals from different substances for the detachment of metabolite signals, for example, intra- and extramyocellular lipids (2).

Proton magnetic resonance spectroscopy or ^{1}H-MRS has become the favoured approach in the musculoskeletal imaging for both clinical and research fields (3,4). When whole-body MRI magnets equipped with gradients are made available, radiologist or spectroscopist can insert a small voxel position in the skeletal muscle. Although ^{1}H-MRS is very complicated, it gives so much information, including some aspects of the physiological changes like in exercise and physical activity (2). ^{1}H-MRS shows a high-resolution spectrum from muscles, and the peaks available are choline (CHO), creatine (Cr), intramyocellular lipid (IMCL), and extracellular lipid (EMCL) metabolites.

Metabolites have different functions in the human skeletal system. In the ^{1}H-MRS technique, metabolites that available to be quantified in the musculoskeletal system are CHO, Cr, and IMCL. CHO is known as a marker of malignancy and potentially differentiate malignant from benign musculoskeletal tumours. When

a person is running or cycling, a reduction of CHO occurs and leads to a decrease in acetylcholine release; thus, affect the person's performance (5). As for Cr, this metabolite acts as a tool to detect and characterise biochemical abnormalities in the muscle. It can access the anomaly in muscle anatomy and function. A study reported that after a vigorous exercise, a replenishment of Cr took from less than five minutes to 15 minutes depending on the reduction of Cr store (6). Meanwhile, IMCL is chosen instead of EMCL (extramyocellular lipid) because IMCL has a broad spectrum that potentially contributes to clinical and research fields. Besides, the metabolite is essential to evaluate muscle conditions in exercise physiology (7).

American Spinal Injury Association or ASIA has issued the American Spinal Injury Association Impairment Scale (ASIA-AIS), which is known as a grading and classification system that helps in identifying a group of key muscle and sensory levels. This system can accurately categorise the patient of either the injury is complete or incomplete (8), and it has become a gold standard in evaluating spinal cord injuries (9). Incomplete spinal cord injury (SCI) refers to a patient presented with some degrees of preserved sensory or motor function below the injury level. Incomplete SCI can further be graded as incomplete SCI B, C, and D. Each of these grades indicates incomplete SCI lesions with consideration of motor and sensory functions (10).

Functional electrical stimulation (FES) cycle is a cycling procedure that uses electrical flow to excite motor functions throughout an operative activity such as cycling. FES exercise helps in returning of type II fast-twitch muscle fibres back to type I slow-twitch muscle fibres (11). After SCI, people are less active because of the lack of functions in motor and the sensory level below the injury, thus resulting in decreased type I muscle fibres. FES exercise repetition can lead to improvements in sensory and motor functions, along with cortical reorganisation. This reorganisation occurs when movement is generated in paralysed muscle and may help in returning to ambulation (12). Therefore, this study aimed to non-invasively quantify choline (CHO), creatine (Cr) and intramyocellular lipid (IMCL) metabolites in human skeletal muscles at pre- and post-functional electrical stimulation (FES) exercise among incomplete spinal cord injury (SCI) of American Spinal Injury Association Impairment Scale (ASIA-AIS) D patients using proton magnetic resonance spectroscopy (^{1}H-MRS).

MATERIALS AND METHODS

Spinal cord injury human subjects

The pre- and post-FES exercise sessions were conducted at University Malaya Medical Centre (UMMC), and three incomplete SCI AIS D patients were recruited (mean age = 48 ± 18 years). Prior to conducting the data collection, an institutional ethical approval of human research was obtained (MREC ID NO: 201682-4100). All subjects were made to clearly understand the purpose of this research study and written consent and MRI safety screening form were recorded for each of them before proceeding with the imaging procedure.

MRI and ^{1}H-MRS protocols

MRI, as well as ^{1}H-MRS scans, had been performed at the Centre for Diagnostic Nuclear Imaging (CDNI), Universiti Putra Malaysia (UPM) using a Siemens Magnetom Prisma 3 Tesla (T) MRI scanner with a four-element 'body' coil on upper legs to include quadriceps (vastus lateralis), and hamstrings (semitendinosus) muscles. The reason why metabolites were evaluated from these muscles because both are lower limb muscles and needed to do regular activities such as standing and sitting (13). The subject was in supine with feet first, and the coil was placed over the thigh, which covered the anterior superior iliac spine or ASIS down until knee joints (from the pelvic area to knees). All three SCI patients underwent through less than 25 seconds localiser to localise and plan for the following sequences. T2 axial MRI weighted anatomical imaging with TR of 3500ms and TE of 100ms, the field-of-view (FOV) of 20cm, and slice thickness of 6mm. These parameters had been chosen for accurately ^{1}H-MRS voxel localisation inside the selected muscle. ^{1}H-MRS voxel was cautiously planned on chosen muscles to steer clear of blood vessels, adjacent muscle areas, and fats like extramyocellular lipid and the femur bone. A single-voxel point-resolved spectroscopy sequence or PRESS with TR of 2000ms, TE of 31ms, and a voxel size of 20mm x 20mm x 35mm was chosen and planned within the selected muscles (vastus lateralis and semitendinosus). Choline (CHO), creatine (Cr), and intramyocellular lipid (IMCL) metabolite values were recorded and evaluated. ^{1}H-MRS metabolite results are in the form of individual peaks of spectra and stated as parts-per-million (ppm). The metabolite values in both muscles were compared pre- and post-FES exercise to discover the distribution design in the muscles. By evaluating these metabolite signals and values, ^{1}H-MRS can provide information for muscles characterisation and physiology.

Exercise protocol

All subjects were recruited and did the FES cycling exercise at UMMC. They were aware of this study as the researcher advertised it through UMMC staffs and flyers. The volunteers called the researcher and planned for dates to scan and exercise. After all subjects underwent pre-MRI and ^{1}H-MRS scans at the resting stage, they were required to perform FES cycling exercise as an intervention. The setup included four sets of electrodes placed over quadriceps and hamstring muscles. There were 40 minutes duration of FES exercise with 20 minutes of stimulation and a 10 minute warm-up and cool-down periods with no stimulation during each FES exercise session. The intensity of stimulation was

adjusted according to the subject's comfortability level. Initially, each subject had a higher intensity FES value. With continued FES exercise in two days per week for six weeks, the intensity was reduced to a lower FES value. After the subject finished the 40 minutes FES exercise on the sixth week, the post-MRI and ^{1}H-MRS imaging were done again with similar protocols as of the pre-scans at CDNI.

Statistical analysis

Statistical paired t-test was applied in this study to compare differences in measured CHO, Cr, and IMCL metabolites in pre- and post-FES exercised muscle groups in all three subjects. The statistical analysis was conducted at the 95% confidence level and a p-value less than 0.05 was considered to indicate a statistically significant difference using IBM SPSS Statistics Base version 23.

RESULTS

Fig. 1 and Fig. 2 provided examples of the ^{1}H-MRS spectra as well as the MRI images of voxel localisation acquired from a subject at 3T in the vastus lateralis and semitendinosus muscles. All three subjects completed the pre- and post- MRI and ^{1}H-MRS scanning and FES exercise that was done between both scanning. From a subject, the vastus lateralis and semitendinosus muscles were assessed, and each muscle contributed to three metabolites. The scanning successfully generated a total of 18 spectra for all subjects. The spectra were shown in parts per million (ppm), and all used the same spectral resolution. Table I tabulates the metabolite values of choline (CHO), creatine (Cr), and intramyocellular lipid (IMCL) for pre- and post-FES exercise in the vastus lateralis and semitendinosus muscles. Out of all metabolites, only the CHO metabolite value in vastus lateralis showed a

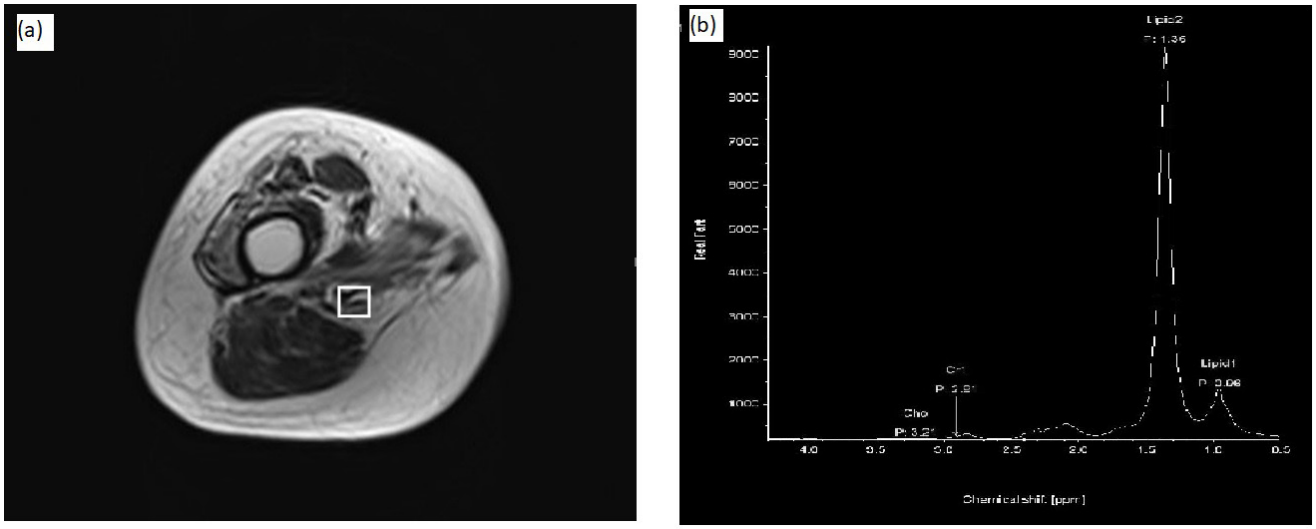

Figure 1: MRI and ^{1}H-MRS images of right vastus lateralis muscle. Representative (a) MRI T2 weighted image and the voxel localisation (white box) followed with (b) the vastus lateralis curve from ^{1}H-MRS of a SCI ASIA-AIS D subject with 9 years injury. ^{1}H-MRS graph showing peaks of the CHO, Cr, and IMCL metabolite values.

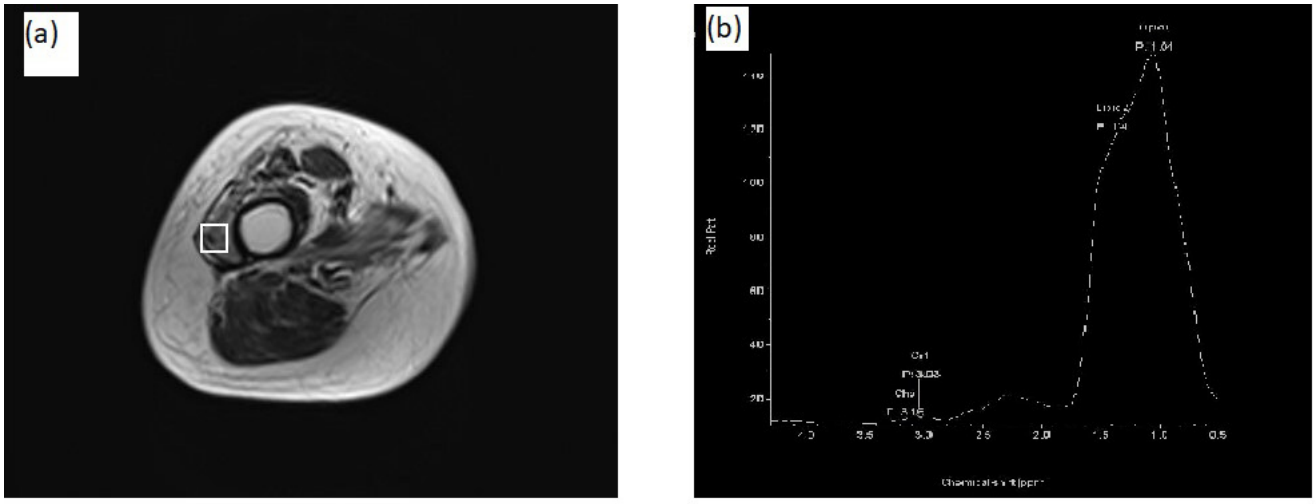

Figure 2: MRI and ^{1}H-MRS images of right semitendinosus muscle. Representative (a) MRI T2 weighted image and the voxel localisation (white box) followed with (b) the semitendinosus curve from ^{1}H-MRS of a SCI ASIA-AIS D subject with 9 years injury. ^{1}H-MRS graph showing peaks of the CHO, Cr, and IMCL metabolite values.

Table I: CHO, Cr, and IMCL metabolite values at pre- and post-exercise in the vastus lateralis and semitendinosus muscles

Muscles – Metabolites	Mean ± Standard Deviation (SD)		p-value	Remark
	Pre-exercise	Post-exercise		
Vastus lateralis – CHO	3.34 ± 0.01	3.16 ± 0.03	0.01	Significant
Vastus lateralis – Cr	3.14 ± 0.01	3.02 ± 0.05	0.05	Not Significant
Vastus lateralis – IMCL	1.63 ± 0.22	1.52 ± 0.14	0.43	Not Significant
Semitendinosus – CHO	3.34± 0.00	3.30 ± 0.08	0.42	Not Significant
Semitendinosus – Cr	3.14 ± 0.01	3.10 ± 0.08	0.42	Not Significant
Semitendinosus – IMCL	1.61 ± 0.14	1.42 ± 0.12	0.26	Not Significant

statistically significant difference between pre and post FES exercise (p = 0.01). However, the metabolites values of all three selected muscles depicted a decreasing trend after the completion of FES exercise. This could indicate the effectiveness of FES exercise to SCI.

There are differences between metabolite peaks at pre-scanning in vastus lateralis and semitendinosus muscles obtained from the three incomplete SCI ASIA-AIS D patients. IMCL metabolite from the ^{1}H-MRS peak curves showed deficient levels at 1.24 ppm in the semitendinosus muscle. However, the IMCL value was higher in the vastus lateralis muscle. Meanwhile, CHO amplitude from the ^{1}H-MRS peak curves at 3.14 ppm showed low levels in the vastus lateralis differed from the semitendinosus. However, Cr metabolite showed the same value in both muscles at the pre-scanning at the resonance of 3.14 ppm.

DISCUSSION

Since there were differences in the metabolite values between pre-and post-scanning, this signifies that ^{1}H-MRS at 3T is feasible in evaluating the strength of lower leg muscles for incomplete SCI subjects. Indeed, some studies have proposed that ^{1}H-MRS can be an alternative technique that is non-invasive to interpret the information about the metabolites in the human muscles (14,15). One of the studies believes that muscle biopsy is not suitable for repetitive assessment since the methodology required metabolite's readings before and after the training process (15). Furthermore, a muscle biopsy is also susceptible to human error, specifically the process of specimens (16).

In the clinical area, ^{1}H-MRS imaging is a more convenient setting as compared to other elements like phosphorus (31P), carbon (12C) or sodium (23Na) spectroscopy since most of MRI protocols depend on hydrogen signals and new MRI scanner is already equipped with the hardware system for ^{1}H-MRS. The ^{1}H-MRS water spectral has become a reference in quantifying metabolite concentration (17). Besides, ^{1}H-MRS provides accurate metabolic information regarding the diagnosis of muscular disorders, and it offers an opportunity of the intramyocellular lipid quantification and other functional metabolites like phosphocreatine or creatine and choline that act as a marker for cell membrane recreation (18). ^{1}H-MRS is also sensitive to metabolite changes that occur after exercise (19).

The increase and decrease of CHO, Cr, and IMCL metabolite values in this present study might result from the technical aspects of ^{1}H-MRS itself. The geometric ordering of the ^{1}H-MRS macroscopic scale helps the separation of IMCL from extramyocellular lipid (EMCL) signals. A relation of muscle orientation to the MRI magnetic field differentiates the IMCL and EMCL peaks and estimates to the highest ppm, approximately 0.2 ppm when the magnetic field is parallel to the muscle (20). A considerable effect of magnetic susceptibility caused by EMCL layers along the central axis of the tissue leads to this chemical shift dissimilarity; meanwhile, IMCL characteristic is spherical droplets inside the muscle cell cytoplasm (21). Several other metabolites like Cr, taurine, and lactate also produce peaks that result in the muscle position to the direction of the magnetic field (21). Thus, the metabolites provide a critical residual dipolar coupling, which indicates the incomplete motional averaging between the dipolar influence of nearby protons (20). The selection of CHO, Cr, and lipids metabolites for this study also depends on other measurement parameters such as repetition time (TR), echo time (TE), and localisation sequence to provide sufficient protocol to detect those metabolite peaks. Besides, it is also crucial to notice that the connection of muscle orientation and ^{1}H-MRS peaks possible to affect the TE value of low molecular weight metabolite signals such as Cr signal from muscles (20). Furthermore, single-voxel spectroscopy (SVS) of ^{1}H-MRS automatically produces signal intensity values for the metabolite resonances (22).

The decrease of CHO, Cr, and IMCL metabolite values from this study after the FES cycling exercise in skeletal muscles was in good agreement accompanied by the previous studies. The metabolite decreased in amounts in the human skeletal muscles had previously been discussed where the creatine (Cr) or phosphocreatine (PCr) signal decreased and recovered similar to PCr in the 31P-MRS technique during and after heavy exercise. The depletion of this Cr2 and Cr3 in Cr has resulted from a study that uses electrical stimulation in the muscle (19,23).

Besides Cr, intramyocellular lipid (IMCL) is also a customarily studied metabolite within human tissue muscle after exercises. IMCL values at baseline vary individually depending on diet, weight, gender, age, and

insulin sensitivity (24). IMCL values also vary between muscle fibre types; about three times higher IMCL concentration in type I fibre such as vastus lateralis muscle as compared to type II muscle fibres (24,25). IMCL acts as a fuel for the increased energy demand of exercise during physical training; hence, IMCL decreased in value. The training level determines the IMCL utilisation throughout the exercise, the strength of the practice, and the amount of IMCL values at pre-exercise (26). Previous studies showed that IMCL metabolite value depleted during a high-intensity exercise (27,28). There was about a 40% drop of IMCL in the anterior tibialis muscle after the completion of strenuous exercise (14). A study from 2017 also reported that activity such as running and cycling caused IMCL depletion, but not during other activities such as sprints or repetitive bouts of dynamic exercise (29). Furthermore, a study that involved four months of aerobic training has also caused a 39% decrease in IMCL values (30). The deficiency is because IMCL metabolite is more approachable for mitochondrial aerobic metabolism and efficaciously used at a high intensity in particular. Some studies pointed out that long-term exercises could make lipids overlay the substrate needs about 70-90% (14,31).

However, one of the subjects in this study showed an increase in IMCL values after the post-FES exercise for both vastus lateralis and semitendinosus muscles. A previous study stated that the rise of IMCL values in younger adults was the first reaction to training (32). However, other research extended the findings and noted that the IMCL increased in value does not restrict to intensive practices, nor is it finite to younger adults (32,33). Exercise effect on IMCL value is still ambiguous where a study reported there was no effect on IMCL metabolite after exercise (34), while the other studies found that endurance training can increase IMCL values (33,35).

As for choline (CHO), there were researches years back showed that strenuous and prolonged exercise could decrease the circulation of CHO stores (36). There was a study indicated that plasma CHO levels in runners begin to drop after about 15 miles have been covered up, and also a study showed that trained runners reported a 40% decline in CHO values after a 26 miles marathon (37,38). The reduction of CHO values in skeletal muscle occurred throughout physiological stress when there are increased demands for CHO as a methyl-group donor, thus leads to a decreasing level of CHO (39). The decline of CHO values during strenuous exercise is afterwards hindering the optimal performance of muscle by reducing the CHO amount available for acetylcholine synthesis, and this effect inhibits the excitation-contraction coupling at the junction of the neuromuscular (37).

Furthermore, a decline in CHO values following exercise may reflect a transient shift in the CHO pool due to alteration of fluid compartments during training and may not cause a change in production and release of neurotransmitters (39). A significant post-exercise decrease in CHO values depends on research protocols that are arduous (36). The depletion of CHO values does not depend on the exercise mode, but it relies on the exercise period and intensity. More extended exercise period at lower intensities or shorter exercise period at higher intensities is not sufficient enough to reduce free CHO values below baseline concentration. In order to significantly reduce the CHO values in the body, a person must be exposed to a long training period (>2 hours) at a high work intensity (40).

The result of metabolite values obtained from this study showed that the FES cycling exercise covered the vastus lateralis muscle better compared to the semitendinosus muscle. This observation can be related to the p-value of the statistical analysis at pre- and post FES exercise. Furthermore, this suggests high-intensity training plays a definite role in metabolites, such as on IMCL metabolite's values that depend on fibre composition in the muscle (41).

Finally, some potential limitations can be listed in this study. First, the sample size of incomplete SCI ASIA-AIS D subjects in this study was small; thus, limits the conclusion concerning the impact of ^{1}H-MRS scanning before and after the FES exercise. As one of the muscle metabolites, which is CHO, showed a significance reading in one of the muscles, future studies with a larger sample size might add value. Secondly, there was no standard gold technique used in this study, which is the muscle biopsy for validating the metabolite measurement. The muscle biopsy has not been proposed in this study since the method is painful and invasive to the subjects.

CONCLUSION

Based on our results, there was a decreasing trend for all three metabolite values between pre- and post-FES exercise in both vastus lateralis and semitendinosus muscles. Therefore, this preliminary finding has postulated that the quantification of muscle metabolites using non-invasively ^{1}H-MRS imaging could be used as a potential indicator in evaluating the muscle strength for incomplete SCI ASIA-AIS D patients after the completion of FES cycling exercise. However, to validate the benefits of ^{1}H-MRS imaging, further investigations in a more significant number of incomplete SCI patients are warranted.

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