

Proton magnetic resonance spectroscopy (¹H-MRS) of human skeletal muscle at 1.5 Tesla: Potential applications in exercise - A pilot study

Nur Syahfinaz Hidayah Rusli ^a, Faikah Zakaria ^a, Farahnaz Ahmad Anwar Basha ^a, Hairil Rashmizal Abdul Razak ^{b,*}

^a Centre of Medical Imaging, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Campus, Malaysia

^b Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia

* Corresponding author: faikah@uitm.edu.my

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Abstract

This research study aimed to evaluate metabolites in human skeletal muscles pre- and post-exercise non-invasively via proton magnetic resonance spectroscopy (¹H-MRS). The upper legs of 6 lightly active male subjects underwent imaging pre- and post-exercise via 1.5 T MRI (TR/TE = 3500ms/100ms, FOV = 20cm, slice thickness = 6mm) and ¹H-MRS (TR/TE = 2000ms/31ms, VOI = 20mm x 20mm x 35mm). The researchers measured the pre- and post-exercise metabolic readings (NAA, CHO, and Cr metabolites) for the vastus lateralis and semitendinosus muscles. A paired t-test was performed. In the vastus lateralis muscle, NAA, CHO, and Cr metabolites values decreased with no significant difference after the exercise. Similarly, in the semitendinosus muscle, NAA, CHO, and Cr metabolites values were also decreased with CHO (p<0.02) and Cr (p<0.01) showed the significant difference after the exercise. Evaluating human skeletal muscles via ¹H-MRS at 1.5 T is feasible.

Keywords: ¹H-MRS; muscle; Cr, CHO, NAA

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INTRODUCTION

Magnetic resonance spectroscopy (MRS) is an advanced MR imaging technique which acts as a complementary to magnetic resonance imaging (MRI) in characterising muscle tissues in musculoskeletal (MSK) imaging. MRS is a non-invasive and non-ionising tool to study the metabolite changes in the brain, breast, prostate, and muscles (Boesch & Kreis, 2016; Howe & Peet, 2016; Mueller-Lisse & Scherr, 2007; Shin *et al.*, 2012). It allows clinicians to determine the metabolite concentrations in selected regions or parts whether it is a neurological and metabolic abnormality. For example, a study was done to evaluate the metabolite concentration of optical radiation in humans (Sidek *et al.*, 2016).

In comparison to diffusion-weighted imaging (DWI), MRS provides information related to chemical microenvironment from atomic nuclei in various functional groups and helps to detect changes in metabolites concentration between healthy and unhealthy tissues. MRS is also practical in evaluating the biomechanical and functional properties of the skeletal muscle such as the management of mitochondrial activity and the complex linkage between muscle fibre organisation and contractile function. Moreover, it is valuable to identify the changes in muscle due to exercise or drug induction (Fatehi *et al.*, 2015).

Proton magnetic resonance spectroscopy (¹H-MRS) is the most employed MRS technique as it is easily integrated and requires no specialised equipment (Deshmukh *et al.*, 2014). The ¹H-MRS helps to assess the biochemical process of the MSK system, and in conjunction with conventional MRI, it can also be employed for anatomic imaging. Besides, ¹H-MRS reflects the metabolism of selected muscle by detecting and measuring signals of water and other metabolites (Deshmukh *et al.*, 2014). There are enormous research findings on the

effectiveness of MRS. However, research studies on the MSK system by using ¹H-MRS are minimal. ¹H-MRS for the brain is the most common research study done, and currently, there is no study available on MSK in Malaysia.

A rest-exercise-recovery procedure can maximise biochemical muscle information. The exercise procedure performed on muscle mass must be standardised to remunerate muscle atrophy or other degenerative changes. Besides, the combination of MRS measures metabolites from rest, exercise, and recovery enhances the diagnostic capability. This method also shows a good separation between healthy subjects, subjects with congenital muscular disorders, and subjects with muscle phosphorylase deficiency (Taylor & Phil, 2000). Thus, this study aimed to measure muscle metabolites, N-acetylaspartate (NAA), Choline (CHO), and Creatine (Cr) in the skeletal muscles at pre- and post-exercise using ¹H-MRS. It is reasoned that if exercise was predominantly influencing metabolites changes, NAA, CHO, and Cr values should not vary from their reference values. This statement would support the arguments that exercise influences metabolites in the skeletal muscle and provides additional insight into the mechanisms by which ¹H-MRS enhances metabolites information.

METHOD

Healthy human subjects

The pre-test and post-test experimental studies were conducted at Clinical Training Centre (CTC) Faculty of Medicine, Universiti Teknologi Mara (UiTM) Sungai Buloh and was performed on six (6) lightly active male subjects (mean age = 23 ± 1.5 years) and had no medical history. The research study was approved by the institutional research ethics board and the study procedure was explained to all subjects. Written informed consent and MRI contraindication and

safety screening were also provided and conducted before the study was conducted.

Exercise protocol

After initial imaging of MRI and ^1H -MRS at rest (pre-scanning), the subject performed the simple exercise (hopped on one leg of preference) until the subject reached fatigue. The mean exercise time was 17.5 ± 5.2 min. Then, immediately the subjects were rescanned with similar MRI and ^1H -MRS protocol (post-scanning).

MRI and ^1H -MRS protocols

MRI and ^1H -MRS imaging of the upper legs which include quadriceps and hamstrings were performed with a 1.5 T whole-body MRI scanner (Siemens Aera) using a four-element "body" coil. The subject was positioned with feet first, and the coil was placed over the thigh covered from the anterior superior iliac spine down to knee joints (from pelvis to knees). The coil was secured by using straps, and the laser beam was centred over the mid-thigh. All subjects underwent less than 25 seconds to complete this process to localise and plan for the following sequences. Axial T2 weighted spin echo (SE) anatomic images with TR=3500 ms, TE=100 ms, the field of view (FOV) = 20 cm, slice thickness = 6 mm, and acquisition (Acq) time = 4 minutes were used to ensure accurate spectroscopy voxel localisation within the selected muscle. The voxel was carefully positioned on the selected muscles to avoid blood vessels, adjacent muscles area, subcutaneous, and other fats like extramyocellular lipid and the femur bone. Single-voxel point-resolved spectroscopy sequence (PRESS) with TR=2000 ms, TE=31 ms, and voxel size = 20 mm x 20 mm x 35 mm were placed within the selected muscles (vastus lateralis and semitendinosus). The size and position of the voxel were uniformly maintained for all the subjects. The manual interactive shimming was performed for five to ten minutes before the spectral data collection. The shimming is the most critical step in ensuring high-quality spectra acquisition.

The metabolite values of Choline (CHO), Creatine (Cr), and N-acetylaspartate (NAA) were recorded for this research study. The metabolite results of ^1H -MRS are in the form of individual peaks of spectra and expressed as parts-per-million (ppm). The values were compared between pre- and post-exercise to determine the distribution and depletion design in the selected muscles. By evaluating these metabolites signal and value, ^1H -MRS can provide information for muscles characterisation and physiology. Statistical analysis was performed with IBM SPSS Statistics Base version 22. The paired t-test was done to evaluate the statistical significance of differently measured metabolites in pre-exercise and post-exercise muscle groups of all the subjects

RESULTS AND DISCUSSION

Muscle ^1H -MRS post-exercise in lightly active subjects

Fig. 1 shows an example of the ^1H -MRS spectra acquired pre- and post-exercise from a subject at 1.5 T. The spectra are shown in parts per million (ppm) and used the same spectral resolution. Table 1 shows the statistical results of NAA, CHO, and Cr metabolites values obtained from the paired t-test for both muscles at pre- and post-exercise. As expected, the values of metabolites were different between pre- and post-exercise at 1.5 T. Statistically significant differences with $p < 0.05$ were only found for CHO and Cr except for NAA metabolite in the semitendinosus muscle. Meanwhile, NAA, CHO, and Cr metabolites values for the vastus lateralis muscle were not statistically significant.

The paired t-test was conducted to evaluate the impact of the leg exercise intervention on metabolites' values at pre- and post-exercise. There was a statistically decrease for NAA, CHO, and Cr metabolites values in the vastus lateralis and semitendinosus muscles. This research study signifies the feasibility of performing ^1H -MRS on human skeletal muscles at 1.5 T. Specifically, and the results demonstrated the metabolites value changes in human skeletal muscle after exercise by using ^1H -MRS. The standard method for evaluating muscle metabolites is the needle biopsy. However, the technique is invasive, painful, and vulnerable to human error, especially with the specimen processing (Finanger et al., 2012). On the contrary, the new and advanced ^1H -MRS is sensitive and contributes the accurate metabolic information. Other

advantages of ^1H -MRS include the simplicity of its application, a short scan time, and easy to maintain its homogeneity of the magnetic field within the volume of interest. Besides, the higher field strengths give a better signal-to-noise ratio (SNR) and thus improves the spectral resolution (Deshmukh et al., 2014). There are metabolic perturbations before and after an endurance training because of the muscle oxidative capacity is increased in accordance with the biogenesis of mitochondria and increased aerobic performance (Befroy & Shulman, 2011).

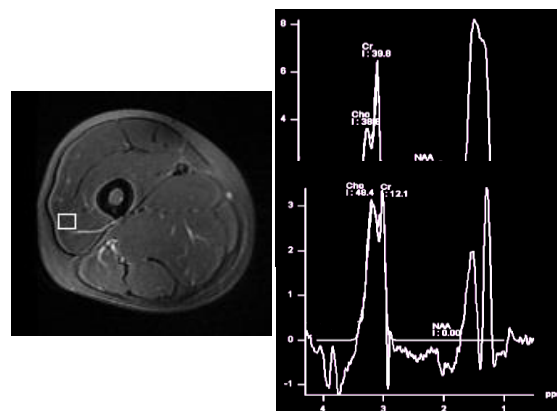


Fig. 1 Representative MRI T2 weighted image (left) of the vastus lateralis muscle and the voxel location (white box) from a subject. The peak height of ^1H -MRS spectra was used to estimate the values for NAA, CHO, and Cr metabolites. The upper right image is the metabolites spectra from pre-exercise while the bottom right is the metabolites spectra from post-exercise. The corresponding values for NAA, CHO, and Cr in the vastus lateralis muscle at pre-exercise was 2.18 ppm, 3.19 ppm, and 3.03 ppm, respectively. Meanwhile, NAA, CHO, and Cr values at post-exercise were 1.95 ppm, 3.17 ppm, and 3.01 ppm, respectively.

Table 1 NAA, CHO, and Cr metabolites values at pre- and post-exercise in the vastus lateralis and semitendinosus muscles at 1.5 T.

Muscles – Metabolites	Mean \pm Standard Deviation (SD)		p-value	Remark (Significant/No Significant Difference)
	Pre-exercise	Post-exercise		
Vastus Lateralis – NAA	2.20 \pm 0.01	2.16 \pm 0.10	0.39	NSD
Vastus Lateralis – CHO	3.23 \pm 0.05	3.21 \pm 0.03	0.47	NSD
Vastus Lateralis – Cr	3.06 \pm 0.05	3.05 \pm 0.05	0.67	NSD
Semitendinosus – NAA	2.10 \pm 0.17	2.06 \pm 0.20	0.63	NSD
Semitendinosus – CHO	3.27 \pm 0.02	3.22 \pm 0.02	0.02	SD
Semitendinosus – Cr	3.15 \pm 0.05	3.07 \pm 0.04	0.01	SD

This study compared NAA, CHO, and Cr metabolites values in the skeletal muscles of lightly active subjects who underwent pre- and post-exercise intervention. Most metabolites values measured decreased after the post-exercise and the trend of changes in metabolites values was upon the principle of ^1H -MRS itself. The electrons around an atom were distributed, and this caused nuclei in different molecules to undergo the magnetic field. This situation leads to different resonance frequencies and later, signals were provided. Then, the raw signals were processed, and spectra which influenced by water would be compressed so that all other spectra become visible (Bell & Gaillard, 2018). In ^1H -MRS imaging, the primary source that causes fluctuation in exercise-induced signal intensity is the changes in T2 relaxation values of tissue water and water content (Ploutz-Snyder et al., 1997; Price et al., 1995). Besides, T2 relaxation values of ^1H -MRS help to estimate patterns of muscle activation (Cannon et al., 2013).

N-Acetylaspartate (NAA) is a metabolite that is predominantly found in the brain. However, a role of NAA outside of the brain has been reported such as the role of mammary gland in converting NAA into lipids (Bogner-Strauss, 2017; D'Adamo & Yatsu, 1966). Based on results of this study, most of NAA values at pre- and post-exercise showed no difference because according to Bogner-Strauss (2017), NAA is found in the skeletal muscle in low amount. N-acetyltransferase 8-like (NAT8L) catalyses NAA from acetyl-CoA and aspartate (Pessentheiner *et al.*, 2013). NAT8L can be found in brown adipose tissue, but its expression is negligible in the skeletal muscle, heart, or liver (Bogner-Strauss, 2017).

NAA is related to mitochondrial viability, and under a normal condition, NAA values fluctuate due to the link of neuronal to mitochondria activity (Chawla *et al.*, 2016; Gonzales *et al.*, 2014). The increase in NAA values in this study indicates mitochondrial health and metabolic efficiency resulted from the exercise. In the brain studies, NAA in the frontal grey matter showed an increase in value after cardiorespiratory exercise (Gonzales *et al.*, 2014). Meanwhile, the other study showed that higher exercise levels helped to moderate the declining value of NAA in aged adults (Erickson *et al.*, 2012). However, some of the subjects in this study showed decreased NAA values at post-exercise. This decrease might have been due to a less vigorous exercise regime for those subjects (Chang *et al.*, 2010).

Choline (CHO) is responsible in synthesising acetylcholine, a signalling molecule. During vigorous activities, the CHO level decreases, resulting in reduced acetylcholine release. Previous studies have shown that people engaging with sports such as cyclists, runners, and marathoners experience reduction in circulatory CHO (Buchman *et al.*, 1999; Conlay, Sabounjian, 1992; von Allwörden *et al.*, 1993). However, based on this study, some of the subjects showed increased values at post-exercise for both the vastus lateralis and semitendinosus muscles. This increase has been due to the muscle recovery process from the exercise regime (Kreis *et al.*, 1999).

Creatine (Cr) is an essential compound found in the skeletal muscle for healthy energy metabolism (Clark, 1997). In humans, most of the Cr is stored in the skeletal muscles, and about 60–70% of total Cr is stored as phosphocreatine. When a person does a high-intensity exercise, the adenosine triphosphate (ATP) in the muscle cells are depleted and with the presence of adenosine 5'-diphosphate (ADP), ATP can be produced with the help of an enzyme called creatine kinase (CK) through an anaerobic pathway.

The fluctuation of muscle Cr metabolites intensity after exercise in healthy subjects is in consensus with previous studies where exhaustion in a specific muscle causes CH2 resonances of Cr/phosphocreatine disappeared in ¹H-MRS spectra (Kreis *et al.*, 1999). Based on the result obtained, there was a subject had elevated Cr metabolite values at post-exercise compared to others where their Cr values depleted after the exercise. This result is due to the muscles had undergone a recovery process from the dynamic exercise to the pre-exercise occurrence within minutes under the aerobic conditions (Kreis *et al.*, 1999).

Finally, the results of this study suggest that the exercise regime covers the semitendinosus muscle more effective compared to the vastus lateralis muscle. One possible explanation for this observation may be related to the p-value of metabolites at pre- and post-exercise. There are several limitations to this study. Firstly, the number of subjects was small even it was a pilot study. Secondly, even though all subjects performed the same exercise regime, there is a possibility that different intensities were shown by them which could affect the metabolite results. Lastly, there was no gold standard which is the muscle biopsy for measurement validation since the technique is invasive, probably dangerous and not ethical in this population of subjects.

CONCLUSION

In conclusion, this study showed the feasibility of ¹H-MRS to evaluate metabolites in human skeletal muscles pre- and post-exercise at 1.5 T. Compared with the metabolites value changes that occur in both the vastus lateralis and semitendinosus muscles at pre- and post-exercise, CHO and Cr values in the semitendinosus muscle shows more significant difference. These data are essential for a suggestion that ¹H-

MRS might be able to detect changes in muscle physiology by providing a suitable set of exercise for subjects with a health problem. Finally, ¹H-MRS could be used as a non-invasive method to provide metabolic and physiological information regarding human skeletal muscles.

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REFERENCES

- Befroy, D. E., Shulman, G. I. (2011). Magnetic resonance spectroscopy studies of human metabolism. *Diabetes*, 60(5), 1361-1369.
- Bell, D. J., Gaillard, F. (2018). MR Spectroscopy. Retrieved from <https://radio-paedia.org/articles/mr-spectroscopy-1>
- Boesch, C., Kreis, R. (2016). Muscle studies by ¹H MRS. *EMagRes*, 5(2), 1097–1108.
- Bogner-Strauss, J. G. (2017). N-acetylaspartate metabolism outside the brain: Lipogenesis, histone acetylation, and cancer. *Frontiers in Endocrinology*, 8(SEP), 1–5.
- Buchman, A. L., Jenden, D., Roch, M. (1999). Plasma free, phospholipid-bound and urinary free choline all decrease during a marathon run and may be associated with impaired performance. *Journal of the American College of Nutrition*, 18(6), 598–601.
- Cannon, D. T., Howe, F. A., Whipp, B. J., Ward, S. A., McIntyre, D. J., Ladroue, C., Rossiter, H. B. (2013). Muscle metabolism and activation heterogeneity by combined ³¹P chemical shift and T2 imaging, and pulmonary O2 uptake during incremental knee-extensor exercise. *Journal of Applied Physiology*, 115(6), 839–849.
- Chang, G., Wang, L., Schweitzer, M. E., Regatte, R. R. (2010). 3D ²³Na MRI of human skeletal muscle at 7 Tesla: Initial experience. *European Radiology*, 20(8), 2039–2046.
- Chawla, S., Ge, Y., Lu, H., Marshall, O., Davitz, M. S., Soher, B. J., Hopkins, J. (2016). Whole-brain N-acetylaspartate concentration is preserved during mild hypercapnia challenge. *American Journal of Neuroradiology*, 36(11), 2055–2061.
- Clark, J. F. (1997). Creatine and phosphocreatine: A review of their use in exercise and sport. *Journal of Athletic Training*, 32(1), 45–51.
- Conlay L. A., Sabounjian L. A., Wurtman, R. J. (1992). Exercise and neuromodulators: Choline and acetylcholine in marathon runners. *International Journal of Sports Medicine*, 13(Suppl 1), S141–S142.
- D'Adamo, A. F., Yatsu, F. M. (1966). Acetate metabolism in the nervous system. N-acetyl-l-aspartic acid and the biosynthesis of brain lipids. *Journal of Neurochemistry*, 13(10), 961-965.
- Deshmukh, S., Subhawong, T., Carrino, J., Fayad, L. (2014). Role of MR spectroscopy in musculoskeletal imaging. *Indian Journal of Radiology and Imaging*, 24(3), 210-216.
- Erickson, K. I., Weinstein, A. M., Sutton, B. P., Prakash, R. S., Voss, M. W., Chaddock, L., Kramer, A. F. (2012). Beyond vascularization: Aerobic fitness is associated with N-acetylaspartate and working memory. *Brain and Behavior*, 2(1), 32-41.
- Fatehi, D., Naleini, F., Salehi, M. G., Afshari, D., Mirfendereski, S., Farzizadeh, M., Rostamzadeh, A. (2015). Traumatic spinal cord injury; Theranostic applications of advanced MRI techniques. *Biomedical and Pharmacology Journal*, 8(2), 891-903.
- Finanger, E. L., Russman, B., Forbes, S. C., Rooney, W. D., Walter, G. A., Vandenberg, K. (2012). Use of skeletal muscle MRI in diagnosis and monitoring disease progression in Duchenne Muscular Dystrophy. *Physical Medicine and Rehabilitation Clinics of North America*, 23(1), 1-12.
- Gonzales, M. M., Haley, A., Tarumi, T., Kaur, S., Nualnim, N., Fallow, B., Tanaka, H. (2014). Aerobic fitness and the brain: Increased N-acetyl-aspartate and choline concentrations in endurance-trained middle-aged adults. *Brain Topography*, 26(1), 126–134.
- Howe, F. A., Peet, A. C. (2016). Characterizing brain tumours by MRS. *EMagRes*, 5(1), 859-874.
- Kreis, R., Jung, B., Slotboom, J., Felblinger, J., Boesch, C. (1999). Effect of exercise on the creatine resonances in ¹H MR spectra of human skeletal muscle. *Journal of Magnetic Resonance*, 137(2), 350-357.
- Mueller-Lisse, U. G., Scherr, M. K. (2007). Proton MR spectroscopy of the prostate. *European Journal of Radiology*, 63(3), 351-360.
- Pessentheiner, A. R., Pelzmann, H. J., Walenta, E., Schweiger, M., Groschner, L. N., Granier, W. F., Bogner-Strauss, J. G. (2013). NAT8L (N-acetyltransferase 8-like) accelerates lipid turnover and increases energy

- expenditure in brown adipocytes. *Journal of Biological Chemistry*, 288(50), 36040-36051.
- Ploutz-Snyder, L. L., Nyren, S., Cooper, T. G., Potchen, E. J., Meyer, R. A. (1997). Different effects of exercise and oedema on T2 relaxation in skeletal muscle. *Magnetic Resonance in Medicine*, 37(5), 676-682.
- Price, T. B., McCauley, T. R., Duleba, A. J., Wilkens, K. L., Gore, J. C. (1995). Changes in magnetic resonance transverse relaxation times of two muscles following standardized exercise. *Medicine & Science in Sports & Exercise*, 27(10), 1421-1429. Retrieved from <http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Changes+in+magnetic+resonance+transverse+relaxation+times+of+two+muscles+following+standardized+exercise#0>
- See, H. H., Schmidt-Marzinkowski, J., Pormsila, W., Morand, R., Krähenbühl, S., Hauser, P. C. (2012). Determination of creatine and phosphocreatine in muscle biopsy samples by capillary electrophoresis with contactless conductivity detection. *Analytica Chimica Acta*, 727, 78-82.
- Shin, H. J., Baek, H.-M., Cha, J. H., Kim, H. H. (2012). Evaluation of breast cancer using proton MR spectroscopy: Total choline peak integral and signal-to-noise ratio as prognostic indicators. *AJR. American Journal of Roentgenology*, 198(5), 488-497.
- Sidek, S., Ramli, N., Rahmat, K., Ramli, N. M., Abdulrahman, F., Kuo, T. L. (2016). In vivo proton magnetic resonance spectroscopy (1H-MRS) evaluation of the metabolite concentration of optic radiation in primary open angle glaucoma. *European Radiology*, 26(12), 4404-4412.
- Taylor, D. J., Phil, D. (2000). Clinical utility of muscle MR spectroscopy. *Seminars in Musculoskeletal Radiology*, 4(4), 481-502.
- Von Allwörden, H. N., Horn, S., Kahl, J., Feldheim, W. (1993). The influence of lecithin on plasma choline concentrations in triathletes and adolescent runners during exercise. *European Journal of Applied Physiology and Occupational Physiology*, 67(1), 87-91.