ORIGINAL ARTICLE

Magnetic Resonance Spectroscopy (MRS) Assessment of Vastus Lateralis Muscle Among Lightly Active Subjects: A Pilot Study

Faikah Zakaria[1], Farahnaz Ahmad Anwar Bashah[1], Abdull Fatah Firdaus Abdull Manaf[2], Wan Hassanuddin Wan Abdul Halim[3]

[1] Centre For Medical Imaging Studies, Faculty of Health Sciences, Universiti Teknologi MARA (UiTM) Selangor, Bandar Puncak Alam,42300, Selangor, Malaysia.
[2] Diagnostic Imaging and Intervention, UKM Specialist Children's Hospital, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia
[3] Department of Radiology, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

ABSTRACT

Introduction: Proton magnetic resonance spectroscopy (^{1}H-MRS) is an advanced magnetic resonance imaging (MRI) technique that permits the molecular characterization by detecting signals of the water, lipids, and metabolites such as lactate, N-acetyl aspartate (NAA), glutamine, creatinine (Cr), and choline (Cho) in the region of interest. ^{1}H-MRS technique has widely explored the area in the brain, breast, prostate, and optical radiation. However, there are limited literature on MR spectroscopy techniques in musculoskeletal (MSK). The primary purpose of this pilot study is to evaluate lower limb muscles strength pre-and post-resistance straining in lightly active subjects using single-voxel ^{1}H-MRS with different voxel sizes. **Methods:** The study examined the vastus lateralis muscle of nine male adults 18-26 years of age, body mass index (BMI) = 21.9-38.0 kg/m^2. Each subject underwent 1.5 T single voxel, short echo time, point resolved proton MRS at pre- and post- resistance training. **Results:** The Wilcoxon signed-rank test was performed and was found no significant difference between 20 mm^2 and 30mm^2 voxel size for NAA, Cr, and Cho for pre- and post-resistance training. **Conclusion:** The finding of this study shows no improvement of the metabolite's quantification from two different sizes of a voxel. However, it may be helpful to explore a different aspect of technique in ^{1}H-MRS imaging to investigate the muscle size, volume, and musculoskeletal properties with significant conditions such as musculoskeletal diseases, muscle injuries as well as in sports sciences.
Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP15): 21-27. doi:10.47836/mjmhs18.s15.4

Keywords: Magnetic Resonance Spectroscopy, ^{1}H-MRS, Musculoskeletal, Exercise, Resistance Training, Sport Science

Corresponding Author:
Faikah Zakaria, MSc
Email: faikah@uitm.edu.my
Tel: +603-3258 4410

INTRODUCTION

Magnetic resonance imaging (MRI) has a distinctive ability to delineate soft tissues with excellent contrast and measure chemical and structural changes non-invasively. It is also promising and potentially being used to develop imaging biomarkers of physiological processes and the assessment of pathologies. Therefore, MRI has become a chosen modality for musculoskeletal tumor assessments, mainly in defining tumor extent using conventional pulse sequences and its ability to characterise the distribution pattern and the severity of changes (1). Moreover, the latest advanced MR sequences provide quantitative measurements for analyzing disease progression, response to therapy, and evaluating the pathophysiological level of disease processes. In addition to that, advanced MR techniques such as proton spectroscopy can provide metabolic information by measuring metabolites produced in a large quantities by malignant tumors, thus, MR spectroscopy can differentiate benign from malignant tumors in both pre-surgical and post-treatment settings (2).

MR spectroscopy (MRS) can evaluate the microstructural of water diffusivity and intramyocellular lipid content (3). MRS enables non-invasive molecular characterization of a region of interest by detecting water, lipids, and other metabolite signals such as N-acetyl aspartate (NAA), Creatine (Cr), and Choline (Cho) (2). In comparison to other MR techniques, MRS can provide information relating to the chemical microenvironment from atomic nuclei in various groups (4). This property helps detect changes in metabolites concentration to evaluate healthy tissues and pathological processes (5). Kachramanoglou

et al (2014) reported that MRS can demonstrate the abnormal concentration of metabolites caused by pathological processes. MRS is proficient in measuring mitochondrial function where it can determine the energetic state of the cell. These studies promised that MRS is useful in evaluating metabolites changes in the musculoskeletal (6).

The movement of a human body depends heavily on the integrity of the muscle and skeletal bones. Muscle and bones demanded metabolism mechanisms from the mitochondria to boost movement energy. The muscles take up almost 30% of the metabolism rate in the human body at the resting state (7). Resistance training is effective at increasing muscle mass (5). A study has been conducted using electromyography (EMG) assessment to take advantage of the muscle manifestations have been conducted between the vastus lateralis and medialis muscles which reported consistent with the finding from biopsy studies (8). Wcisto et al. (2014) piloted a study using 31-Phosphorus MRS before and after exercise on the lower calf and reported no significant difference in the concentration of the remaining metabolites (9). However, 31-P MRS is considered a promising tool but required improvement in its techniques. Proton magnetic resonance spectroscopy (^{1}H-MRS) is widely used for the heart muscle, brain, breast, and prostate(1, 10-12), but there are limited works of literature on skeletal muscle (13,14).

The cell metabolism of the vastus lateralis muscle relies on the enzyme of the activity between males and females (15) .The microvascular function in the lower leg and slight brief of contraction could altered the metabolites involves during the exercise due to muscle oxidative capacity (16). The main importance of using ^{1}H-MRS is to identify and quantify the metabolites (17) that peaks other than water and fat. Using a bigger voxel on a subject with a small size of vastus lateralis muscle could bring a false reading of metabolites. The smaller voxel is favorable to avoid overlapping adjacent structures from the region of interest (18) . It also comes in handy to prevent the voxel placement near the area artifact or implant. The variation of voxel sizes is also desirable to compensate in-cooperative patients, and when time is limited. However, different voxel size affects the scanning time. Besides, a larger voxel deteriorates when echo time (TE) is short.

Numerus MR spectroscopy studies in Malaysia focused on evaluating neuro-metabolite changes in patients with mild traumatic brain injury and metabolite concentration in human optical radiation (19,20). To date, there is no research on the use of MR spectroscopy in the muscular-skeletal system and no study on MRS scanning parameters that highlight the benefits of different voxel sizes of ^{1}H-MRS spectra acquisition from the cell metabolism responsible for musculoskeletal movement from exercises. Therefore, the primary purpose of this pilot study is to assess the effectiveness of single-voxel magnetic resonance spectroscopy (^{1}H-MRS) in assessing patients' lower limb muscles with different voxel sizes. The study focused to measure metabolite changes, N-acetyl aspartate (NAA), Choline (Cho) and Creatine (Cr) in the vastus lateralis muscles pre- and post-resistance training using 1.5T MRI system in lightly active male subjects. This study will benefit as a preliminary finding in determining the usefulness of ^{1}H-MRS imaging protocol in the musculoskeletal system and substantiating the assertion that exercise affect metabolites in the skeletal muscle.

MATERTIALS AND METHODS

Subject recruitment

This was a prospective study that involved pre- and post- resistance training. All the subjects volunteered to participate in the study. The recruitment was conducted via social media and instant messaging. A total of nine lightly active males between the ages 18–26-year-old with ideal body mass index (BMI) at approximately 18.90- 29.9 kg/m2. All the subjects self-reported no regular intense exercise other than walking around the campus and being lightly active. Before the subject recruitment, a subject information sheet and consent form were provided to all subjects. The subjects were brief about the MR safety and the possible risk and injury from performing the resistance training. The safety screening was conducted verbally and recorded on the MR safety checklist. The study was approved by the Universiti Teknologi MARA (UiTM) research ethics committee and adhered to the Declaration of Helsinki 1964.

Study setting

The MR images and spectra were acquired using a 1.5 Tesla MRI machine (Magnetom Aera, Siemens, Erlangen, Germany) using a four-element body coil available at Clinical Training Centre (CTC) Faculty of Medicine, Universiti Teknologi MARA (UiTM) Sungai Buloh. The integrity of the coil was secured using a strap and a laser beam was centered from the knee joint down to the ankle joint. Subject performed the exercises at the gymnasium available at the residential hostel near the MR facility. The nearest gym was selected to ensure the subjects undergo MR scanning immediately after the workout to preserve the data acquired from the post-training scanning. Pre-training MR scanning held with the subject in resting condition. Then, the subjects were brought into the gym. They must complete several sets of an exercise to stimulate the cell metabolism of the lower limb muscles. After the completion of the resistance training, subjects were informed to go to the MR scanner room for the image acquisition.

Resistance Training

With a supervision and advise by a certified physiotherapist, subjects were instructed to do strength

training of the lower leg. Resistance training is an efficient method to improve muscle strength, muscle mass and combat metabolic syndrome (19,20). The exercise was open chained exercises that must include vastus lateralis muscle, predominantly in the lower limbs. The subjects begin by doing leg muscle warm-up exercises and strength training. Warming up exercise is vital to make sure the excellent blood flow to the muscles, helps loosens the joints, and prevent them from acute injuries such as sprains. During the warm-up exercise, subjects were required to lift their right foot and begin to swing it back and forth in standing position. Subjects were advised to ensure the abdominal muscle contracted and the affected leg was fully extended and straight without moving the torso part. The exercise was repeated for 20 times before switching to the left leg. The standing leg curl exercise was incorporated in the strength training. In standing position, both legs of the subject are required to extended straight. This position was the starting position for the subjects. Then, subjects were required to exhale whilst maximizing their lung capacity as their right leg raise. Subjects must hold the position for a couple 10 seconds and then inhale as they slowly lower their right leg back to the starting position. The procedure was repeated until it was completed, with each set consisting of 10 repetitions for 30 minutes per subject or until the subject became fatigued (19, 21). Table I contains a list of the exercises.

Table 1: Resistance training. Exercises performed by the subject.

	Description of the exercise	Number of repetitions
Warm-up exercise		
Leg swings	Stand up straight and raise one leg. Gently swing the leg back and forth while contracting the abs and not moving the torso.	20 x/side
Strength training		
Standing leg curl	Remain standing with both legs extended. Raise one leg and hold for 10 seconds while exhaling. Then, inhale slowly as subject lower his leg back to the starting position.	10 x/side for 30 minutes

MRI and ^{1}H-MRS protocols

The MRI and ^{1}H-MRS scans were performed on the thigh with a four-element body coil to include the vastus lateralis muscle. The scan performed pre- and post-resistance training. Within this study, several acquisition parameters were fixed, and these included the use of fish oil as a marker, patient positioning, type of coil, and the post-acquisition image processing. Localizer scanning were performed on all the subjects to localize and planning for the subsequence MR sequence

A Single-voxel spectra used in this study by using the point-resolved spectroscopy sequence (PRESS) and the parameters for ^{1}H-MRS are TR of 2000 ms and TE of 31 ms, eight acquisitions and eight phase steps (13). A voxel size of 30mm^3 and 20mm^3 was placed within the vastus lateralis of subjects. The voxel position determined by using corresponding axial T2 spin echo (SE) weighted MR images with TR of 3500 ms, TE of 100 ms, slice thickness of 6 mm, field of view (FOV) of 20 cm and acquisition (Acq) time of 4 minutes. Those voxel sizes and the position maintained uniformly for all subjects. The location of the voxel was placed on the pre-determined muscle to deviate from the blood vessels, subcutaneous, adipose tissue, and adjacent muscles area (Figure 1). The values of NAA, Cr and Cho metabolites spectral peaks were recorded. The spectral peaks from the individual metabolites are expressed as parts-per-million (ppm). The values for the targeted metabolites was compared for pre- and post-training to identify any changes to the vastus lateralis muscles mass (13).

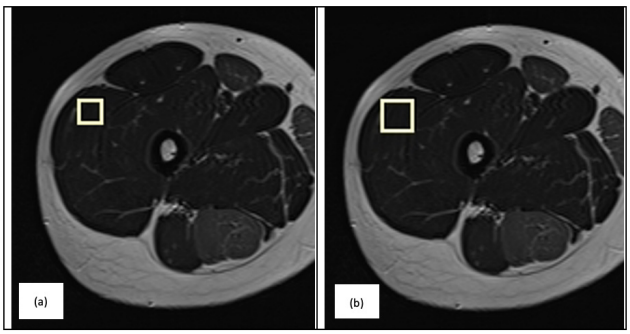

Figure 1: Size and Location of the voxel. (a) A MRI T2 weighted image with voxel size of 20 mm^3 (white box) and (b) voxel size of 30 mm^3 (white box) in the vastus lateralis muscle.

Statistical Analysis

All the data were analyses using IBM Statistical Package for Social Science (SPSS) (Armonk, NY) version 24.0 software. Descriptive analysis and a Shapiro-Wilk test was performed to assess the data normality. A Wilcoxon signed ranks test was conducted to compare the voxel size for each metabolite (NAA, Cr and Cho) pre- and post-training for the subjects. The statistical significance was defined by $p<0.05$.

Ethical Clearance

This study was approved by Universiti Teknologi MARA (UiTM) Research Ethics Committee with the reference number REC/257/19.

RESULTS

The result from this study comprised of the differences between the pre- and post-resistance training metabolites reading obtained using the 20 mm^3 and 30 mm^3 voxel size (Table II), along Figure 2 show an axial MRI image of voxel localization in the vastus lateralis muscle, and the ^{1}H-MRS spectra in parts per million (ppm).

N-acetyl aspartate (NAA) pre-exercise and post-exercise of 20mm^3 and 30mm^3 voxel size

The N-acetyl aspartate (NAA) metabolite value of

Table II: Pre- and post-resistance training metabolite values. NAA, Cr and Cho metabolite values at pre- and post-resistance training in the vastus lateralis muscle using 20 mm^3 and 30 mm^3 voxel size.

Voxel Size	20 mm^3		p-value
Muscle - Metabolites	Mean ± Standard Deviation (SD)		
	Pre-training	Post-training	
Vastus lateralis - NAA	2.15 ± 0.07	2.16 ± 0.012	0.49
Vastus lateralis - Cr	3.04 ± 0.01	3.06 ± 0.03	0.13
Vastus lateralis - Cho	3.24 ± 0.04	3.22 ± 0.018	0.29

Voxel Size	30 mm^3		p-value
Muscle - Metabolites	Mean ± Standard Deviation (SD)		
	Pre-training	Post-training	
Vastus lateralis - NAA	2.16 ± 0.12	2.19 ± 0.02	0.34
Vastus lateralis - Cr	3.05 ± 0.03	3.04 ± 0.02	0.28
Vastus lateralis - Cho	3.24 ± 0.04	3.22 ± 0.018	0.24

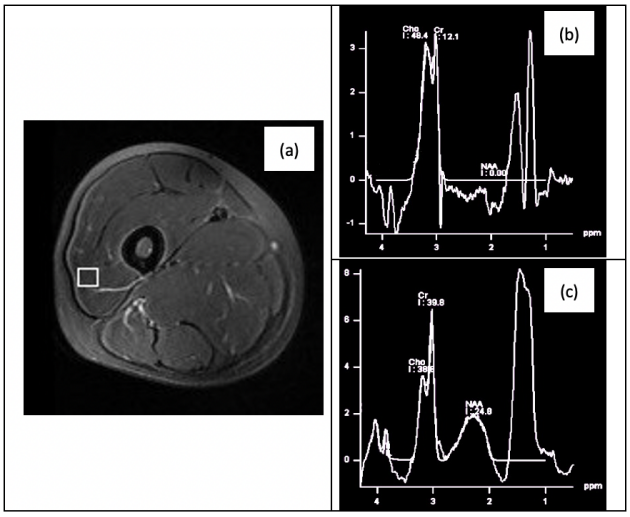

Figure 2: Axial MRI and 1H-MRS images of vastus lateralis muscle. (a) A MRI T2 weighted image and the voxel localization (white box) are shown and the vastus lateralis curve from 1H-MRS, showing peaks of the NAA, Cr and CHO metabolite of a lightly active subject pre-resistance training (b) and post-resistance training (c)

pre- and post-training using 20 mm^3 voxel size were 2.15 ppm and 2.16 ppm, respectively. The standard deviation of pre- and post-training was 0.07 ppm and 0.012 ppm, respectively. Whereas for scanning utilizing the 30 mm^3 voxel size demonstrated the metabolite value of pre- and post-training at 2.16 ppm and 2.19 ppm and the standard deviation at 0.12 ppm and 0.02 ppm, respectively. There was no significant difference for NAA concentration for voxel size at 20 mm^3 and 30mm^3. This result suggests that metabolite reading of NAA for pre- and post-training is deviated from the normality. A Wilcoxon signed-rank test was performed and found no statistically significant difference in NAA concentration between pre- and post-training 20 mm^3 (Z = -0.677, p = 0.498) and for 30 mm^3 voxel size (Z = -0.949, p = 0.343).

Creatine (Cr) Pre-Exercise and Post-Exercise of 20mm^3 and 30mm^3 Voxel size

The metabolite value of Creatine (Cr) collected for pre- and post- training was 3.04 ppm and 3.06 ppm with the reported standard deviation at 0.01 ppm and 0.03ppm for voxel size at 20 mm^3. However, at 30 mm^3 voxel size, the Cr concentration was reported at 3.05 ppm and 3.04 ppm, and standard deviation 0.03 and 0.02. A Shapiro-Wilk test was performed and showed that the distribution of Cr for voxel size at 20 mm^3 is not normally distributed (p = 0.01) but show evidence of normality for voxel size at 30 mm^3 (p = 0.353). Since the assumption is disagree, Wilcoxon Signed Rank Test was performed and reported no significant difference of Cr spectral peak between 20 mm^3 (Z = -1.49, p = 0.136) and 30 mm^3 (Z = -1.06. p = 0.288) voxel sizes in this study.

Choline (Cho) pre-exercise and post-exercise of 20mm^3 and 30mm^3 voxel size

The metabolite value of Choline (Cho) pre- and post-training using 20mm^3 and 30mm^3 voxel size was 3.24 ppm and 3.22 ppm, respectively. The standard deviation of 20mm^3 and 30mm^3 voxel size was 0.04 ppm and 0.018 ppm, respectively. There was not a significant difference in the spectral peak of Cho for pre- and post-training of 20mm^3 and 30mm^3 voxel size voxel size because it is not normally distributed. A Wilcoxon signed-rank test was conducted to determine the amount of Cho peaks and found that there was no significant changes in each participant's lower leg for pre-and post-exercise for 20 mm^3 (Z = -1.057, p = 0.29) and 30 mm^3 (Z = -1.17, p = 0.24).

DISCUSSION

The Wilcoxon Signed Ranks Test result of the statistical test showed that there was no significant difference in metabolites reading between pre- and post-resistance training MRS scanning for both 20 mm^3 and 30 mm^3 voxel sizes. Presence of mitochondria in the muscle has enabled the measurement of the metabolites changes that help in energy metabolism (22). However, during exercise, both anaerobic and aerobic channels activated. According to Saltin et al., (2010) the activation of the channels results in difficulty to measure the ATP production that leads to more complex channels of phosphocreatine (PCr) hydrolysis, oxidative phosphorylatin, and glycolysis(23). Due to small chemical shift range in which the metabolites resonate, the changes in post-exercise metabolite readings became insignificant because many of the metabolite's resonances overlapped, obscuring their presence (24). The visibility of acetylcarnitine has been observed using long TE for the skeletal muscle (25). Based on the research, in the conventional short-TE spectroscopy, the peak of metabolites at 2.13 ppm was covered by broad lipid resonances (25). However,

the spectrum of the metabolites appears as a single, sharp, and symmetrical peak when a long TE used in the spectroscopy scanning protocol. It also recommended using at least 3T MRI machine to make sure the higher sensitivity of metabolites detection. It was also suggested that at least a 3T MRI machine be used to ensure higher sensitivity of metabolite detection. Nonetheless, higher magnetic strength does not guarantee absolute quantification of metabolites (26).

The Wilcoxon Signed Ranks Test result of the statistical test showed that there was no significant difference of metabolites reading between pre-training 20 mm^3 to 30 mm^3 voxels sizes scanning and post-training 20 mm^3 to 30 mm^3 voxels sizes scanning. The finding in this study shows that the size of voxel has a significant cost to the signal to noise (SNR) ratio of the scanning. The relationship between the SNR and the metabolites is directly proportional. SNR of metabolites increases with an increase in the voxel size (27) . In this research, both sizes of voxel were chosen according to the SNR range. The 20 mm^3 and 30 mm^3 voxel size produced SNR that lies within the optimum range. The importance of the different size of a voxel is to compensate the size of the voxel with the size of the volume of interest (VOI) and avoiding voxel bleeding from occurring. A study utilizing phosphor MR spectroscopy (31P-MRS) reported that the adjacent temporal lobe structures cannot be avoided during the placement of bigger size voxel (27). As a recommendation to improve the diagnostic accuracy of the MRS scanning, data acquisition with higher SNR but with smaller voxel size is needed. This current MRS with semi-quantitation study using metabolites ratio is limited to the relation of SNR and size of the voxel (27). This variation size of voxels is essential to compensate the variation of the size of the lower limb muscles and to improve the performance of MRS as a useful and promising diagnostic tool in the evaluation of abnormal of MSK. The study of MRS of MSK profoundly affected by the size of the voxel. As mentioned before, the small size of the voxel is required to be able to examine the structure correctly according to the VOI. Hence, the SNR reduces as the size of voxel reduces.

Using a bigger sample size is recommended in improving the result of the statistical test. Participating more subjects involved in this study can better understand the effectiveness of MRS in evaluating vastus lateralis muscle. Due to the long scanning time, the study needed a more extended time from the recruitment until the scanning procedure. The proper planning and scheduled scanning need to be prepared to compensate for the increasing number of subjects and the hospital schedule.

MRI and ^{1}H-MRS showed the impressive result in the assessment of vastus lateralis muscle among the lightly active subject. The purpose of this study was to add to the assumption that ^{1}H-MRS is one of the modalities capable of diagnosing lower limb muscle abnormalities, in addition to invasive tissue biopsy. The improvement to the ^{1}H-MRS scanning protocol has proven to be useful in evaluating metabolite changes. The study shows that ^{1}H-MRS can read metabolites changes in vastus lateralis muscle with different voxel sizes. MRI can measure the different size of lower limb muscle before and after exercise, which is also evident in another study that was conducted to evaluate semitendinosus muscle (13). Even though the metabolite values pre- and post-resistance training in this study were not statistically significant, implementing the recommendations given may improve the data in a future study, and ^{1}H-MRS can be a non-invasive method for providing metabolite and physiological information concerning MSK anomaly.

CONCLUSION

The protocol of this study used the basic principle of MRS by applying short TE and long TR to acquire data. Due to the overlapping of the signal of metabolites by the activities of mitochondria after the exercise in the muscle, the metabolites reading cannot be detect correctly. Thus, causing the indifferences of metabolites reading during pre- exercise and post-exercise. Longer TE in the protocol of MRS of MSK in future studies could potentially improve the metabolites reading and reduce the overlapping signal between the metabolites and mitochondria activities in the muscle. It is suggested that future studies manipulate the small voxel size with high SNR and incorporate different software to analyse the metabolites peak. A future study could also compare metabolites in the lower limbs of males and females, pre- and post-resistance training, or high intensity interval training to gain a better understanding of the effectiveness of MRS in evaluating cell metabolism in the vastus lateralis muscle. Currently gold standard in evaluating lower limb muscle injuries is muscle biopsies, however with robust and advanced technology from the MR spectroscopy it could ameliorate the need for invasive biopsy procedure.

ACKNOWLEDGEMENT

The authors would like to express our gratitude to the Head of Department of Radiology, Clinical Training Centre, Faculty of Medicine, Universiti Teknologi MARA for the permission to use the MRI scanner, and a special thanks to Mr Mohd Faqrul Ghani for performing the MRI examination.

REFERENCES

1. Vilanova JC, Baleato-Gonzalez S, Romero MJ, Carrascoso-Arranz J, Luna A. Assessment of Musculoskeletal Malignancies with Functional MR Imaging. Magn Reson Imaging Clin N Am. 2016 Feb 1;24(1):239–59. doi: 10.1016/j.mric.2015.08.006
2. Deshmukh S, Subhawong T, Carrino JA, Fayad

L. Role of MR spectroscopy in musculoskeletal imaging. Indian J Radiol Imaging [Internet]. 2014;24:210. doi: 10.4103/0971-3026.137024.
3. Komoroski RA, Kotrla KJ, Lemen L, Lindquist D, Diaz P, Foundas A. Brain metabolite concentration ratios in vivo: multisite reproducibility by single-voxel ^{1}H MR spectroscopy. Magn Reson Imaging. 2004 Jun 1;22(5):721–5. doi: 10.1016/j.mri.2003.12.001.
4. Tadano K, Okamoto Y, Isobe T, Mori S, Suzuki H, Minami M, et al. Changes in skeletal muscle diffusion parameters owing to intramyocellular lipid. Magn Reson Imaging. 2020 Nov 1;73:70–5. doi: 10.1016/j.mri.2020.08.002.
5. Hata J, Endo K, Tsuji O, Arakawa S, Sato M, Yagi K, et al. Analysis of skeletal-muscle condition after excessive loading of the lower legs by sequential magnetic resonance imaging. J Orthop Sci. 2019 Sep 1;24(5):873–80. doi: 10.1016/j.jos.2019.01.016
6. Julia-Sapй M, Coronel I, Majys C, Candiota AP, Serrallonga M, Cos M, et al. Prospective diagnostic performance evaluation of single-voxel ^{1}H MRS for typing and grading of brain tumours. NMR Biomed [Internet]. 2012 Apr 1;25(4):661–73. doi: 10.1002/nbm.1782
7. Krššák M, Lindeboom L, Schrauwen-Hinderling V, Szczepaniak LS, Derave W, Lundbom J, et al. Proton magnetic resonance spectroscopy in skeletal muscle: Experts' consensus recommendations. NMR Biomed [Internet]. 2020/02/05. 2021 May;34(5):e4266–e4266. doi: 10.1002/nbm.4266
8. Rainoldi A, Falla D, Mellor R, Bennell K, Hodges P. Myoelectric manifestations of fatigue in vastus lateralis, medialis obliquus and medialis longus muscles. J Electromyogr Kinesiol. 2008 Dec 1;18(6):1032–7. doi: 10.1016/j.jelekin.2007.05.008
9. Wcisłoe B, Cichockac M, Urbanika A. Phosphorus Spectroscopy of Calf Muscles before and after Exercise. Polish J Radiol [Internet]. 2014;79:328–32. doi: 10.12659/PJR.890601
10. Sourdon J, Roussel T, Costes C, Viout P, Guye M, Ranjeva JP, et al. Comparison of single - voxel H - cardiovascular magnetic resonance spectroscopy techniques for in vivo measurement of myocardial creatine and triglycerides at 3T. J Cardiovasc Magn Reson [Internet]. 2021;1–13. doi:10.1186/s12968-021-00748-x
11. Soares AF, Gruetter R, Lei H. Technical and experimental features of Magnetic Resonance Spectroscopy of brain glycogen metabolism. Anal Biochem. 2017 Jul 15;529:117–26. oi: 10.1016/j.ab.2016.12.023.
12. Tayari N, Heerschap A, Scheenen TWJ, Kobus T. In vivo MR spectroscopic imaging of the prostate, from application to interpretation. Anal Biochem [Internet]. 2017;529:158–70. doi:10.1016/j.ab.2017.02.001
13. Syahfinaz N, Rusli H, Zakaria F, Ahmad F, Basha A, Abdul R. Proton magnetic resonance spectroscopy (1 H-MRS) of human skeletal muscle at 1 . 5 Tesla : Potential applications in exercise - A pilot study. 2019;15(6):772–5. doi:10.11113/mjfas.v15n6.1509
14. Wang X, Salibi N, Fayad LM, Barker PB, Phil D, Morgan RH. Proton Magnetic Resonance Spectroscopy of Skeletal Muscle: A Comparison of Two Quantitation Techniques. J Magn Reson. 2014;243:81–4. doi: 10.1016/j.jmr.2014.03.010.
15. Green HJ, Fraser IG, Ranney DA. Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. J Neurol Sci. 1984 Sep 1;65(3):323–31. doi: 10.1016/0022-510x(84)90095-9.
16. Molbo L, Hansen RK, Østergaard LR, Frøkjær JB, Larsen RG. Sex differences in microvascular function across lower leg muscles in humans. Microvasc Res. 2022 Jan 1;139:104278. doi: 10.1016/j.mvr.2021.104278.
17. Graaf M Van Der. In vivo magnetic resonance spectroscopy : basic methodology and clinical applications. Eur Biophys J 2010; 39(4): 527–40. doi: 10.1007/s00249-009-0517-y.
18. Chang KH, Kim HD, Park SW, Song IC, Yu IK, Han MH, et al. Usefulness of Single Voxel Proton MR Spectroscopy in the Evaluation of Hippocampal Sclerosis. Korean J Radiol. 2000;1(1):25–32. doi: 10.3348/kjr.2000.1.1.25.
19. Franco, Cristine Maria de Castro, Carneiro, Marcelo Augusto Da Silva, Alves, Lucas Tatsuo Horinouchi, Junior, Gersiel Nascimento de Oliveira, De Sousa, Jairo de Freitas Rodrigues O. Lower load is more effective than higher load resistance training in increasing muscle mass in young women. J Strength Cond Res. 2019;00(00):1–7. doi: 10.1519/JSC.0000000000002970.
20. Sundell J. Resistance Training Is an Effective Tool against Metabolic and Frailty Syndromes. Adv Prev Med. 2011;2011:1–7. doi: 10.4061/2011/984683
21. Cannell LJ, Taunton JE, Clement DB, Smith C, Khan KM. A randomised clinical trial of the efficacy of drop squats or leg extension/leg curl exercises to treat clinically diagnosed jumper's knee in athletes: Pilot study. Br J Sports Med. 2001;35(1):60–4. doi: 10.1136/bjsm.35.1.60.
22. Liu Y, Gu Y, Yu X. Assessing tissue metabolism by phosphorous-31 magnetic resonance spectroscopy and imaging : a methodology review. Quant Imaging Med Surg. 2017;7(6):707-726. doi: 10.21037/qims.2017.11.03.
23. Yoshida T, Abe D, Fukuoka Y. Phosphocreatine resynthesis during recovery in different muscles of the exercising leg by 31P-MRS. Scand J Med Sci Sports [Internet]. 2013 Oct 1;23(5):e313–9. doi:10.1111/sms.12081
24. Sato S, Dyar KA, Treebak JT, Jepsen SL, Ehrlich AM, Ashcroft SP, et al. Atlas of exercise metabolism

reveals time-dependent signatures of metabolic homeostasis. Cell Metab [Internet]. 2022 Jan 13 34(2):329-345.e8 [cited 2022 Jan 30] . doi: 10.1016/j.cmet.2021.12.016.

25. Lindeboom L, Nabuurs CI, Hoeks J, Brouwers B, Phielix E, Kooi ME, et al. Long – echo time MR spectroscopy for skeletal muscle acetylcarnitine detection. J Clin Invest. 2014;124(11):4915-25. doi: 10.1172/JCI74830
26. Torriani M, Thomas BJ, Bredella MA, Ouellette H. Intramyocellular lipid quantification: comparison between 3.0- and 1.5-T ^{1}H-MRS. Magn Reson Imaging. 2007 Sep 1;25(7):1105–11. doi: 10.1016/j.mri.2006.12.003
27. Park JH, Niermann KJ, Ryder NM, Nelson AE, Das A, Lawton AR, et al. MUSCLE ABNORMALITIES IN JUVENILE DERMATOMYOSITIS PATIENTS P-31. Arthritis Rheum. 2000;43(10):2359-67. doi: 10.1002/1529-0131(200010)43:10<2359::AID-ANR25>3.0.CO;2-D.