Received:
18 July 2017

Revised:
05 December 2017

Accepted:
05 December 2017

<https://doi.org/10.1259/bjr.20170536>

Cite this article as:

Takashima H, Takebayashi T, Ogon I, Yoshimoto M, Morita T, Imamura R, et al. Analysis of intra and extramyocellular lipids in the multifidus muscle in patients with chronic low back pain using MR spectroscopy. *Br J Radiol* 2018; **91**: 20170536.

FULL PAPER

Analysis of intra and extramyocellular lipids in the multifidus muscle in patients with chronic low back pain using MR spectroscopy

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Objective: To analyse the intra- (IMCL) and extramyocellular lipids (EMCL) concentration in the multifidus muscle (Mm) using MR spectroscopy (MRS) in patients with low back pain (LBP), and to evaluate the correlation between those lipid concentrations and age, obesity, atrophy of the Mm and LBP intensity.

Methods: 60 LBP patients underwent routine diagnostic MRI of the lumbar spine before undergoing imaging for the study. Body mass index, as an indicator of obesity and visual analogue scale, as an indicator of LBP were also measured. Proton MRS was acquired with a single-voxel point-resolved spectroscopy sequence. Furthermore, the MRS volume of interest for measuring the IMCL and EMCL concentration at L4/5 for the right Mm was determined, and we measured the cross-sectional area of Mm as an indicator of muscle atrophy.

Results: Age showed correlation with EMCL concentration ($r = 0.314$, $p = 0.008$). The body mass index showed correlation with EMCL concentration ($r = 0.358$, $p = 0.005$). The cross-sectional area of Mm showed correlation with EMCL concentration ($r = -0.543$, $p < 0.001$). Moreover, the LBP visual analogue scale showed correlation with IMCL concentration ($r = 0.367$, $p = 0.004$).

Conclusion: There were correlations between age, obesity, muscle atrophy, and EMCL concentration in Mm. IMCL concentration in Mm showed a correlation with LBP intensity. This may suggest that IMCL concentration could become an effective objective indicator of chronic LBP intensity.

Advances in knowledge: We investigated the characteristics of fat content in Mm with LBP patients. This study was demonstrated the association of the IMCL and EMCL concentration in Mm with various patient parameters.

INTRODUCTION

Low back pain (LBP) is one of the most common cause of job-related disability in developed countries, leading to social and health-related high expenses.^{1,2} LBP can be caused by variety of problems in any part of the complex, such as interconnected network of spinal muscles, bones, discs, nerves, or tendons in the lumbar spine. In addition, LBP can be due to a specific cause (e.g. trauma, infection, and tumour) or to a nonspecific cause. In most cases, the cause of LBP remains unknown or cannot be clearly defined.^{1,3}

The trunk muscles, erector spinal and posterior paraspinal muscles, are reportedly involved in the aetiology of LBP.⁴ In particular, the multifidus muscle (Mm) is responsible for providing two-thirds of the spinal segmental stability and

plays an important role in the functioning of the trunk.^{5,6} Previous studies have reported the relationship between LBP and fat degeneration or atrophy in the paraspinal muscles.^{7–9} Imaging techniques such as ultrasonography, CT, and MR imaging are often used to evaluate fat degeneration in paraspinal muscles.^{7,8,10} Among the MR techniques, the Dixon technique,^{11,12} and MR spectroscopy (MRS) have been used in previous studies.^{13,14} Proton MRS analysis of muscle physiology has been used in fields such as sports medicine and analyses of metabolism, thereby possibility to detailed analyses of muscular fat content,^{15,16} e.g. recording an intra- (IMCL) and extra-myocellular lipids (EMCL), which in turn has helped clarify the relationship between lipid content and insulin resistance.^{17,18} In our previous study, we applied MRS to the investigation of LBP. We compared IMCL and EMCL in paraspinal

muscles between asymptomatic volunteers and LBP patients and reported that IMCL in Mm of patients with LBP was higher than that in Mm of asymptomatic volunteers.¹⁹ However, the association of the concentration of these lipids in Mm with various patient parameters or indicators such as LBP intensity remains unclear.

The aim of the present study was to analyse IMCL and EMCL in Mm using MRS in patients with LBP and to evaluate the correlation between the concentration of these lipids and age, obesity, atrophy of Mm, and LBP intensity.

METHODS AND MATERIALS

Study participants

The institutional review board approved this prospective and cross-sectional study, and written informed consent was obtained from study participants. 76 consecutive patients with LBP who underwent medical examination at the LBP clinic of our hospital were prospectively included in the study if they met the following inclusion criteria: (a) more than 3 months of continuous or recurrent LBP and (b) referral for MRI given by a spine surgeon at Sapporo Medical University because of LBP. The exclusion criteria were as follows: (i) prior spine surgery; (ii) systemic inflammatory disease; (iii) neurological disorder; (iv) acute trauma, neoplasm, or infection; (v) spinal deformities; and (vi) diabetes, hypertriglyceridemia, or other metabolic disorders. All the patients underwent routine diagnostic MRI examination of the lumbar spine before participating in the study. Following this, spine surgeons confirmed that there were no specific cause of LBP identifiable on the MR images. 16 patients were excluded by those criteria from 76 patients. Finally, the subjects were 60 patients [35 females and 25 males; mean age of 64.3 ± 12.4 years (standard deviation), age range 22–83 years]. Body mass index (BMI) as an indicator of obesity and visual analogue scale (VAS, 0–100 mm) score as an indicator of LBP were also measured.

MR imaging protocol and measurement of muscle atrophy

The Signa HDx 1.5 T MRI system (GE Healthcare, Milwaukee, WI) with a spine coil was used to obtain T_1 weighted coronal [repetition time (TR), 420 ms; echo time (TE), 8.4 ms; field of view (FOV), 30 cm; matrix, 352×224 ; slice thickness, 5 mm;

number of excitations (NEX), 2], T_2 weighted sagittal (TR, 4000 ms; TE, 100 ms; FOV, 30 cm; matrix, 320×256 ; slice thickness, 4 mm; NEX, 2), and T_2 weighted transverse (TR, 5100 ms; TE, 85 ms; FOV, 16 cm; matrix, 256×192 ; slice thickness, 4 mm; NEX, 2) MR images. From these images, the position of the volume of interest (VOI) for proton MRS acquisition at the midpoint of the L4/5 disc for the right Mm was determined (Figure 1a). Single-voxel point-resolved spectroscopy sequence was set with parameters as follows: TR, 2000 ms; TE, 35 ms; average number of signals, 64; voxel of interest, $15 \times 15 \times 15 \text{ mm}^3$ (3.4 ml); and acquisition time, 164 s. Furthermore, the cross-sectional area (CSA) of the right Mm at L4/5 (same level as for MRS) was measured as an indicator of muscle atrophy using the Ziostation2 workstation (ZIOSoft, Tokyo, Japan) (Figure 1b).

Analysis of MR spectroscopic data

The recorded spectral data were used to measure IMCL and EMCL using the LCModel software (Stephen Provencher, Inc., Oakville, ON).²⁰ Data were transferred from the scanners to a Linux workstation, and metabolite quantification was performed by Eddy current correction and water scaling. The methylene signals of IMCL (1.3 ppm) and EMCL (1.5 ppm) were used for quantification. Estimates of IMCL and EMCL concentrations were automatically scaled to an unsuppressed water peak (4.7 ppm) and the total creatine peak (approximately 3.03 ppm) to determine the lipid concentrations in mmol l^{-1} .^{21,22} A typical MR spectrum is presented in Figure 2.

Statistical analysis

The correlative relationships between subject age, BMI, VAS, CSA of Mm and both IMCL and EMCL concentrations were evaluated in patients with LBP. The associations were analysed using Pearson's correlation coefficient. A p -value of <0.05 was considered statistically significant. All statistical analyses were performed with commercially available software (SPSS software, v. 20.0; IBM, SPSS, Chicago, IL).

RESULTS

In patients with LBP, age showed no correlation with IMCL but showed correlation with EMCL ($r = 0.314$, $p = 0.008$) (Figure 3). The mean BMI of subjects was 23.7 kg m^{-2} (range, 16.0–32.1 kg m^{-2}), which showed no correlation with IMCL concentration

Figure 1. Positioning of the volume of interest in the –Mm for MR spectroscopy measurements and cross-sectional area on the MR images. (a) The volume of interest for MR spectroscopy measurements was positioned in the right Mm as indicated on the coronal T_1 and transverse T_2 weighted image at the intervertebral level L4 through L5. (b) The cross-sectional area of the right Mm was measured using transverse T_2 weighted image at the intervertebral level L4 through L5. Mm, multifidus muscle.

Figure 2. The proton MR spectrum of the Mm was analysed using LCModel software (Stephen Provencher, Inc., Oakville, ON). The following metabolites are identified: IMCL methylene ($-\text{CH}_2-$) protons at 1.3 ppm; and EMCL ($-\text{CH}_2-$) protons at 1.5 ppm. EMCL, extramyocellular lipids; IMCL, intramyocellular lipids; Mm, multifidus muscle.

but showed correlation with EMCL concentration ($r = 0.358$, $p = 0.005$) (Figure 4). The mean CSA of Mm was 520.5 mm^2 (range, $134.2\text{--}1138.7 \text{ mm}^2$), which showed no correlation with IMCL concentration but showed correlation with EMCL concentration ($r = -0.543$, $p < 0.001$) (Figure 5). The mean LBP VAS value was 62.8 mm (range, $14\text{--}99 \text{ mm}$), which showed correlation with IMCL concentration ($r = 0.367$, $p = 0.004$) but no correlation with EMCL concentration (Figure 6).

DISCUSSION

Previous studies have investigated an association between chronic LBP patients and fat degeneration in the paraspinal muscles using several indicators and imaging techniques, and all studies have reported an increase in fat degeneration.^{12,14} The majority of those studies^{11–14} used the fat-fraction of the

multipoint Dixon in MRI techniques to evaluate degeneration and reported that fat degeneration and atrophy of the paraspinal muscles is common in adults with LBP. Moreover, previous studies^{13,14} using MRS comprehensively have evaluated fat degeneration as the overall amount of fat content. Previously, we reported that IMCL concentration in Mm of patients with chronic LBP are significantly higher than those of asymptomatic volunteers.¹⁹ In the present study, quantitative analysis of IMCL and EMCL concentrations in Mm revealed correlations of IMCL concentration with LBP, VAS and EMCL concentration with age, BMI and CSA. These findings were consistent with those of the relationship between LBP, obesity, and atrophy of paraspinal muscles observed in previous reports.^{23,24}

Our data were recorded in the Mm at the L4/5 level because many pathological changes in Mm reportedly develop at that location.^{13,14,25} Mengiardi et al¹³ used proton MRS to evaluate the changes in the fat content of Mm. These patients with LBP exhibited a significant increase in the fat content of Mm. We reported that the increased fat content in Mm of patients with LBP corresponds to increased IMCL concentration.¹⁹ Meanwhile, peak of EMCL signal increase greatly as soon as the VOI touches the adipose tissue, because EMCL in adipose tissue is about 200 times more concentrated than IMCL in muscle.²⁶ However, as long as the VOI comprises muscular tissue only, a shift or expansion of the VOI entails no changes in IMCL per volume. Signals of EMCL are very position-dependent; thus, they are, at most, an indicator of the reproducibility of the VOI position and should not be interpreted physiologically. Inter-l and intraindividual reproducibility studies have indicated that the inherent error of IMCL measurements is about 6%.²⁶ Therefore, we believe that IMCL was accurately assessed in the current study.

IMCL are stored inside skeletal muscle cells as small intramuscular lipid droplets that are located close to the mitochondria possibly associated with aerobic metabolism.^{15,17} They are used as an available energy source for muscle fatty acid oxidation.^{15,27,28} It has been reported that IMCL are associated with insulin resistance and accumulation of free fatty acid, and elevated IMCL levels have been observed in patients with Type 2 diabetes.^{27,29}

Figure 3. Relationship between subject age and IMCL, EMCL in Mm. (a) No correlation was observed between age and IMCL. (b) The subject age was positively correlated with EMCL ($r = 0.314$, $p = 0.008$).

Figure 4. Relationship between BMI and IMCL, EMCL in Mm. (a) No correlation was observed between BMI and IMCL. (b) The BMI was positively correlated with EMCL ($r = 0.358$, $p = 0.005$). BMI, body mass index; EMCL, extramyocellular lipids; IMCL, intramyocellular lipids; Mm, multifidus muscle.

Because of the observed association between IMCL and LBP, it is possible that mitochondrial activity in Mm and other muscles is reduced, leading to an increase in levels of inflammatory cytokines such as tumour necrosis factor- α and interleukin-6 in Mm of patients with chronic LBP, regardless of the aetiology.^{30,31}

In contrast, EMCL exist as both subcutaneous and extracellular adipose tissues between muscle cells along muscle fibres and provide long-term energy storage. Therefore, we consider the results of the present study to be consistent with those of previous studies^{17,32} because we observed a relationship between EMCL and CSA as an indicator of muscle atrophy. Moreover, EMCL are considered to be metabolically inactive lipid deposits that are factors involved in reduced functionality associated with obesity and a sedentary lifestyle.^{27,33} Lost muscle strength is associated with the accumulation of EMCL, which can interfere with sufficient muscle nutrition.^{33,34} In the present study, we, therefore, assumed that the relationship of EMCL with age and BMI would be reflected in our subjects and increased levels of EMCL would correlate with aging or reduced functionality.

The present study had several limitations that should be addressed. First, the daily physical activity of subjects in the present study was not assessed. Hence, it is possible that daily activity-associated changes in IMCL occurred in our subjects. Next, the confidence and reproducibility of the localization of the VOI was unidentified. The VOI fully within muscle was able to position a $15 \times 15 \times 15$ mm cube carefully within muscle, but also the slice selects in the point-resolved spectroscopy sequence, particularly, we were not able to confirm the precision of two 180-pulses. Finally, because the present study had a cross-sectional design, it is unclear whether the increase in IMCL was the cause or the result of LBP. Furthermore, the VAS is subjective, it is difficult for us to evaluate objective measures of LBP. Therefore, future longitudinal studies are warranted to address these limitations.

In conclusion, there were correlations between age, obesity, muscle atrophy, and EMCL in Mm. IMCL in Mm showed a correlation with LBP intensity. This may suggest that IMCL could become an effective objective indicator of chronic LBP.

Figure 5. Relationship between CSA and IMCL, EMCL in Mm. (a) No correlation was observed between CSA and IMCL. (b) The CSA was moderately negatively correlated with EMCL ($r = -0.543$, $p < 0.001$). CSA, cross-sectional area; EMCL, extramyocellular lipids; IMCL, intramyocellular lipids; Mm, multifidus muscle.

Figure 6. Relationship between VAS and IMCL, EMCL in Mm. (a) The VAS was moderately positively correlated with IMCL ($r = 0.367$, $p = 0.004$). (b) No correlation was observed between VAS and EMCL. EMCL, extramyocellular lipids; IMCL, intramyocellular lipids; Mm, multifidus muscle; VAS, visual analogue score.

ACKNOWLEDGEMENTS

We thank Ms Mika Yanagida and Mr Yuki Sakurai for their help regarding the patient treatment and MRI.

FUNDING

This work was supported by Grant of Japan Orthopaedics and Traumatology Research Foundation, Inc. No. 230.

REFERENCES

- Berenguera A, Pujol-Ribera E, Rodriguez-Blanco T, Violan C, Casajuana M, de Kort N, et al. Study protocol of cost-effectiveness and cost-utility of a biopsychosocial multidisciplinary intervention in the evolution of non-specific sub-acute low back pain in the working population: cluster randomised trial. *BMC Musculoskelet Disord* 2011; **12**: 194. doi: <https://doi.org/10.1186/1471-2474-12-194>
- Balagué F, Mannion AF, Pellisé F, Cedraschi C. Non-specific low back pain. *Lancet* 2012; **379**: 482–91. doi: [https://doi.org/10.1016/S0140-6736\(11\)60610-7](https://doi.org/10.1016/S0140-6736(11)60610-7)
- Hong J, Reed C, Novick D, Happich M. Costs associated with treatment of chronic low back pain: an analysis of the UK general practice research database. *Spine* 2013; **38**: 75–82. doi: <https://doi.org/10.1097/BRS.0b013e318276450f>
- Renkawitz T, Boluki D, Grifka J. The association of low back pain, neuromuscular imbalance, and trunk extension strength in athletes. *Spine J* 2006; **6**: 673–83. doi: <https://doi.org/10.1016/j.spinee.2006.03.012>
- Hebert JJ, Kjaer P, Fritz JM, Walker BF. The relationship of lumbar multifidus muscle morphology to previous, current, and future low back pain: a 9-year population-based prospective cohort study. *Spine* 2014; **39**: 1417–25. doi: <https://doi.org/10.1097/BRS.0000000000000424>
- Hebert JJ, Koppenhaver SL, Teyhen DS, Walker BF, Fritz JM. The evaluation of lumbar multifidus muscle function via palpation: reliability and validity of a new clinical test. *Spine J* 2015; **15**: 1196–202. doi: <https://doi.org/10.1016/j.spinee.2013.08.056>
- Danneels LA, Vanderstraeten GG, Cambier DC, Witvrouw EE, De Cuyper HJ. CT imaging of trunk muscles in chronic low back pain patients and healthy control subjects. *Eur Spine J* 2000; **9**: 266–72. doi: <https://doi.org/10.1007/s005860000190>
- Kader DF, Wardlaw D, Smith FW. Correlation between the MRI changes in the lumbar multifidus muscles and leg pain. *Clin Radiol* 2000; **55**: 145–9. doi: <https://doi.org/10.1053/crad.1999.0340>
- Kjaer P, Bendix T, Sorensen JS, Korsholm L, Leboeuf-Yde C. Are MRI-defined fat infiltrations in the multifidus muscles associated with low back pain? *BMC Med* 2007; **5**: 2. doi: <https://doi.org/10.1186/1741-7015-5-2>
- Chen YY, Pao JL, Liaw CK, Hsu WL, Yang RS. Image changes of paraspinal muscles and clinical correlations in patients with unilateral lumbar spinal stenosis. *Eur Spine J* 2014; **23**: 999–1006. doi: <https://doi.org/10.1007/s00586-013-3148-z>
- Paalanen N, Niinimäki J, Karppinen J, Taimela S, Mutanen P, Takatalo J, et al. Assessment of association between low back pain and paraspinal muscle atrophy using opposed-phase magnetic resonance imaging: a population-based study among young adults. *Spine* 2011; **36**: 1961–8. doi: <https://doi.org/10.1097/BRS.0b013e3181fef890>
- Yanik B, Keyik B, Conkbayir I. Fatty degeneration of multifidus muscle in patients with chronic low back pain and in asymptomatic volunteers: quantification with chemical shift magnetic resonance imaging. *Skeletal Radiol* 2013; **42**: 771–8. doi: <https://doi.org/10.1007/s00256-012-1545-8>
- Mengiardi B, Schmid MR, Boos N, Pfirrmann CW, Brunner F, Elfering A, et al. Fat content of lumbar paraspinal muscles in patients with chronic low back pain and in asymptomatic volunteers: quantification with MR spectroscopy. *Radiology* 2006; **240**: 786–92. doi: <https://doi.org/10.1148/radiol.2403050820>
- Fischer MA, Nanz D, Shimakawa A, Schirmer T, Guggenberger R, Chhabra A, et al. Quantification of muscle fat in patients with low back pain: comparison of multi-echo MR imaging with single-voxel MR spectroscopy. *Radiology* 2013; **266**: 555–63. doi: <https://doi.org/10.1148/radiol.12120399>
- Schrauwen-Hinderling VB, Hesselink MK, Schrauwen P, Kooi ME. Intramyocellular

- lipid content in human skeletal muscle. *Obesity* 2006; **14**: 357–67. doi: <https://doi.org/10.1038/oby.2006.47>
16. Bredella MA, Ghomi RH, Thomas BJ, Miller KK, Torriani M. Comparison of 3.0 T proton magnetic resonance spectroscopy short and long echo-time measures of intramyocellular lipids in obese and normal-weight women. *J Magn Reson Imaging* 2010; **32**: 388–93. doi: <https://doi.org/10.1002/jmri.22226>
 17. Boesch C. Musculoskeletal spectroscopy. *J Magn Reson Imaging* 2007; **25**: 321–38. doi: <https://doi.org/10.1002/jmri.20806>
 18. Takashima H, Shishido H, Imamura R, Akatsuka Y, Taniguchi K, Nakanishi M, et al. Effect of ankle flexion on the quantification of MRS for intramyocellular lipids of the tibialis anterior and the medial gastrocnemius. *Radiol Phys Technol* 2015; **8**: 209–14. doi: <https://doi.org/10.1007/s12194-015-0309-2>
 19. Takashima H, Takebayashi T, Ogon I, Yoshimoto M, Terashima Y, Imamura R, et al. Evaluation of intramyocellular and extramyocellular lipids in the paraspinal muscle in patients with chronic low back pain using MR spectroscopy: preliminary results. *Br J Radiol* 2016; **89**: 20160136. doi: <https://doi.org/10.1259/bjr.20160136>
 20. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993; **30**: 672–9. doi: <https://doi.org/10.1002/mrm.1910300604>
 21. Torriani M, Thomas BJ, Halpern EF, Jensen ME, Rosenthal DI, Palmer WE. Intramyocellular lipid quantification: repeatability with 1H MR spectroscopy. *Radiology* 2005; **236**: 609–14. doi: <https://doi.org/10.1148/radiol.2362041661>
 22. Weis J, Johansson L, Ortiz-Nieto F, Ahlström H. Assessment of lipids in skeletal muscle by LCModel and AMARES. *J Magn Reson Imaging* 2009; **30**: 1124–9. doi: <https://doi.org/10.1002/jmri.21900>
 23. Tabaraee E, Ahn J, Bohl DD, Phillips FM, Singh K. Quantification of multifidus atrophy and fatty infiltration following a minimally invasive microdiscectomy. *Int J Spine Surg* 2015; **9**: 25. doi: <https://doi.org/10.14444/2025>
 24. Chou L, Brady SR, Urquhart DM, Teichtahl AJ, Cicuttini FM, Pasco JA, et al. The association between obesity and low back pain and disability is affected by mood disorders: a population-based, cross-sectional study of men. *Medicine* 2016; **95**: e3367. doi: <https://doi.org/10.1097/MD.0000000000003367>
 25. Huang Q, Zhang Y, Li D, Yang D, Huo M, Maruyama H. The evaluation of chronic low back pain by determining the ratio of the lumbar multifidus muscle cross-sectional areas of the unaffected and affected sides. *J Phys Ther Sci* 2014; **26**: 1613–4. doi: <https://doi.org/10.1589/jpts.26.1613>
 26. Boesch C, Kreis R. Observation of intramyocellular lipids by 1H-magnetic resonance spectroscopy. *Ann N Y Acad Sci* 2000; **904**: 25–31. doi: <https://doi.org/10.1111/j.1749-6632.2000.tb06417.x>
 27. Srikanthan P, Singhal A, Lee CC, Nagarajan R, Wilson N, Roberts CK, et al. Characterization of intra-myocellular lipids using 2D localized correlated spectroscopy and abdominal fat using MRI in type 2 diabetes. *Magn Reson Insights* 2012; **5**: MRI.S10489–36. doi: <https://doi.org/10.4137/MRI.S10489>
 28. Wijnsman CA, van Opstal AM, Kan HE, Maier AB, Westendorp RG, Slagboom PE, et al. Proton magnetic resonance spectroscopy shows lower intramyocellular lipid accumulation in middle-aged subjects predisposed to familial longevity. *Am J Physiol Endocrinol Metab* 2012; **302**: E344–E348. doi: <https://doi.org/10.1152/ajpendo.00455.2011>
 29. Lawrence JC, Gower BA, Garvey WT, Muñoz AJ, Darnell BE, Oster RA, et al. Relationship between insulin sensitivity and muscle lipids may differ with muscle group and ethnicity. *Open Obes J* 2010; **2**: 137–44. doi: <https://doi.org/10.2174/1876823701002010137>
 30. Dyck DJ, Heigenhauser GJ, Bruce CR. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. *Acta Physiol* 2006; **186**: 5–16. doi: <https://doi.org/10.1111/j.1748-1716.2005.01502.x>
 31. Amouzou C, Breuker C, Fabre O, Bourret A, Lambert K, Birot O, et al. Skeletal muscle insulin resistance and absence of inflammation characterize insulin-resistant grade I obese women. *PLoS One* 2016; **11**: e0154119. doi: <https://doi.org/10.1371/journal.pone.0154119>
 32. Dahlqvist JR, Vissing CR, Hedermann G, Thomsen C, Vissing J. Fat replacement of paraspinal muscles with aging in healthy adults. *Med Sci Sports Exerc* 2017; **49**: 595–601. doi: <https://doi.org/10.1249/MSS.0000000000001119>
 33. Velan SS, Said N, Durst C, Frisbee S, Frisbee J, Raylman RR, et al. Distinct patterns of fat metabolism in skeletal muscle of normal-weight, overweight, and obese humans. *Am J Physiol Regul Integr Comp Physiol* 2008; **295**: R1060–R1065. doi: <https://doi.org/10.1152/ajpregu.90367.2008>
 34. Boesch C, Machann J, Vermathen P, Schick F. Role of proton MR for the study of muscle lipid metabolism. *NMR Biomed* 2006; **19**: 968–88. doi: <https://doi.org/10.1002/nbm.1096>