

Article title

Degeneration of the cartilage quality is correlated with a higher intramuscular fat infiltration of the vastus medialis in older adults with pre-to-mild knee osteoarthritis

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31

32 **Acknowledgments**

33 We would like to thank all the study participants and Editage (www.editage.jp) for
34 English language editing. This study was conducted using the MRI scanner and related
35 facilities of Institute for the Future of Human Society, Kyoto University.

36

37 **Competing interests**

38 The authors declare that they have no competing interests to declare.

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Keywords:

Knee osteoarthritis; T2 mapping; Cartilage; Quadriceps; Muscle quality; Dixon

Abstract

Purpose: To quantitatively verify whether degeneration in the quality of the medial femoral cartilage is correlated with muscle volume loss and intramuscular adipose tissue (IntraMAT) infiltration in quadriceps using magnetic resonance imaging (MRI).

Methods: Of the 66 older adult participants ≥ 60 years old (74.5 ± 6.9 years), 29 were female. All participants were patients with pre-to-mild knee osteoarthritis. Muscle volume and IntraMAT infiltration of the four individual muscles, the rectus femoris (RF), vastus medialis (VM), vastus intermedius, and vastus lateralis were assessed, using proton density-weighted and 2-point Dixon MR images. Based on the MRI T2 mapping, the T2 relaxation times of the weight-bearing surface of the medial femoral cartilage were calculated. Simple linear regression analyses were performed, evaluating the correlations between the T2 relaxation time of the medial femoral cartilage and quadriceps muscle volume and IntraMAT. Multiple linear regression analysis using a stepwise method was performed, determining which indicators of the four individual muscles correlated with cartilage quality degeneration.

Results: Simple linear regression analyses revealed that a longer T2 relaxation time of the medial femoral cartilage was positively correlated with IntraMAT infiltration of the entire quadriceps muscle, RF, and VM, however not with the quadriceps muscle volume. Multiple linear regression analysis revealed that VM IntraMAT was particularly correlated with the extension of T2 relaxation time of the medial femoral cartilage.

Conclusions: In older adults with pre-to-mild knee osteoarthritis, IntraMAT infiltration

73 of the quadriceps muscles, particularly of the VM was correlated with degeneration in the
74 quality of the medial femoral cartilage.

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Introduction

Quadricep muscle degeneration is common in patients with knee osteoarthritis (OA)[1]. To evaluate muscle degeneration quantitatively and non-invasively, measuring muscle volume loss using magnetic resonance imaging (MRI) is essential[2]. In addition, recently, intramuscular adipose tissue (IntraMAT) infiltration also has been quantified, using chemical shift-based 2-point Dixon water-fat separation MRI techniques[3]. Both decreasing muscle volume and worsening IntraMAT infiltration are distinctive in patients with knee OA[4, 5]. These characteristics of muscle degeneration are related to the progression of knee OA, presenting with the aggravation of knee symptoms, functional disability, and increased risk of artificial joint replacement[6-9]. In particular, recently, IntraMAT infiltration of the quadriceps muscles has garnered attention, because the changes thereof precede muscle volume loss in the early stages of knee OA[10].

Degeneration of the quadriceps muscles, caused by IntraMAT infiltration is associated with structural joint abnormalities in patients with knee OA. Previous studies have revealed that severe IntraMAT infiltration of the quadriceps muscles, particularly of the vastus medialis (VM) is related to the future progression of cartilage degeneration, such as the loss of thickness and worsening of the morphological score (whole-organ magnetic resonance imaging score: WOMS)[11-13]. However, all the aforementioned studies have included patients with terminal-stage knee OA who have presented with severe cartilage damage and IntraMAT infiltration of the quadriceps muscles; only focusing on the association with cartilage volume loss. Cartilage compositional changes, such as proteoglycan loss or increased water content of the cartilage tissue occur in the early stages of knee OA, particularly without remarkable cartilage thinning[14-16]. However, no studies have investigated whether degeneration of cartilage quality, caused

by compositional changes in early knee OA is associated with quadriceps muscle degeneration.

MRI T2 mapping is one of the gold standards for assessing cartilage quality[17]. This is an assessment of the increasing water content of the cartilage and can quantitatively detect the degeneration of cartilage quality, which is difficult to observe with the naked eye, as an extension of T2 relaxation times[18]. To the best of our knowledge, no study has investigated whether T2 relaxation times of the cartilage are correlated with quadriceps muscle volume and quality. Moreover, whether differences exist in the association with the degeneration of the cartilage quality among the four individual quadriceps muscles has not been elucidated. Thus, theoretically, necessity for further research lies in the early assessment of quadriceps muscle degeneration, to prevent the progression of knee OA and provide important findings for clinical settings.

Consequently, the purpose of this study was to quantitatively verify whether degeneration in the quality of the medial femoral cartilage is correlated with quadriceps muscle degeneration, using MRI, in older adults with pre-to-mild knee OA. We hypothesized that IntraMAT infiltration of the quadriceps muscles, particularly of the VM is correlated with the degeneration in the quality of the medial femoral cartilage.

Material and methods

Participants

In total, 66 community-dwelling older adults aged > 60 years old participated in this study. The inclusion criteria were as follows: 1) no diagnosis of severe knee OA (Kellgren-Lawrence [KL] grade ≥ 3 [14, 19]), and 2) could walk without any assistive devices. The exclusion criteria were as follows: 1) past surgical history involving the

lower limb, 2) rheumatoid arthritis or a neuromuscular ailment, and 3) contraindications for MRI.

All participants were informed of the study aims and project briefings, and written informed consent was obtained before the study was initiated. This study was approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (R1746). The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

MRI imaging

MR images of the thigh and knee joints of the right lower limb were acquired in the supine and knee extension position, using 3.0 Tesla scanners (MAGNETOM Verio; Siemens AG, Erlangen, Germany) and body coils. Before scanning, the participants rested on the bed for > 15 min to eliminate the effects of any condition of daily living (e.g. the T2 relaxation time of knee cartilage is immediately decreased after loading [20]). For the whole knee joints, three-dimensional-Sampling Perfection with Application optimized Contrast using different flip angle Evolutions (3D SPACE) with proton density-weighted and multi-echo images were acquired. Additionally, multi-slice, spin-lattice relaxation time (T1)-weighted and two-point Dixon images of the thigh muscles were acquired. The detailed parameters of all MR images are presented in Table 1. The entire scan took 18 min and 25 s. Using the two-point Dixon sequence, images of the water and fat ratios were obtained by computing the difference in signal intensities, based on the chemical shift between the in-phase and opposed-phase. The IntraMAT was calculated using the following equation:

$$\text{IntraMAT (\%)} = \frac{\text{mean signal intensity of fat} \times 100}{\text{mean signal intensity of fat} + \text{mean signal intensity of water}} \quad (1)$$

[Insert Table 1]

[Insert Fig.1]

Evaluation of knee OA severity

The severity of OA of the tibiofemoral joint was assessed by an orthopedic surgeon using 3D SPACE with proton density-weighted images of the knee[10]. The assessment focused on several indicators of OA, including the degeneration of the cartilage and meniscus, presence of osteophytes, bone marrow edema, and subchondral cysts. Knee OA severity was graded on a scale of 0 (no OA) to 4 (severe OA). As per the findings of a previous study, this grading system has been confirmed to have excellent inter- and intra-observer agreements and is highly consistent with radiographic knee OA assessment using the KL grade, which classifies OA in five grades with radiological signs.[21].

Quantification of the quadriceps muscle volume and intramuscular fat

Using OsiriX MD (version 11.0; OsiriX, Geneva, Switzerland), the regions of interest (ROI) of the rectus femoris (RF), VM, vastus intermedius (VI), and vastus lateralis (VL) were manually segmented on the MR images. Muscle volume was calculated using multi-slice T1-weighted images. Subsequent to the ROI segmentation of all slices of the MR images, the muscle cross-sectional area (CSA, cm²) of each slice was

measured. The CSA of each muscle multiplied by a slice thickness of 4 mm was summed as the muscle volume (cm³). The volume of the entire quadriceps muscle was defined as the total value of the volumes of the four individual muscles. IntraMAT was calculated using two-point Dixon imaging. First, we segmented the ROI of the RF, VM, VI, and VL on the slice at the midpoint, between the anterior superior iliac spine and proximal end of the patella. Thereafter, the aforementioned ROIs for each muscle were traced on five consecutive proximal and distal slices, with a total of 10 slices. Finally, we adjusted each ROI to include only the individual target muscles, and the mean values of IntraMAT of each muscle were calculated. Furthermore, the IntraMAT of the entire quadriceps muscle was calculated as follows:

$$\begin{aligned} \text{Quadriceps IntraMAT (\%)} = & \text{RF Intra MAT (\%)} \times \frac{\text{RF Volume (cm}^3\text{)}}{\text{Quadriceps Volume (cm}^3\text{)}} + \\ & \text{VM Intra MAT (\%)} \times \frac{\text{VM Volume (cm}^3\text{)}}{\text{Quadriceps Volume (cm}^3\text{)}} + \text{VI Intra MAT (\%)} \times \\ & \frac{\text{VI Volume (cm}^3\text{)}}{\text{Quadriceps Volume (cm}^3\text{)}} + \text{VL Intra MAT (\%)} \times \frac{\text{VL Volume (cm}^3\text{)}}{\text{Quadriceps Volume (cm}^3\text{)}} \quad (2) \end{aligned}$$

[Insert Fig.2]

We confirmed the validity of the imaging analysis of each quadriceps muscle using the data of eight participants randomly selected in this study. The results indicated that the intraclass correlation coefficient (ICC [1,1]) of muscle volume (cm³) was 0.890–0.995, and that of IntraMAT (%) was 0.788–0.988.

Evaluation of the T2 relaxation time of cartilage

Using the T2 mapping modules in 3D Slicer (<http://www.slicer.org/>), the T2 mapping images were produced from the multi-echo images of the knee. Subsequent to the logarithmic transformation of the signal intensity for each voxel, all values were fitted to the regression line in which the vertical and horizontal axes indicated the signal intensity and echo time (TE), respectively, based on the linear least-squares method. The reciprocal values of the slope of the regression line were subsequently calculated as the T2 relaxation times. Detailed information on the calculations is provided at: <https://github.com/gattia/Slicer-T2mapping>.

To improve the accuracy of the T2 mapping, the TE 10.4 s image was excluded from the calculation of the T2 relaxation time. Previous studies have demonstrated that a high accuracy of T2 mapping is achieved, by excluding the signal intensity of the image obtained from the shortest TE, during the MR sequence[22, 23]. In addition, voxels with T2 relaxation times > 100 ms, or coefficients of determination (R^2) of regression lines, estimating T2 relaxation times < 0.7 were eliminated as outliers, caused by artifacts[24].

Subsequent to T2 mapping, T2 relaxation times of the weight-bearing surface of the medial and lateral femoral cartilage were measured using 3D slicer (<http://www.slicer.org/>). The weight-bearing surface of the femoral was defined as the area between the anterior margin of the anterior horn of the meniscus and posterior capsular attachment of the posterior horn of the meniscus, based on the segmentation of WORMS[25]. On the TE 10.4 ms image, a slice that was clearly visible in the anterior and posterior horns of the meniscus was detected; and the ROIs were manually segmented. The ROIs were subsequently reflected on the T2 mapping image, and the means of the T2 relaxation times of all ROIs were calculated.

We confirmed the validity of the imaging analysis of cartilage using the data of eight participants randomly selected in this study. The results indicated that the intraclass correlation coefficient (ICC [1,1]) of T2 relaxation times (ms) was 0.925.

[Insert Fig.3]

Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 22 (IBM SPSS, Armonk, NY, USA), and the threshold for statistical significance was set at $p < 0.05$. Simple linear regression analyses with and without adjustment for age, sex, height, and body mass were performed, to evaluate the correlations between the T2 relaxation times of the medial femoral cartilage and quadriceps muscle volume and IntraMAT. Furthermore, a multiple linear regression analysis with the T2 relaxation time of the medial femoral cartilage as a dependent variable; in addition to the muscle volume and IntraMAT of the four individual muscles as independent variables was performed. This was done to investigate which indicators of the four individual muscles were particularly correlated with the degeneration in the quality of the medial femoral cartilage. All indicators of the four individual quadricep muscle volumes and IntraMAT were entered using a stepwise method; and the analysis was adjusted for age, sex, height, and body mass. In addition, as supplementary analyses to confirm whether the correlation with quadriceps muscle degeneration was specifically observed in the medial femoral cartilage, simple and multiple linear regression analyses with the T2 relaxation times of the lateral femoral cartilage as a dependent variable were performed. All independent and

adjustment variables were entered using a method similar to that in the analysis of the medial femoral cartilage.

Results

The demographic and clinical characteristics of the 66 participants, means of the four individual quadriceps muscle volumes, and IntraMAT are depicted in Table 2. No participants were graded as severe knee OA in this study as a result of the assessment using MRI [21]. Knee OA severity was distributed as grade 0, n=20 (women, 25.0%); grade 1, n=31 (women, 61.3%); and grade 2, n=15 (women, 33.3%).

[Insert Table 2]

The results of the simple linear regression analyses revealing the correlations of the T2 relaxation times of the medial femoral cartilage and quadriceps muscle volume and IntraMAT are shown in Table 3. The volume of the quadriceps muscles was negatively correlated with the T2 relaxation times of the medial femoral cartilage, and lower volumes of the entire quadriceps and each individual muscle were correlated with a longer T2 relaxation time of the medial femoral cartilage (all $p < 0.001$). However, these correlations were not observed after adjusting for age, sex, height, and body mass. Conversely, the IntraMAT of the quadriceps muscles was positively correlated with the T2 relaxation times of the medial femoral cartilage, and worse IntraMAT infiltration of the VM was correlated with a longer T2 relaxation time of the medial femoral cartilage ($p = 0.005$). After adjusting for age, sex, height, and body mass, worse IntraMAT infiltration of the entire quadriceps muscle, RF, and VM was correlated with a longer T2

relaxation time of the medial femoral cartilage (entire quadriceps muscle, $p= 0.034$; RF, $p= 0.014$; VM, $p= 0.011$).

[Insert Table 3]

Multiple linear regression analysis using a stepwise method revealed that VM IntraMAT was the only independent variable correlated with the extension of the T2 relaxation time of the medial femoral cartilage (Table 4, unstandardized regression coefficient $[B]= 0.876$; 95% confidence interval= 0.209 to 1.543; $p= 0.011$).

[Insert Table 4]

The results of supplemental analyses revealing the correlations of the T2 relaxation times of the lateral femoral cartilage and quadriceps muscle volume and IntraMAT are shown in Table 5. The volume of the quadriceps muscles was negatively correlated with the T2 relaxation times of the lateral femoral cartilage, and lower volumes of the entire quadriceps muscle were correlated with a longer T2 relaxation time of the lateral femoral cartilage (entire quadriceps muscle, $p= 0.001$; RF, $p= 0.011$; VM, $p= 0.002$, VL, $p=0.002$; VI, $p=0.001$). In addition, the IntraMAT of the VM was positively correlated with the T2 relaxation times of the lateral femoral cartilage, and worse IntraMAT infiltration of the VM was correlated with a longer T2 relaxation time of the lateral femoral cartilage ($p=0.019$). However, in contrast to the medial femoral cartilage, these correlations were not observed after adjusting for age, sex, height, and body mass. Multiple linear regression analysis using a stepwise method also revealed that no

independent variables correlated with the T2 relaxation time of the lateral femoral cartilage.

[Insert Table 5]

Discussion

This is the first study to quantitatively verify whether degeneration in the quality of the medial femoral cartilage is correlated with quadriceps muscle degeneration, using MRI in older adults with pre-to-mild knee OA. Our results revealed that IntraMAT infiltration of the quadriceps muscles was correlated with the degeneration in the quality of the medial femoral cartilage, whereas no correlation was observed with muscle volume. Another important finding revealed that, among the quadriceps muscles, VM IntraMAT was particularly correlated with the degeneration in the quality of the medial femoral cartilage. Considering that this correlation was not observed in the lateral femoral cartilage, the VM IntraMAT infiltration may be specifically correlated with degeneration in the quality of the medial femoral cartilage in patients with pre-to-mild knee OA.

Simple linear regression analysis revealed a correlation between the cartilage degeneration and IntraMAT infiltration of the quadriceps muscles. Severe IntraMAT infiltration of the quadriceps muscles is known to affect the cartilage volume loss and worsens WOMS in patients with knee OA[11, 13]. The extension of T2 relaxation times can detect an increase in the water content of the cartilage[18], which is a representative change in the cartilage composition, before thinning[14-16]. Thus, the findings of our study revealed that IntraMAT infiltration of the quadriceps muscles is additionally related to the degeneration of the cartilage quality, which is observable in the early-to-advanced

stages of knee OA. The adipose tissue involved in the decline of muscle quality is known to emit inflammatory cytokines[26, 27]; and an increase in interleukin (IL)-6, which is an inflammatory cytokine affects the degeneration of cartilage[5, 28, 29]. In animal experiments, post-intra-articular injection of IL-6, a reduction in the proteoglycan concentrations of the cartilage tissue was detected, suggesting an association between the changes in cartilage composition and inflammatory biomolecules[29, 30]. The physiological mechanism by which IntraMAT infiltration contributes to cartilage degeneration remains unclear; however, a mutual relation with inflammation in OA may exist.

Simple linear regression analyses adjusted for age, sex, height, and body mass revealed the absence of a correlation between the degeneration of the cartilage quality and quadriceps muscle atrophy. This result is inconsistent with the findings of a previous study by Kumar et al. in 2014 which revealed a negative correlation between the cartilage degeneration and CSA of the quadriceps muscles[6]. However, this inconsistency can be deliberated. First, Kumar et al.[6] included patients with an advanced-to-terminal OA with a KL grade 3–4, whereas our study only targeted healthy older adults and patients with early to-mild knee OA. Second, their study[6] assessed cartilage degeneration, using the semi-quantitative morphological score, WOMBS. Conversely, we assessed the degeneration in cartilage quality, using T2 relaxation times, which reflected the change in the amount of the water content of the cartilage. Both atrophy and IntraMAT infiltration of the quadriceps muscles have been observed in patients with knee OA[4, 5]; nonetheless, only an increasing IntraMAT of the quadriceps muscles without a notable volume loss is a characteristic of the early stage of knee OA[10]. This could be a factor that has no

significant association between degeneration of cartilage quality and quadriceps muscle atrophy.

Multiple linear regression analysis revealed that, among the quadriceps muscles, the VM IntraMAT was particularly correlated with degeneration of cartilage quality. In patients with knee OA, ectopic adipogenesis occurs in the VM[31]; and this degeneration is associated with the future progression of knee joint lesions[11]. In general, the VM is an important muscle that contributes to knee joint stability[11, 32, 33], playing an integral role in the efficient movement of knee joints. A recent anatomical study found that an additional medial-acting force produced by the VM contributes to the contraction of the entire quadriceps muscle, via the myofascial connections. This plays an important role in the effective generation of the quadriceps muscle force, during the entire range of motion[34]. Considering these findings, it can be inferred that VM dysfunction is possible to restrict efficient muscle exertion for knee extension. Moreover, increasing co-contraction of the VM and other knee muscles (e.g. semimembranosus muscle, biceps femoris, and VL) during the stance phase of gait is associated with a greater progression of future cartilage loss in patients with knee OA[35]. Incidentally, muscle co-contraction is one of the compensatory strategies for joint instability caused by muscle weakness[36]. Increased VM IntraMAT inflammation, which is a distinctive change in patients with early knee OA[10] can result in these dysfunctions of knee joint. This could partially explain the correlation found in our study between IntraMAT infiltration of the VM and the degeneration of cartilage quality.

Clinically, maintaining or improving VM IntraMAT, rather than focusing solely on muscular hypertrophy, may contribute to the prevention of cartilage degeneration. Our finding emphasized the importance of VM IntraMAT for cartilage degeneration in not

only the advanced to severe stage of knee OA but also the early stage of knee OA. Recently, in the clinical setting, the compositional degeneration of skeletal muscles, including an increased IntraMAT or non-contractive tissue has been conveniently evaluated, using ultrasound echo intensity or bioelectrical impedance analysis [37, 38], which can be improved by muscle strength exercises [39-41]. Therefore, our findings can potentially contribute to the establishment of a strategy for preventing the progression of cartilage degeneration in knee OA.

This study had several limitations. First, only the cartilage degeneration of the weight-bearing surface of the femoral cartilage was evaluated. However, in the early stages of knee OA, because the femoral cartilage is more markedly degenerated than the tibial cartilage[42, 43], the evaluations approximately encompassed the degeneration of the cartilage quality of the tibiofemoral joint. The correlation between cartilage degeneration in other compartments, such as the patellofemoral joint, and quadriceps muscle degeneration should be investigated. Second, the IntraMAT of the quadriceps muscles was partially evaluated with only 10 slices. However, previous studies have suggested that the distribution of the IntraMAT among the quadriceps muscles is constant [44, 45]. Third, the sample size of our study was relatively small. The post-hoc power analysis for the multiple linear regression analysis, based on the adjusted coefficient of determination $R^2 = 0.294$, yielded a power of 0.985, which confirmed that the statistical power was sufficient. However, our findings should be validated in a larger cohort study to assess their relevance to broader populations. Finally, this was a cross-sectional study; thus, the causal correlation between the cartilage degeneration and IntraMAT infiltration of the quadriceps muscles is unclear. Future longitudinal research is needed to identify this causal correlation.

Conclusion

In conclusion, in older adults with pre-to-mild knee OA, IntraMAT infiltration of the quadriceps muscles, particularly of the VM was correlated with futher , whereas no correlation was observed with muscle volume.

Author contributions

All authors have made substantial contributions to: 1) the conception and design of the study, 2) critical revision of important intellectual content, and 3) final approval of the version to be submitted. Specific contributions of the authors are as follows: 1) analysis and interpretation of the data and 2) drafting of the article: SO, MT, MaY, and NI.

Abbreviations

B: unstandardized regression coefficient

β : standardized regression coefficient

CI: confidence interval

CSA: cross-sectional area

IL-6: interleukin-6

IntraMAT: intramuscular adipose tissue

KL grade: Kellgren-Lawrence grade

MRI: magnetic resonance imaging

OA: osteoarthritis

ROI: regions of interest

408 RF: rectus femoris

409 SD: Standard deviations

410 TE: echo time

411 VM: vastus medialis

412 VL: vastus lateralis

413 VI: vastus intermedius

414 WORMS: whole-organ magnetic resonance imaging score

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416 **Funding sources**

417 This research has been supported by the JSPS KAKENHI Grants-in-Aid for
418 Scientific Research (18H03164 and 20K19376). This grant has been used for the data
419 collection, analysis, and manuscript writing.

420

421 **References**

- 422 [1] K. Yamauchi, C. Kato, T. Kato, Characteristics of individual thigh muscles including
423 cross-sectional area and adipose tissue content measured by magnetic resonance imaging
424 in knee osteoarthritis: a cross-sectional study, *Rheumatol. Int.* 39(4) (2019) 679-687.
- 425 [2] C. Beaudart, E. McCloskey, O. Bruyère, M. Cesari, Y. Rolland, R. Rizzoli, I. Araujo
426 de Carvalho, J. Amuthavalli Thiyagarajan, I. Bautmans, M.C. Bertièrè, M.L. Brandi, N.M.
427 Al-Daghri, N. Burlet, E. Cavalier, F. Cerreta, A. Cherubini, R. Fielding, E. Gielen, F.
428 Landi, J. Petermans, J.Y. Reginster, M. Visser, J. Kanis, C. Cooper, Sarcopenia in daily
429 practice: assessment and management, *BMC Geriatr.* 16(1) (2016) 1-10.

- 430 [3] K. Engelke, O. Chaudry, L. Gast, M.A.B. Eldib, L. Wang, J.D. Laredo, G. Schett, A.M.
 431 Nagel, Magnetic resonance imaging techniques for the quantitative analysis of skeletal
 432 muscle: State of the art, *Journal of Orthopaedic Translation* 42 (2023) 57-72.
- 433 [4] M.B. Conroy, C.K. Kwoh, E. Krishnan, M.C. Nevitt, R. Boudreau, L.D. Carbone, H.
 434 Chen, T.B. Harris, A.B. Newman, B.H. Goodpaster, Muscle strength, mass, and quality
 435 in older men and women with knee osteoarthritis, *Arthritis Care and Research (Hoboken)*
 436 64(1) (2012) 15-21.
- 437 [5] M.G. Pedroso, A.C. de Almeida, J.B. Aily, M. de Noronha, S.M. Mattiello, Fatty
 438 infiltration in the thigh muscles in knee osteoarthritis: a systematic review and meta-
 439 analysis, *Rheumatol. Int.* 39(4) (2019) 627-635.
- 440 [6] D. Kumar, D.C. Karampinos, T.D. MacLeod, W. Lin, L. Nardo, X. Li, T.M. Link, S.
 441 Majumdar, R.B. Souza, Quadriceps intramuscular fat fraction rather than muscle size is
 442 associated with knee osteoarthritis, *Osteoarthritis Cartilage* 22(2) (2014) 226-234.
- 443 [7] M. Taniguchi, Y. Fukumoto, M. Yagi, Y. Motomura, S. Okada, S. Okada, M.
 444 Kobayashi, N. Ichihashi, Enhanced echo intensity in vastus medialis is associated with
 445 worsening of functional disabilities and symptoms in patients with knee osteoarthritis: a
 446 3 years longitudinal study, *Rheumatol. Int.* 43(5) (2023) 953-960.
- 447 [8] A. Teoli, J. Martel-Pelletier, F. Abram, J.P. Pelletier, S.M. Robbins, Vastus medialis
 448 intramuscular fat is associated with reduced quadriceps strength, but not knee
 449 osteoarthritis severity, *Clinical Biomechanics* 96 (2022) 105669.
- 450 [9] Y. Wang, A.E. Wluka, P.A. Berry, T. Siew, A.J. Teichtahl, D.M. Urquhart, D.G. Lloyd,
 451 G. Jones, F.M. Cicuttini, Increase in vastus medialis cross-sectional area is associated
 452 with reduced pain, cartilage loss, and joint replacement risk in knee osteoarthritis,
 453 *Arthritis Rheum.* 64(12) (2012) 3917-3925.

- 454 [10] M. Taniguchi, Y. Fukumoto, M. Yagi, T. Hirono, M. Yamagata, A. Asayama, S.
 455 Okada, R. Nakai, M. Kobayashi, N. Ichihashi, A higher intramuscular fat in vastus
 456 medialis is associated with functional disabilities and symptoms in early stage of knee
 457 osteoarthritis: a case–control study, *Arthritis Res. Ther.* 25(1) (2023) 61.
- 458 [11] D. Kumar, T.M. Link, S.R. Jafarzadeh, M.P. LaValley, S. Majumdar, R.B. Souza,
 459 Association of quadriceps adiposity with an increase in knee cartilage, meniscus, or bone
 460 marrow lesions over three years, *Arthritis Care Res.* 73(8) (2021) 1134-1139.
- 461 [12] J.P. Raynald, J.P. Pelletier, C. Roubille, M. Dorais, F. Abram, W. Li, Y. Wang, J.
 462 Fairley, F.M. Cicuttini, J. Martel-Pelletier, Magnetic Resonance Imaging-Assessed Vastus
 463 Medialis Muscle Fat Content and Risk for Knee Osteoarthritis Progression: Relevance
 464 from a Clinical Trial, *Arthritis Care Res.* 67(10) (2015) 1406-1415.
- 465 [13] A.J. Teichtahl, A.E. Wluka, Y. Wang, P.N. Wijethilake, B.J. Strauss, J. Proietto, J.B.
 466 Dixon, G. Jones, A. Forbes, F.M. Cicuttini, Vastus medialis fat infiltration - a modifiable
 467 determinant of knee cartilage loss, *Osteoarthritis Cartilage* 23(12) (2015) 2150-2157.
- 468 [14] J.W. MacKay, S.B.L. Low, T.O. Smith, A.P. Toms, A.W. McCaskie, F.J. Gilbert,
 469 Systematic review and meta-analysis of the reliability and discriminative validity of
 470 cartilage compositional MRI in knee osteoarthritis, *Osteoarthritis Cartilage* 26(9) (2018)
 471 1140-1152.
- 472 [15] S.J. Matzat, J. van Tiel, G.E. Gold, E.H.G. Oei, Quantitative MRI techniques of
 473 cartilage composition, *Quantitative imaging in medicine and surgery* 3(3) (2013) 162-174.
- 474 [16] S. Reichenbach, M. Yang, F. Eckstein, J. Niu, D.J. Hunter, C.E. McLennan, A.
 475 Guermazi, F. Roemer, M. Hudelmaier, P. Aliabadi, D.T. Felson, Does cartilage volume or
 476 thickness distinguish knees with and without mild radiographic osteoarthritis? The
 477 Framingham Study, *Ann. Rheum. Dis.* 69(1) (2010) 143-9.

- 478 [17] C.A. Emery, J.L. Whittaker, A. Mahmoudian, L.S. Lohmander, E.M. Roos, K.L.
 479 Bennell, C.M. Toomey, R.A. Reimer, D. Thompson, J.L. Ronsky, G. Kuntze, D.G. Lloyd,
 480 T. Andriacchi, M. Englund, V.B. Kraus, E. Losina, S. Bierma-Zeinstra, J. Runhaar, G.
 481 Peat, F.P. Luyten, L. Snyder-Mackler, M.A. Risberg, A. Mobasheri, A. Guermazi, D.J.
 482 Hunter, N.K. Arden, Establishing outcome measures in early knee osteoarthritis, *Nature*
 483 *Reviews Rheumatology* 15(7) (2019) 438-448.
- 484 [18] S. Mittal, G. Pradhan, S. Singh, R. Batra, T1 and T2 mapping of articular cartilage
 485 and menisci in early osteoarthritis of the knee using 3-Tesla magnetic resonance imaging,
 486 *Polish Journal of Radiology* 84 (2019) 549-564.
- 487 [19] K. Rueckl, F. Boettner, N. Maza, A. Runer, U. Bechler, P. Sculco, The posterior-
 488 anterior flexed view is better than the anterior-posterior view for assessing osteoarthritis
 489 of the knee, *Skeletal Radiol.* 47(4) (2018) 511-517.
- 490 [20] S.L. Coburn, K.M. Crossley, J.L. Kemp, S.J. Warden, T.J. West, A.M. Bruder, B.F.
 491 Mentiplay, A.G. Culvenor, Is running good or bad for your knees? A systematic review
 492 and meta-analysis of cartilage morphology and composition changes in the tibiofemoral
 493 and patellofemoral joints, *Osteoarthritis Cartilage* 31(2) (2023) 144-157.
- 494 [21] H.J. Park, S.S. Kim, S.Y. Lee, N.H. Park, J.Y. Park, Y.J. Choi, H.J. Jeon, A practical
 495 MRI grading system for osteoarthritis of the knee: Association with Kellgren-Lawrence
 496 radiographic scores, *Eur. J. Radiol.* 82(1) (2013) 112-117.
- 497 [22] C.F. Maier, S.G. Tan, H. Hariharan, H.G. Potter, T2 quantitation of articular cartilage
 498 at 1.5 T, *J. Magn. Reson. Imaging* 17(3) (2003) 358-364.
- 499 [23] H.E. Smith, T.J. Mosher, B.J. Dardzinski, B.G. Collins, C.M. Collins, Q.X. Yang,
 500 V.J. Schmithorst, M.B. Smith, Spatial variation in cartilage T2 of the knee, *J. Magn.*
 501 *Reson. Imaging* 14(1) (2001) 50-55.

- 502 [24] A.A. Gatti, M.D. Noseworthy, P.W. Stratford, E.C. Brenneman, S. Totterman, J.
 503 Tamez-Peña, M.R. Maly, Acute changes in knee cartilage transverse relaxation time after
 504 running and bicycling, *J. Biomech.* 53 (2017) 171-177.
- 505 [25] C.G. Peterfy, A. Guermazi, S. Zaim, P.F.J. Tirman, Y. Miaux, D. White, M. Kothari,
 506 Y. Lu, K. Fye, S. Zhao, H.K. Genant, Whole-organ magnetic resonance imaging score
 507 (WORMS) of the knee in osteoarthritis, *Osteoarthritis Cartilage* 12(3) (2004) 177-190.
- 508 [26] O. Addison, M.J. Drummond, P.C. Lastayo, L.E. Dibble, A.R. Wende, D.A. McClain,
 509 R.L. Marcus, Intramuscular fat and inflammation differ in older adults: The impact of
 510 frailty and inactivity, *Journal of Nutrition, Health and Aging* 18(5) (2014) 532-538.
- 511 [27] O. Gabay, D.J. Hall, F. Berenbaum, Y. Henrotin, C. Sanchez, Osteoarthritis and
 512 obesity: Experimental models, *Joint Bone Spine* 75 (2008) 675-679.
- 513 [28] K. Amano, J.L. Huebner, T.V. Stabler, M. Tanaka, C.E. McCulloch, I. Lobach, N.E.
 514 Lane, V.B. Kraus, C.B. Ma, X. Li, Synovial Fluid Profile at the Time of Anterior Cruciate
 515 Ligament Reconstruction and Its Association With Cartilage Matrix Composition 3 Years
 516 After Surgery, *Am. J. Sports Med.* 46(4) (2018) 890-899.
- 517 [29] P. Wojdasiewicz, Ł.A. Poniatowski, D. Szukiewicz, The role of inflammatory and
 518 anti-inflammatory cytokines in the pathogenesis of osteoarthritis, *Mediators Inflamm.*
 519 2014 (2014) 561459.
- 520 [30] F.A. Van de Loo, O.J. Arntz, W.B. Van den Berg, Effect of interleukin 1 and
 521 leukaemia inhibitory factor on chondrocyte metabolism in articular cartilage from normal
 522 and interleukin-6-deficient mice: role of nitric oxide and IL-6 in the suppression of
 523 proteoglycan synthesis, *Cytokine* 9(7) (1997) 453-62.
- 524 [31] M. Ikemoto-Uezumi, Y. Matsui, M. Hasegawa, R. Fujita, Y. Kanayama, A. Uezumi,
 525 T. Watanabe, A. Harada, A.R. Poole, N. Hashimoto, Disuse Atrophy Accompanied by

- 526 Intramuscular Ectopic Adipogenesis in Vastus Medialis Muscle of Advanced
527 Osteoarthritis Patients, *Am. J. Pathol.* 187(12) (2017) 2674-2685.
- 528 [32] H. Toumi, G. Poumarat, M. Benjamin, T. Best, S. F'Guyer, J. Fairclough, New
529 insights into the function of the vastus medialis with clinical implications, *Med. Sci.*
530 *Sports Exerc.* 39(7) (2007) 1153-1159.
- 531 [33] A.C. Waligora, N.A. Johanson, B.E. Hirsch, Clinical anatomy of the quadriceps
532 femoris and extensor apparatus of the knee, *Clin. Orthop. Relat. Res.* 467(12) (2009)
533 3297-3306.
- 534 [34] K. Grob, M. Manestar, L. Filgueira, M.S. Kuster, H. Gilbey, T. Ackland, The
535 interaction between the vastus medialis and vastus intermedius and its influence on the
536 extensor apparatus of the knee joint, *Knee Surg. Sports Traumatol. Arthrosc.* 26(3) (2018)
537 727-738.
- 538 [35] P.W. Hodges, W. van den Hoorn, T.V. Wrigley, R.S. Hinman, K.A. Bowles, F.
539 Cicuttini, Y. Wang, K. Bennell, Increased duration of co-contraction of medial knee
540 muscles is associated with greater progression of knee osteoarthritis, *Man. Ther.* 21
541 (2016) 151-158.
- 542 [36] Y. Yoshida, R.L. Mizner, L. Snyder-Mackler, Association between long-term
543 quadriceps weakness and early walking muscle co-contraction after total knee
544 arthroplasty, *Knee* 20(6) (2013) 426-431.
- 545 [37] M.S. Stock, B.J. Thompson, Echo intensity as an indicator of skeletal muscle quality:
546 applications, methodology, and future directions, *Eur. J. Appl. Physiol.* 121(2) (2021)
547 369-380.
- 548 [38] Y. Yamada, B. Buehring, D. Krueger, R.M. Anderson, D.A. Schoeller, N. Binkley,
549 Electrical Properties Assessed by Bioelectrical Impedance Spectroscopy as Biomarkers

550 of Age-related Loss of Skeletal Muscle Quantity and Quality, The journals of gerontology.
 551 Series A, Biological sciences and medical sciences 72(9) (2017) 1180-1186.
 552 [39] P.M. Holm, A.D. Blankholm, J.L. Nielsen, T. Bandholm, W. Wirth, A. Wisser, J.
 553 Kemnitz, F. Eckstein, H.M. Schroder, M. Wernbom, S.T. Skou, Effects of neuromuscular
 554 control and strengthening exercises on MRI-measured thigh tissue composition and
 555 muscle properties in people with knee osteoarthritis - an exploratory secondary analysis
 556 from a randomized controlled trial, Semin. Arthritis Rheum. 65 (2024) 152390.
 557 [40] T. Ikezoe, T. Kobayashi, M. Nakamura, N. Ichihashi, Effects of Low-Load, Higher-
 558 Repetition vs. High-Load, Lower-Repetition Resistance Training Not Performed to
 559 Failure on Muscle Strength, Mass, and Echo Intensity in Healthy Young Men: A Time-
 560 Course Study, J. Strength Cond. Res. 34(12) (2020) 3439-3445.
 561 [41] Z. Wang, M. Taniguchi, J. Saeki, N. Ichihashi, Effects of High-Velocity Versus Low-
 562 Velocity Resistance Training on Muscle Echo Intensity in Healthy Young Women: A
 563 Randomized Controlled Trial, Sports Health (2024) 19417381241257181.
 564 [42] S. Hada, H. Kaneko, R. Sadatsuki, L. Liu, I. Futami, M. Kinoshita, A. Yusup, Y. Saita,
 565 Y. Takazawa, H. Ikeda, K. Kaneko, M. Ishijima, The degeneration and destruction of
 566 femoral articular cartilage shows a greater degree of deterioration than that of the tibial
 567 and patellar articular cartilage in early stage knee osteoarthritis: A cross-sectional study,
 568 Osteoarthritis Cartilage 22(10) (2014) 1583-1589.
 569 [43] G.Y. Yang, H.L. Guo, T. Li, H.B. Shang, Y.F. Zhao, Y.Y. Shi, The medial
 570 compartment and patellofemoral joint degenerate more severely in early stage knee
 571 osteoarthritis: a cross-sectional study, Eur. Rev. Med. Pharmacol. Sci. 24(19) (2020)
 572 9815-9823.

- 573 [44] A. Yoshiko, M. Hioki, N. Kanehira, K. Shimaoka, T. Koike, H. Sakakibara, Y. Oshida,
574 H. Akima, Three-dimensional comparison of intramuscular fat content between young
575 and old adults, BMC Med. Imaging 17(1) (2017) 12.
- 576 [45] A.N. Varanoske, D.H. Fukuda, C.H. Boone, K.S. Beyer, J.R. Stout, J.R. Hoffman,
577 Homogeneity of echo intensity values in transverse ultrasound images, Muscle Nerve
578 56(1) (2017) 93-98.

579

580 **Table 1. Parameters of the magnetic resonance imaging for each sequence**

	proton density weighted image	multi-echo image	T1 weighted image	2-point dixon image
target domain	knee	knee	thigh	thigh
repetition time	1000 ms	2200 ms	2820 ms	4.33 ms
echo time	35 ms	10.4, 20.8, 31.2, 41.6, 52.0, 62.4, 72.8, 83.2 ms	16 ms	1.31, 2.54 ms
flip angle	120°	180°	129°	9°
slice thickness	0.7 mm	2.5mm	4 mm	3 mm
interslice gap	-	2.5mm	-	-
field of view	150 × 150 mm	160 × 300 mm	320 × 240 mm	286.4 × 365 mm
voxel size	0.59 × 0.59 × 0.7 mm ³	0.5 × 0.5 × 2.5 mm ³	0.5 × 0.5 × 4.0 mm ³	0.5 × 0.5 × 3.0 mm ³

581

582 **Table 2. Demographic and clinical characteristics of the participants**

	Mean $\pm$ SD or n (%)
Age, years	74.5 $\pm$ 6.9
Female sex	29 (43.9%)
Height, cm	160.1 $\pm$ 8.9
Body mass, kg	54.6 $\pm$ 9.7
T2 relaxation time of femoral cartilage, ms	44.4 $\pm$ 5.6
Muscle volume, cm ³	
Quadriceps femoris	1144.4 $\pm$ 297.6
Rectus femoris	159.2 $\pm$ 41.8
Vastus medial	287.6 $\pm$ 79.7
Vastus lateralis	369.4 $\pm$ 98.7
Vastus intermedius	328.2 $\pm$ 91.5
IntraMAT, %	
Quadriceps femoris	7.7 $\pm$ 1.7
Rectus femoris	5.9 $\pm$ 1.5
Vastus medialis	7.9 $\pm$ 2.2
Vastus lateralis	8.2 $\pm$ 2.1
Vastus intermedius	7.7 $\pm$ 1.8

583 Intra-MAT: intramuscular adipose tissue, SD: Standard deviations

584

Table 3. Simple linear regression analysis revealing the correlations of the T2 relaxation time of the medial femoral cartilage and quadriceps muscle volume and IntraMAT

	Crude value				Adjusted value			
	B	β	95% CI	<i>p</i> value	B	β	95% CI	<i>p</i> value
Muscle volume, cm ³								
Quadriceps femoris	-0.010	-0.534	-0.014 to -0.006	<0.001	-0.007	-0.379	-0.016 to 0.002	0.127
Rectus femoris	-0.068	-0.504	-0.097 to -0.039	<0.001	-0.033	-0.244	-0.082 to 0.016	0.188
Vastus medialis	-0.036	-0.513	-0.051 to -0.021	<0.001	-0.020	-0.286	-0.053 to 0.012	0.220
Vastus lateralis	-0.029	-0.509	-0.041 to -0.017	<0.001	-0.016	-0.271	-0.039 to 0.008	0.183
Vastus intermedius	-0.031	-0.510	-0.045 to -0.018	<0.001	-0.017	-0.272	-0.045 to 0.012	0.244
IntraMAT, %								
Quadriceps femoris	0.584	0.180	-0.215 to 1.384	0.149	0.871	0.268	0.068 to 1.673	0.034
Rectus femoris	0.752	0.199	-0.170 to 1.675	0.108	1.154	0.306	0.238 to 2.071	0.014
Vastus medialis	0.904	0.345	0.289 to 1.518	0.005	0.876	0.334	0.209 to 1.543	0.011
Vastus lateralis	0.074	0.027	-0.613 to 0.761	0.830	0.463	0.168	-0.216 to 1.142	0.178
Vastus intermedius	0.365	0.119	-0.397 to 1.128	0.342	0.646	0.210	-0.107 to 1.399	0.091

A single linear regression analysis has been conducted with age, sex, height, and body mass, as the adjustment variables. The results with and without adjustments are presented as crude and adjusted values, respectively. IntraMAT: intramuscular adipose tissue, B: unstandardized regression coefficient, β : standardized regression coefficient, CI: confidence interval

590 **Table 4. Multiple linear regression analysis revealing the correlations of the T2 relaxation time of the medial femoral cartilage**
 591 **and quadriceps muscle volume and IntraMAT**

	B	β	95% CI	<i>p</i> value
Vastus medialis IntraMAT, %	0.876	0.334	0.209 to 1.543	0.011
Age, years	0.056	0.068	-0.127 to 0.239	0.546
Sex	-0.578	-0.051	-5.166 to 4.010	0.802
Height, cm	0.072	0.113	-0.155 to 0.298	0.529
Body mass, kg	-0.339	-0.585	-0.532 to -0.145	0.001

592 A multiple linear regression analysis has been conducted using a stepwise method, including the age, sex, height, and body mass, as adjustment
 593 variables. IntraMAT: intramuscular adipose tissue, B: unstandardized regression coefficient, β : standardized regression coefficient, CI: confidence
 594 interval

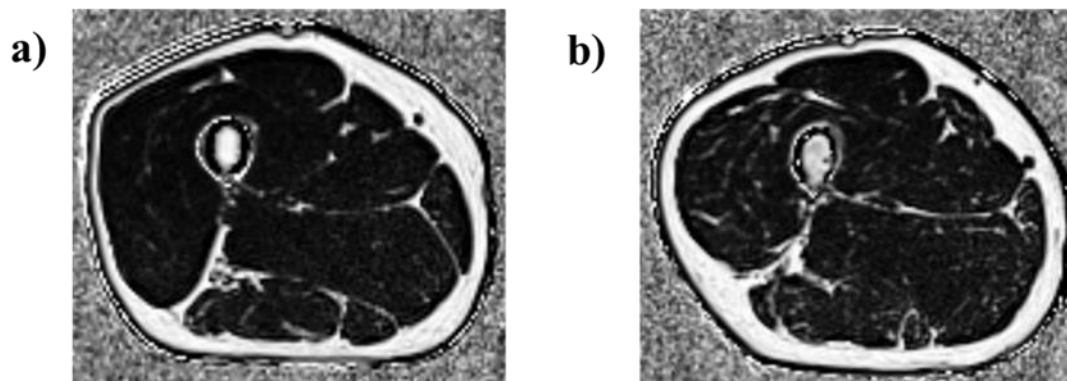


Fig. 1 Representative MR images of quadriceps muscles with low IntraMAT (%) and high IntraMAT (%)

Image (a) is indicated as quadriceps muscles with low IntraMAT (%), and image (b) is indicated as quadriceps muscles with high IntraMAT (%). The knee OA severity of participants (a) and (b) were both grade 1. The voxels with high signal intensity of MR images are equivalent to the intramuscular adipose tissue.

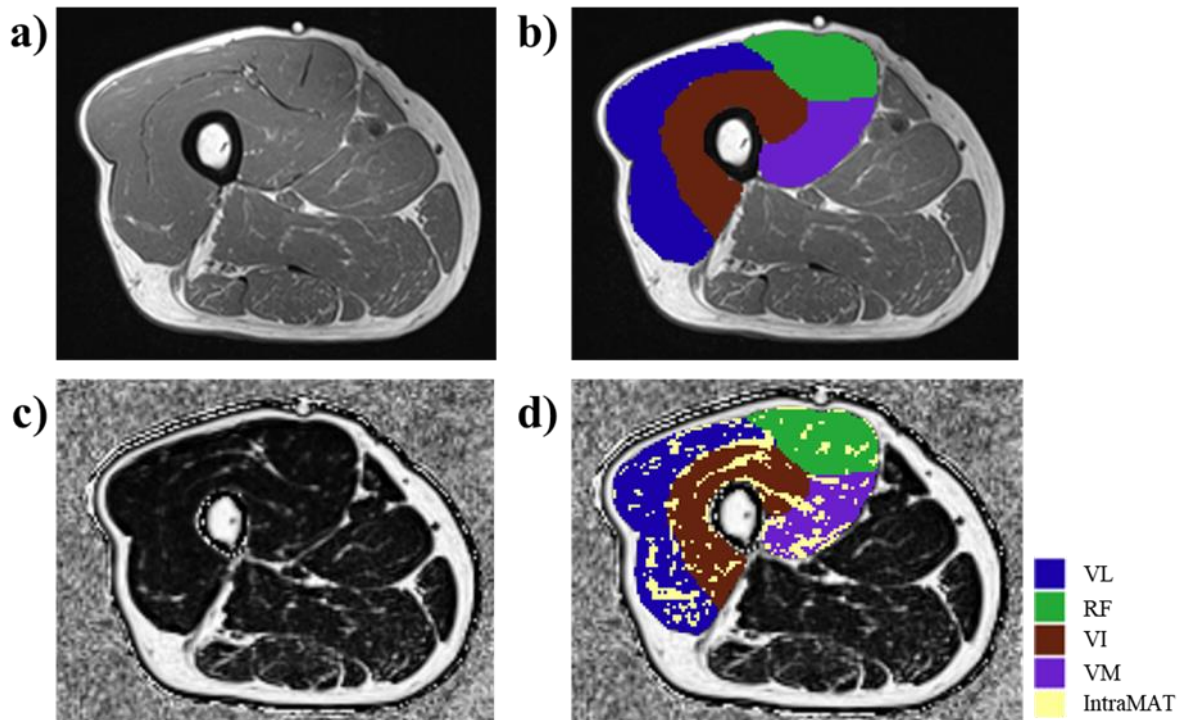


Fig. 2 The MR images to quantify the quadriceps muscle volume and intramuscular fat

a) To calculate the volume of each muscle (VL, RF, VI, and VM), T1 weighted images were used. b) The region of interests (ROIs) segmentation of all slices of the MR images were conducted. After the muscle cross-sectional area (CSA, cm^2) of each slice was measured, The CSA of each muscle multiplied by a slice thickness of 4 mm was summed as the muscle volume (cm^3). c) To calculate the IntraMAT of each muscle (VL, RF, VI, and VM), 2-point Dixon images were used. d) The ROIs were segmented on the slice at the midpoint, between the anterior superior iliac spine and the proximal end of the patella. These ROIs for each muscle were traced on five consecutive proximal and distal slices, with a total of 10 slices, and the mean values of IntraMAT of each muscle were calculated. VL: Vastus lateralis, RF: Rectus femoris, VI: Vastus intermedius, VM: Vastus medialis, IntraMAT: intramuscular adipose tissue

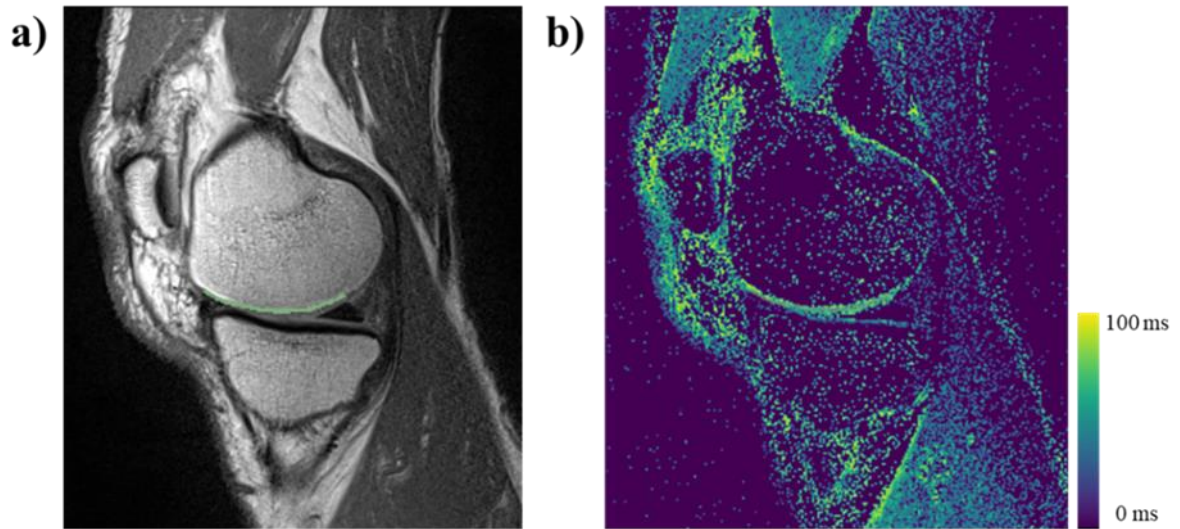


Fig. 3 The MR images to entire the T2 relaxation time of cartilage

a) The weight-bearing surface of the medial femoral was defined as the area between the anterior margin of the anterior horn of the meniscus and the posterior capsular attachment of the posterior horn of the meniscus [25]. The segmentation of region of interest (ROI) was performed manually on all slices of the medial femur. b) All ROIs were reflected on the T2 mapping image, and the means of the T2 relaxation times of all ROIs were calculated.