Osteoarthritis and Cartilage 24 (2016) 129–133

Osteoarthritis and Cartilage

Brief Report

Abnormal perfusion in patellofemoral subchondral bone marrow in the rat anterior cruciate ligament transection model of post-traumatic osteoarthritis: a dynamic contrast-enhanced magnetic resonance imaging study

P.-H. Tsai † ‡ §, H.-S. Lee ‖ ¶, T.Y. Siow # ††, C.-Y. Wang † ‡‡, Y.-C. Chang §§, M.-H. Lin ††, Y.-C. Hsu †, C.-H. Lee |||, H.-W. Chung ¶¶, G.-S. Huang † ## *

† *Department of Radiology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC*
‡ *Department of Radiology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, ROC*
§ *Department of Medical Imaging and Translational Imaging Research Center, Taipei Medical University Hospital, Taipei Medical University, Taipei, Taiwan, ROC*
‖ *Department of Pathology and Laboratory Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan, ROC*
¶ *Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC*
# *Department of Medical Imaging and Intervention, Chang Gung Memorial Hospital at Linkou, Institute for Radiological Research, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC*
†† *Institute of Biomedical Sciences, Academic Sinica, Taipei, Taiwan, ROC*
‡‡ *Department and Graduate Institute of Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan, ROC*
§§ *Department of Mathematics, Tamkang University, Taipei, Taiwan, ROC*
||| *Department of Orthopedics, School of Medicine, College of Medicine, Taipei Medical University and Hospital, Taipei, Taiwan, ROC*
¶¶ *Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan, ROC*
Department of Medical Research, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, ROC

ARTICLE INFO

Article history:
Received 27 February 2015
Accepted 21 July 2015

Keywords:
Post-traumatic osteoarthritis
Patellofemoral joint
Anterior cruciate ligament
DCE-MRI
Perfusion
Subchondral bone marrow

SUMMARY

Objective: Although anterior cruciate ligament (ACL) injury is a well-recognized risk factor for developing knee post-traumatic osteoarthritis (PTOA), the process in the patellofemoral (PF) joint after ACL injury is still under-researched. Our aim was to investigate the perfusion changes in PF subchondral bone marrow in the rat ACL transection (ACLX) model of PTOA using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

Design: Eighteen male Sprague Dawley rats were randomly separated into three groups ($n = 6$ each group): a normal control group and groups receiving ACLX and sham-surgery, respectively, in the right knee. Perfusion parameters in the patellar and femoral subchondral bone marrows of all rats were measured on DCE-MRI at 0, 4, 8, and 16 weeks after respective treatment. After the last MRI at week 16, the rats were sacrificed and their right knees were harvested for histologic examination. In addition, to observe the long-term histologic change in PF joints, 9 additional rats ($n = 3$ in each group) were included and sacrificed at week 32 for histologic examination.

Results: In the ACLX group vs the sham and control groups, the perfusion parameters were significantly changed in both patellar and femoral subchondral bone marrows at week 16. Histologic examination revealed cartilage defects in ACLX rats at 32 weeks after surgery.

Conclusions: These data point to a possible functional relationship between subchondral bone marrow perfusion abnormalities and cartilage breakdown in PTOA. Moreover, the perfusion parameters derived from DCE-MRI can potentially serve as biomarkers of early OA.

* Address correspondence and reprint requests to: G.-S. Huang, Department of Radiology, Tri-Service General Hospital, National Defense Medical Center, No. 325, Sec. 2, Cheng-kung Rd., Neihu 114, Taipei, Taiwan, ROC. Tel: 886-2-8792-7244; Fax: 886-2-8792-7245.
E-mail address: gsh5@seed.net.tw (G.-S. Huang).

http://dx.doi.org/10.1016/j.joca.2015.07.015
1063-4584/

Introduction

Post-traumatic arthritis (PTA) is a broad clinical term used to describe articular degeneration after any kind of joint injury, such as joint fractures, joint dislocations, ligament injuries, and cartilage injuries[1]. The progression of PTA may give rise to OA-like changes of joint cartilage, referred to in the literature as post-traumatic osteoarthritis (PTOA)[2]. Anterior cruciate ligament (ACL) injury is by far the most well known risk factor for developing PTOA. ACL insufficiency after the initial trauma causes chronic instability of the knee and leads to OA, most recognizably in the tibiofemoral (TF) joint where the major stress develops. TF joint PTOA after an ACL tear has been studied extensively; in contrast, the patellofemoral (PF) joint PTOA associated with ACL injury is relatively under-researched. However, this disease entity may be more common and important than once thought[3]. In one prospective cohort study[4], PF joint OA was present in 16% of knees 15 years after the acute ACL injury. PF joint PTOA is associated with increased functional deficits (i.e., flexion and extension) in ACL-deficient patients[4]. PF joint OA is even more prevalent (47%) than TF joint OA (31%) in patients receiving autograft ACL reconstruction[5,6]. The long-term symptoms and functional deficits in these patients correlate with PF joint OA severity but independent of the TF joint OA severity. In a broader sense, regardless of the etiology, PF joint OA is an important source of anterior knee pain[7].

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) provides a valuable noninvasive method to measure bone marrow hemodynamics and derive several crucial perfusion parameters. A previous DCE-MRI study successfully demonstrated significant alteration of perfusion in a spontaneous TF joint OA animal model[8]. A similar change can be anticipated in TF joints after ACL injury based on the radiographic similarity of PTOA and primary OA. A preliminary study[9] has already shown that the DCE-MRI derived hemodynamic parameters change significantly in the TF joints of ACL-injured human subjects. Nonetheless, it would be more intriguing to assess hemodynamic change in the more subtle, late onset, yet equally important PF joint OA after ACL injury using DCE-MRI, in the hope of developing hemodynamic change as an early biomarker for PF joint PTOA. Therefore, the purpose of present study is to use DCE-MRI to investigate the subchondral bone marrow hemodynamics of the PF joints in rat model of knee OA induced by ACL transection (ACLX) and to correlate DCE-MRI findings with histologic examination.

Materials and methods

Ethics statement

The experiments were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The applied protocol was approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center (Permit Number: IACUC-06-103).

Animal preparation

Eighteen male Sprague Dawley rats aged 8 weeks and weighing around 300 g were randomly separated into three groups ($n = 6$ for each group): the normal control group without intervention, the ACLX group where the right ACL was transected as previously described[10] while the left ACL was left intact, and the sham-control group where the skin of the right knee was surgically wounded, while the left knee was left intact. The rats were housed (two rats per cage) in a sanitary ventilated room on a 12-h light–dark cycle within a temperature range of 21 ± 2°C, with free access to tap water and standard chow. The time when the rats received respective treatment was designated as week 0. The right knees of all rats were assessed using DCE-MRI at week 0, 4, 8, and 16. After the last MRI, all the rats were sacrificed and their right knees harvested for histologic examination. In addition, to observe the long-term histologic change of PF joint, 9 supplementary rats ($n = 3$ in each group) were included in the study, with DCE-MRI performed at week 0, 4, 8, 16, and 32 and sacrifice performed at the end of the MRI experiments for histologic examination.

DCE-MRI

The rats were first anesthetized by inhalation of an isoflurane–oxygen mixture. A birdcage coil with an inner diameter of 72 mm was used as the transmitter coil, and a separate quadrature surface coil (Bruker, Ettlingen, Germany) was placed above both knee joints to achieve maximum signal reception. The entire device was placed in an Oxford Instruments (Bruker) 200/300 magnet (4.7 T, 33 cm clear bore) equipped with an actively shielded Oxford gradient coil (16 cm inner diameter, 18 G/cm, 200 μs rise time).

After three-plane tripilot imaging, 10 contiguous sagittal T_2-weighted images (T2WIs) were acquired for the purpose of later slice positioning. In the subsequent imaging, four contiguous axial imaging planes (slices) were placed nearly perpendicular to the patella cartilage with the prior obtained right knee mid-sagittal T2WIs as reference images [Fig. 1(a)].

DCE-MRI was performed as dynamic T_1-weighted images (T1WIs) using a fast gradient echo sequence with repetition time = 100 ms, echo time = 3.5 ms, slice thickness = 1 mm, matrix size = 128 × 128 (zero-filled to 256 × 256), in-plane resolution = 156 × 156 μm^2, number of excitation = 1, flip angle = 90°, bandwidth = 37.879 kHz, and acquisition time = 6 min 24 s. The rats received a bolus injection of gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA) (Magnevist; Bayer Healthcare Pharmaceuticals, Wayne, NJ, USA) via jugular vein at a dose of 0.2 mmol/kg during the 10th image acquisition. The temporal resolution is 6.4 s.

Data analysis

The Gd-DTPA-enhanced kinetic signals were analyzed based on the Brix two-compartment pharmacokinetic model[11–13]. Regions of interest (ROIs) were drawn manually on the patella and femoral subchondral bone marrows with reference to the first DCE-MRI image acquired at the middle level of the PF joint as shown in Fig. 1(b). To minimize manual discrepancies in the positioning of the ROIs, the ROIs were drawn by two image analysts well trained in knee MRI (PHT, CYW) and their positions were confirmed by an experienced musculoskeletal radiologist (GSH). Results shown in this study are the mean of two measurements. Mean signal intensities of the ROIs at each imaging frame were calculated. Three perfusion parameters were obtained from the time–intensity curve fitting: amplitude (A), rate constant (k_{ep}), and elimination constant (k_{el}) as previously described[13].

Histologic examination

All rats were sacrificed at the end of the MRI experiments (18 rats at week 16 and 9 rats at week 32). Right PF joints were removed, fixed in neutral formalin, and decalcified in a rapid decalcifier (Nihon Shiyaku Industries, Osaka, Japan). After decalcification, the PF joint tissues were cut in half along the mid-axial plane. Samples from each joint were paraffin-embedded, and cut into 5-μm sections for hematoxylin and eosin (H&E) staining.

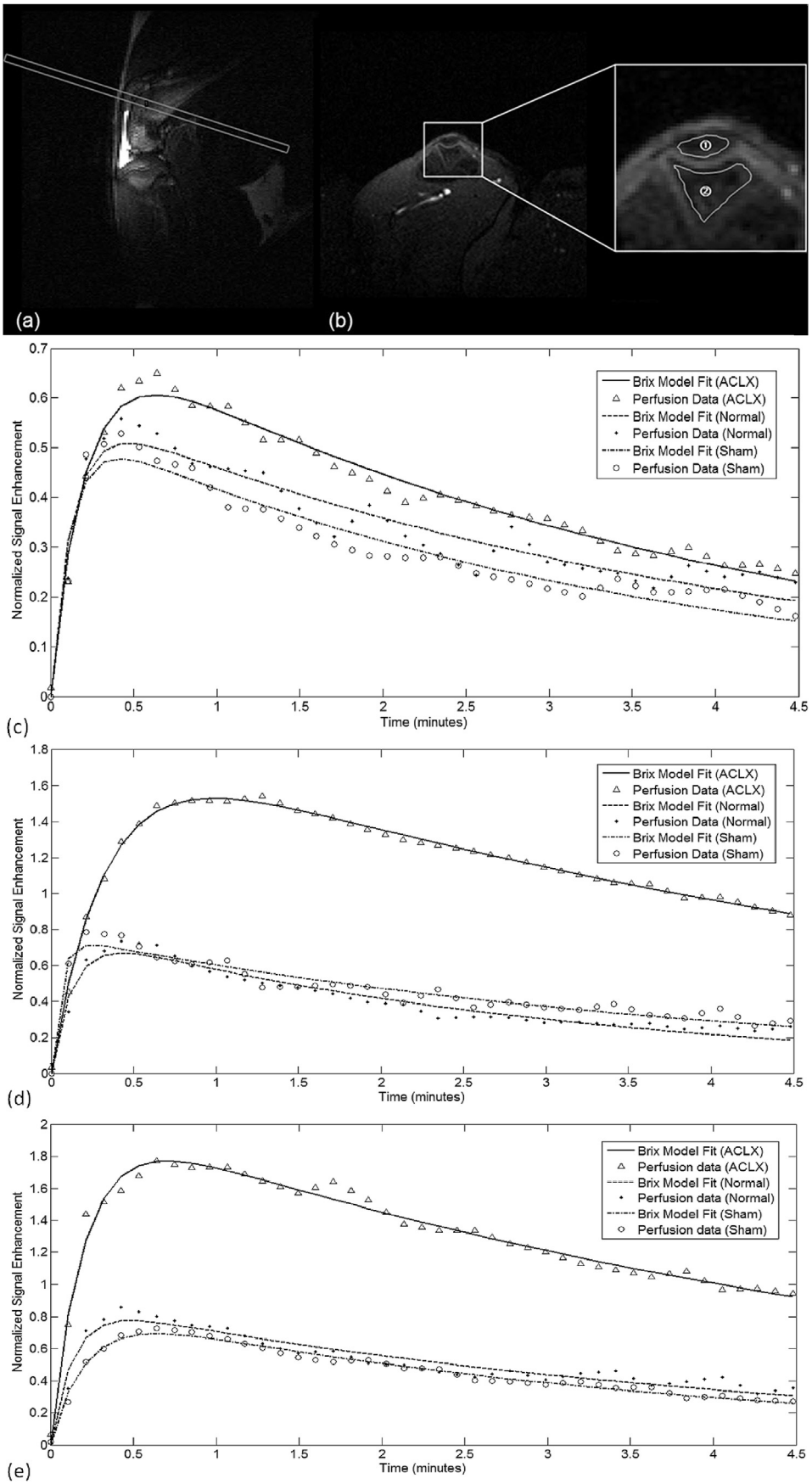

Fig. 1. DCE-MRI study. (a) Target slice for the perfusion measurement in the mid-sagittal plane and (b) ROIs selected for subsequent calculations of the perfusion parameters: ① patellar bone marrow ② femoral bone marrow. Moreover, curve fitting of the normalized MR dynamic signal enhancement data of the patellar bone marrow in rats from the three groups at 0 weeks (c), 16 weeks (d), and 32 weeks (e) are shown respectively. Compared to baseline values, inflow is altered and outflow reduced at 16 and 32 weeks after ACL surgery.

Statistical analysis

The mean and the standard deviation (SD) of the derived parametric perfusion data were first calculated in each group. To compare differences in the longitudinal effects of the parametric perfusion indices among groups due to their dependence on repeated measurements, the GEE (Generalized Estimating Equations) method's multiple linear regression was used to assess the interaction of groups (ACLX, normal, and sham) with time (0, 4, 8, and 16 weeks). SPSS v21.0 software (SPSS, Chicago, IL, USA) was used to analyze the results of GEE with a first-order autoregressive correlation structure. The normality of the data and homogeneity of variances were checked using the Shapiro–Wilk test and Leven's test, respectively. *P*-values < 0.05 were regarded as statistically significant.

Results

DCE-MRI

Fig. 1(c–e) shows representative contrast enhancement kinetic curves of the patellar subchondral bone marrow in ACLX rats at week 0 [Fig. 1(c)], week 16 [Fig. 1(d)] and week 32 [Fig. 1(e)]. As shown in Fig. 1(c), peak signal enhancement was rapid (i.e., reached within 1 min after contrast medium injection) and followed by a gradual fade, indicating a rapid inflow and fairly slow washout of the contrast medium in the patellar subchondral bone marrow. On the other hand, perfusion MRI at week 16 resulted in a slightly delayed time to peak enhancement with more gradual and slower washout, indicating altered rates of contrast medium inflow and washout rate in the subchondral bone marrow. A similar perfusion pattern was observed in the PF joint of ALCX rats at week 32 [Fig. 1(e)].

The derived perfusion parameters, i.e., femoral A, k_{ep}, k_{el} and patellar A, k_{ep}, and k_{el} values, were analyzed statistically (Supplemental materials). For the femoral subchondral bone marrow, the perfusion parameters (A, k_{ep}, k_{el}) were initially similar between groups (all *P*-values >0.05), not significantly changed at week 4, 8, and 16 compared to baseline values in the control and sham groups (all *P*-values >0.05), and significantly changed at week 16 (*P*-values <0.001, =0.012, and =0.013, respectively), but not at week 4 and 8 (all *P*-values >0.05) in the ACLX group. The perfusion parameters were similar between sham and control groups at all time points. Similarly for the patellar subchondral bone marrow, A and k_{el} values were significantly changed at week 16 ($P = 0.002$, and =0.024, respectively) but not at week 4 and 8 (all *P*-values >0.05), and k_{ep} values were notably similar between the ACLX and control groups (*P*-value = 0.201) at week 16.

Histologic examination

Fig. 2(a) shows representative H&E-stained sections of PF joints in control, sham, and ACLX rats at week 16. In both the patellar and femoral subchondral bone marrow, pale-staining zones indicative of accumulated edema fluid and plasma were present in ACLX rats but not in control and sham rats. The PF joint cartilage surface was intact in all three groups at week 16. Fig. 2(b) shows histologic sections of PF joints at week 32. Notably at 32 weeks, the joint in ACLX rats showed matrix degeneration with irregular cartilage surface, hypertrophic chondrocyte, as well as subchondral bone marrow edema. The joint synovial membrane showed mild synovitis with inflammatory infiltration of a few lymphocytes.

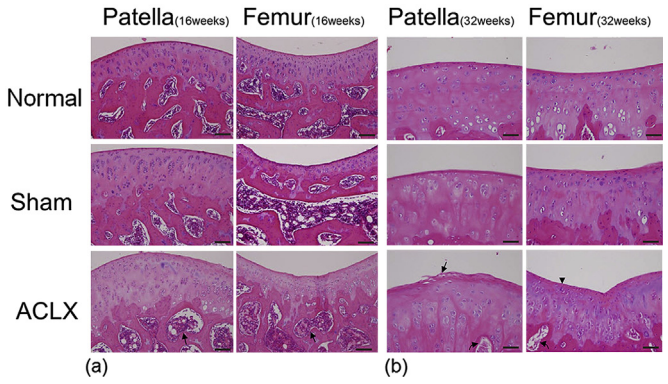

Fig. 2. Histopathology of PF joint cartilage. (a) Subchondral bone marrow edema with plasma accumulation (arrows) in the patella and femur was seen only in ACLX rats at 16 weeks after transection. The overlying cartilage was relatively intact. Original magnification 200× (scale bars, 50 μm) (b) At 32 weeks after ACLX, matrix degeneration with irregular cartilage surface (arrow), and hypertrophic chondrocytes (arrowhead), as well as subchondral bone marrow edema (curved arrows) were recognized in ACLX rats, when compared to normal and sham-control rats. Original magnification, 400× (scale bars, 25 μm).

Discussion

In the rat ACLX model, our study demonstrates a perturbation of perfusion in the PF subchondral bone marrow using DCE-MRI and corresponding edematous change using histopathological methods. The perfusion alteration in PF subchondral bone marrow precedes the change in cartilage morphology first observed at week 32 after ACLX. These findings echo the previous reported results[14], which consistently show that perfusion abnormalities of subchondral bone marrow developed in the early stage of OA.

In the present study, the elimination constant k_{el} decreased significantly in both patellar and femoral subchondral bone marrow in the ACLX rat. The reduced k_{el} indicates prolonged retention of contrast medium in the interstitium, which most likely reflects impaired venous outflow. This is consistent with previous work[8,14–16] showing venous outflow obstruction as the primary perfusion parameter change in human and animal OA studies. In addition, the present study also revealed a significant alteration of the inflow-related parameters in the PF joint of the ACLX rat, including elevated amplitude A and reduced rate constant k_{ep}. The amplitude A is a relatively non-specific perfusion parameter, which could be affected by several factors[8]. On the other hand, the rate constant k_{ep} represents a ratio of permeability surface area product per unit volume relative to the size of the interstitial space, and its reduction can be readily attributed to decreased capillary endothelial permeability or increased intraosseous pressure, both of which limit the transfer of molecules from the capillary bed to interstitial space[8]. Collectively, alterations in these perfusion parameters could infer that high intraosseous pressure, possibly related to venous outflow obstruction, result in impaired vascular inflow to the PF subchondral bone marrow in ACLX rats.

Despite their differing pathogenesis, PTOA and primary OA share similar histologic and radiographic features. Nonetheless, extrapolation of the “PTOA” condition in the ACLX model to that in primary OA, or vice versa, should be treated with caution. Further perfusion study in PTOA models as well as other OA models involving injury to both TF and PF joints could provide more insight into these disease entities.

Several limitations in the present study should be noted. First, manual injection of contrast medium during DCE-MRI acquisition may introduce variability in the lag time, rate and total dose of contrast medium administered. Second, the ROI-based approach

used in the present study for analysis of DCE-MRI data precludes a voxel wise comparison; therefore, subtle variation within the same ROI may be masked. Third, the present study did not carry out histology at each point to correlate histologic change with DCE-MRI change. In addition, the sample size used to determine the long-term histologic change in the PF joint after ACLX was relatively small. Fourth, the investigation of the perfusion change in TF joint PTOA was not included in the present study, and a comprehensive DCE-MRI study of TF joints after ACLX will be carried out in the future.

In conclusion, the present study demonstrated an early perfusion perturbation in the PF joint of rat after ACLX, which precede the morphological destruction of the articular cartilage. These data add to the growing body of evidence shedding light on a possible functional relationship between subchondral bone marrow perfusion abnormalities and cartilage breakdown. Moreover, the perfusion parameters derived from DCE-MRI can potentially serve as imaging biomarkers to enable early diagnosis of OA.

Contributions

Conception and design: Guo-Shu Huang.

Collection and assembly of data: Ping-Huei Tsai, Herng-Sheng Lee, Chao-Ying Wang, Ming-Huang Lin, Guo-Shu Huang.

Analysis and interpretation of data: all authors.

Statistical analysis: Ping-Huei Tsai, Yue-Cune Chang.

Drafting of the article: Ping-Huei Tsai, Tiing Yee Siow, Guo-Shu Huang.

Critical revision: Ping-Huei Tsai, Herng-Sheng Lee, Tiing Yee Siow, Guo-Shu Huang.

Final approval: Ping-Huei Tsai, Herng-Sheng Lee, Tiing Yee Siow, Guo-Shu Huang.

Obtaining funding: Guo-Shu Huang.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

This study was supported by a grant from the National Science Council of Taiwan (NSC96-2628-B-016-008-MY3).

Supplemental materials

Supplemental materials related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.07.015.

Reference

1. Marsh JL, Buckwalter J, Gelberman R, Dirschl D, Olson S, Brown T, *et al*. Articular fractures: does an anatomic reduction really change the result? J Bone Joint Surg Am 2002;84-A:1259–71.
2. Buckwalter JA, Anderson DD, Brown TD, Tochigi Y, Martin JA. The roles of mechanical stresses in the pathogenesis of osteoarthritis: implications for treatment of joint injuries. Cartilage 2013;4:286–94.
3. Lohmander LS, Ostenberg A, Englund M, Roos H. High prevalence of knee osteoarthritis, pain, and functional limitations in female soccer players twelve years after anterior cruciate ligament injury. Arthritis Rheum 2004;50:3145–52.
4. Neuman P, Kostogiannis I, Friden T, Roos H, Dahlberg LE, Englund M. Patellofemoral osteoarthritis 15 years after anterior cruciate ligament injury–a prospective cohort study. Osteoarthritis Cartilage 2009;17:284–90.
5. Culvenor AG, Lai CC, Gabbe BJ, Makdissi M, Collins NJ, Vicenzino B, *et al*. Patellofemoral osteoarthritis is prevalent and associated with worse symptoms and function after hamstring tendon autograft ACL reconstruction. Br J Sports Med 2014;48:435–9.
6. Cohen M, Amaro JT, Ejnisman B, Carvalho RT, Nakano KK, Peccin MS, *et al*. Anterior cruciate ligament reconstruction after 10 to 15 years: association between meniscectomy and osteoarthrosis. Arthroscopy 2007;23:629–34.
7. Hinman RS, Crossley KM. Patellofemoral joint osteoarthritis: an important subgroup of knee osteoarthritis. Rheumatology (Oxford) 2007;46:1057–62.
8. Lee JH, Dyke JP, Ballon D, Ciombor DM, Rosenwasser MP, Aaron RK. Subchondral fluid dynamics in a model of osteoarthritis: use of dynamic contrast-enhanced magnetic resonance imaging. Osteoarthritis Cartilage 2009;17:1350–5.
9. Zuo J, Majumdar S, Li X. Perfusion abnormalities of bone marrow edema-like lesions in knees with anterior cruciate ligament injury using dynamic contrast-enhanced MRI (Abstract). Proc Intl Soc Mag Reson Med 2010;18:802.
10. Tsai PH, Lee HS, Siow TY, Chang YC, Chou MC, Lin MH, *et al*. Sequential change in T2* values of cartilage, meniscus, and subchondral bone marrow in a rat model of knee osteoarthritis. PLoS One 2013;8:e76658.
11. Seah S, Wheaton D, Li L, Dyke JP, Talmo C, Harvey WF, *et al*. The relationship of tibial bone perfusion to pain in knee osteoarthritis. Osteoarthritis Cartilage 2012;20:1527–33.
12. Hoffmann U, Brix G, Knopp MV, Hess T, Lorenz WJ. Pharmacokinetic mapping of the breast: a new method for dynamic MR mammography. Magn Reson Med 1995;33:506–14.
13. Brix G, Semmler W, Port R, Schad LR, Layer G, Lorenz WJ. Pharmacokinetic parameters in CNS Gd-DTPA enhanced MR imaging. J Comput Assist Tomogr 1991;15:621–8.
14. Aaron RK, Dyke JP, Ciombor DM, Ballon D, Lee J, Jung E, *et al*. Perfusion abnormalities in subchondral bone associated with marrow edema, osteoarthritis, and avascular necrosis. Ann N Y Acad Sci 2007;1117:124–37.
15. Imhof H, Breitenseher M, Kainberger F, Rand T, Trattnig S. Importance of subchondral bone to articular cartilage in health and disease. Top Magn Reson Imaging 1999;10:180–92.
16. Kiaer T, Pedersen NW, Kristensen KD, Starklint H. Intraosseous pressure and oxygen tension in avascular necrosis and osteoarthritis of the hip. J Bone Joint Surg Br 1990;72:1023–30.